PHARMACEUTICAL ANALYSIS AND DIABETES MELLITUS - A REVIEW

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Received: 10 October 2018, Revised and Accepted: 11 December 2018

ABSTRACT

Context: Current review focuses on the importance of pharmaceutical analytical techniques in the diagnosis of diabetes and the study of antidiabetic drugs.

Objective: The main objective of the review is to compile the different analytical tools such as UV-spectroscopy, Fourier-transform infrared (FT-IR), nuclear magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS), gas chromatography (GC)-MS, and electrophoresis that are used to diagnose diabetes using different analytical methods. It also attempts to provide information on the assay of antidiabetic drugs by various analytical techniques and thus helps in the development of novel methods for the study of newer drugs molecules having antidiabetic activity.

Methods: The blood sample is subjected to centrifugation to collect the serum before any analytical estimation of antidiabetic drugs. The different types of diabetes were diagnosed using various analytical techniques such as UV-spectroscopy, FT-IR, NMR, LC-MS, GC-MS, and electrophoresis.

Results: Different analytical methods that were reported in the literature, were succinctly discussed about the quantification of various antidiabetic drugs. These methods were also employed for the determination of antidiabetic drugs in the research field for the study of active pharmaceutical ingredients, marketing dosage forms, and post-marketed dosage forms.

Conclusion: This review is compiled in such a manner such that it helps the analysts to diagnose diabetes using different analytical techniques and also to study various antidiabetic drugs in the pharmaceutical research field. It also attempts to provide information about different active pharmaceutical ingredients, marketing dosage forms, and post-marketed dosage forms.

Keywords: Diabetes, Pharmaceutical analysis, Diagnosis, Antidiabetic drugs, UV spectroscopy, Fourier-transform infrared, Nuclear magnetic resonance, High-performance liquid chromatography, Electrophoresis.

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INTRODUCTION

Diabetes mellitus (DM) is caused due to the insufficient secretion of insulin. DM is considered as a metabolic disorder due to the long-term tissue or organ damage. It may also occur due to the damage of kidney or vascular system or nervous system [1]. In diabetes, blood sugars levels are increased for a prolonged period due to the group of metabolic diseases [2]. Diabetes is also characterized by a reduction in the protein, carbohydrate, and fat metabolism due to the insulin deficiency, thus causing chronic hyperglycemia [3].

According to the WHO guidelines, diabetes is classified into several types.

DM
Regardless of reasons DM is divided into two types.

a. Insulin-dependent DM (IDDM): To control the body metabolism insulin is required. Insulin secreted in the body is not sufficient to control the metabolism; hence, insulin is administered into the body externally to get normal glycemic levels.
b. Non-IDDM: This type of diabetes can be controlled with the help of oral hypoglycemic drugs which, in turn, can control blood glucose levels.

Impaired glucose tolerance (IGT) or impaired glucose regulation and impaired fasting glycemia (IFG)
The terms IGT and IFG, both are different, where IGT measure glucose levels in post-prandial state and IFG measures glucose levels in fasting conditions. A stage of IGT indicates the progress in the risk of diabetes, macrovascular, and cardiovascular disorders. IFG indicates cardiovascular disorder. IFG levels are always less than the normal glucose levels because it is usually measured under fasting condition [3].

If IFG levels 100–125 mg/dl or 5.6 mmol-6.9 mmol/L and IGT levels are in the range of 140–199 mg/dl are considered acceptable [4].

Normoglycemia
Fasting glucose levels around 6 mmol/L are considered as normal levels. Such results also may have IGT or normal glucose tolerance. The values beyond this level lead to macro- or micro-vascular diseases [3].

Etiological types
Based on a-etiological factors diabetes is divided into the following types:

Type-1 diabetes
Type-1 diabetes is usually progresses due to the dysfunction of β cells present in the pancreas, leads to a deficiency in insulin production. β-Cells are destroyed by anti-glutamic acid decarboxylase, islet cell or insulin antibodies. Due to this condition absolute insulin deficiency occurs [3,5].

Type-II diabetes
Insulin resistance and deficiency of relative quantities of insulin are characteristic features of Type-II diabetes. Both can be present at a
time and either of the conditions predominant clinically. The reason for this type is unknown even though this is a common type of diabetes [3].

**Gestational hyperglycemia**

It occurs during the pregnancy and may improve or recover after the delivery. Gestational hyperglycemia condition is similar to that of Type-II diabetes. Gestational diabetes causes health issues in the fetus or pregnant women. After the birth, the child may have congenital cardiac and central nervous system disorders, malformations of skeletal muscle, heavy weight and respiratory distress syndrome. Sometimes perinatal death may also occur due to the hyperglyburinemia [2,3]. In the first trimester of pregnancy, the risk of gestational diabetes is very much high. In general, the diagnosis of gestational diabetes is carried out between 24 and 28 weeks of pregnancy [3].

**Complications of diabetes**

Based on the complications diabetes can be divided into two types.
1. Acute problems
2. Chronic complications.

**Acute problems**

It also termed as a medical emergency in diabetes, for example, diabetes ketoacidosis and hypoglycemia.

**Chronic complications**

It includes microvascular complications and macrovascular complications. It includes hyperglycemia and hypoinsulinemia or other metabolic disorders associated with them.
1. Microvascular problems: This also called as microangiopathic. This includes diabetes retinopathy, diabetic neuropathy, and some diabetic skin problems.
2. Macrovascular problems: This includes hypertension, arteriosclerosis, and cerebrovascular disease.
3. Other associated metabolic abnormalities: Other abnormalities include hypercholesterolemia.

**Signs and symptoms**

Polyuria, weight loss, polydipsia, and polyphagia are common symptoms of diabetes. Along with this blurry vision, headache, fatigue, reduced healing capacity, and itchy skin are also observed. Sometimes dehydration may also occur [2].

**Prevention**

It is always said that prevention is better than cure. Type-II diabetes can be prevented by physical exercise, maintaining normal weight, and diet regulation. Healthy diet includes rich in whole grain, fiber, good fats present in vegetable oils, nuts, and fish. Smoking and consumption of alcohol increase the occurrence of diabetes and hence should be avoided [2].

**Treatment**

a. Type-I diabetes: Insulin or semi-synthetic insulin is administered according to the weight, age, and sex of the patient.
b. Type-II diabetes: Metformin is the most widely used drug for this type of diabetes. Other types of oral hypoglycemic drugs are also prescribed depending on the conditions. In addition, angiotensin-converting enzyme inhibitors can also be used in the treatment of this type of diabetes [2].

treatment

a. Type-I diabetes: Insulin or synthetic insulin is administered according to the weight, age, and sex of the patient (Fig. 1).
b. Type-II diabetes: Metformin is the most preferred drug for this type of diabetes (Fig. 2). Angiotensin-converting enzyme inhibitors are also used for the treatment Type-II diabetes [2,6].

**Study of antidiabetic drugs**

Study of antidiabetic drugs can be carried out by various analytical techniques in the pharmaceutical research field from drug development to marketing and post-marketing. Study of active pharmaceutical ingredients, dosage forms, impurities, intermediate products, diagnosis of diseases, biological samples, and degradation products can also be performed. Analytical techniques include titrmetry, spectroscopy, chromatography, electrophoresis, and other electrical methods [7].

Pharmaceutical analytical techniques used in the study of drugs.

**Titrimetry and electrical methods**

Titrmetric methods include acid-base titration, oxidation-reduction, complexometry, precipitation titration, non-aqueous titrations, and diazotization titration. These methods are conventional methods and were used in the analysis in earlier days. Now, they have been replaced by electrical methods such as potentiometry, conductometry, and amperometry. Using these methods then there is an improvement in the endpoint detection, accuracy, and precision; in addition, it saves the labor, cost incurred, and time. Titrimetric methods can also be used for the estimation of degradation products [7]. for example, metformin hydrochloride (non-aqueous titration) [8], tolazamid (non-aqueous titration) [9], and pioglitazone HCl (Acid-base titration) [10].

**Chromatographic techniques**

Chromatography is an important analytical tool used for the separation of compounds according to their affinity toward the stationary phase. No single molecule have the same affinity due to difference in their molecular structure or composition [11].

**Thin-layer chromatography (TLC)**

In the TLC method, a thin layer of 0.25 mm thickness solid adsorbents acts as a stationary phase. To increase the adherence capacity of adsorbent to plate’s binders is mixed with adsorbents as listed in Table 1 while preparing the slurry. The plates or sheets used for the preparation of TLC plates are made out of glass, plastic, or aluminum. Sample molecules are dissolved in suitable solvents, and the sample solution is applied to TLC plates with the help of micropipette at the bottom of the plate. TLC plate is introduced into the chamber which consists of mobile phase usually as a mixture of solvents. Mobile phase moves over the plate with the help of capillary action, and as a result, separation of molecules occurs according to the affinity.

This method used for the high degree of separation and purification of organic and inorganic samples with low cost. It also used for determination of impurities [7,11], for example, metformin [12-14], pioglitazone hydrochloride [15], glibencamide, glimepiride [15,16], dasatinib [17], chlorpropamide [18], rosiglitazone [19], and nateglinide [14].

**High-performance liquid chromatography (HPLC)**

HPLC is the most preferred method of analysis in recent times. HPLC is a powerful qualitative and quantitative analytical technique, which are able to separate similar analytes present in the mixtures. Separation of molecules occurs in the column in short time under high pressure [7,11]. Simultaneous estimation of compounds also possible using HPLC, for example, tolbutamide [20], glibencamide [21,22], glipizide [22], glimepiride [22], acarbose [23], miglitol [24], bromocriptine [25], alogliptin [26], metformin [26,27], abilidn [28], liraglutide [29], linaglipin [30], saxagliptin [27], sitagliptin [31], nateglinide [32,33], exenatide [34], repaglinide [35], dapagliflozin [36], canagliflozin [37], glimepiride [38], glyburide [39], chlorpropamide [40], tolbutamide [41], rosiglitazone [42], and pioglitazone hydrochloride [43].

**Gas chromatography (GC)**

GC is used for the separation of volatile compounds. Gas LC plays an important role in the analysis of pharmaceutical dosage forms. Impurities and residual solvents present in traces amount also detected by GC [11], for example, metformin [44] and chlorpropamide [45].
SPECTROSCOPIC METHODS

Ultraviolet-visible spectroscopy
In UV spectrophotometry, the excitation of electrons present in the sample molecules occurs easily by absorption of high energy of light in the range of 200–800 nm. The molecules with conjugated π-electrons are able to absorb the radiation in this region [11]. In this type of spectroscopy, reflection or transmission properties of molecules are used for the quantitative analysis as a function of wavelength, for example, glibenclamide [46,47], metformin [8,48], acarbose [49], miglitol [49,50], alogliptin [50], linagliptin [51], saxagliptin [52], sitagliptin [53], nateglinide [54], repaglinide [54], miglitol [55], canagliflozin [57], empagliflozin [58], glimepiride [59], glipizide [60], and chlorpropamide [61].

Infrared (IR) spectroscopy
Molecules or atoms produce vibrational and rotational excitation by absorbing energy radiation in the IR region. The different bands produced correspond to characteristic absorption of radiation and thus helps to identify different functional groups present in the drug molecules. Qualitative, quantitative, multi component analysis and simultaneous estimation are possible by using IR spectroscopy [7,11], for example, metformin [62].

Fluorimetry
Fluorescence spectroscopy is used for the quantitative analysis of drug molecules present in drug products and biological samples. This method gives precise and sensitive results [11], for example, rosiglitazone maleate [63] and nateglinide [64].

Electrophoresis
Electrophoresis method has become an important technique in pharmaceutical analysis. Capillary electrophoresis (CE) is the best method to separate charged particles through the capillary tube in the presence of an electric field. CE is used for the qualitative and quantitative analysis of charged molecules. This method requires small amount of sample in aqueous condition. Biopolymers and inorganic ions can be separated by CE [7], for example, sitagliptin [65] and metformin [65].

HYHPHENATED TECHNIQUES

HPLC–mass spectrometry (MS)
HPLC connected with MS with various interfaces. Effluent from the column of HPLC enters into the MS through the interface. In MS, ions are generated and sent to electron multiplier tubes. Ionization occurs using two methods, namely Atmospheric pressure chemical ionization and ESI or APESI: (Atmospheric pressure) or electrospray ionization. Drugs molecules, degradants identification, characterization of impurities, and separation of components occur by LC-MS method [7,11], for example, acarbose [66], miglitol [24], glimepiride [67], dapagliflozin [68], canagliflozin [69], Linagliptin [70], sitagliptin [71], saxagliptin [72], and bromocriptine [73].
Table 1: Types of adsorbent

<table>
<thead>
<tr>
<th>S. No</th>
<th>Type of adsorbent</th>
<th>Adsorbent</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Most strong adsorbent</td>
<td>Alumina</td>
<td>Al₂O₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Charcoal</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>Least strong adsorbent</td>
<td>Silica gel</td>
<td>SiO₂</td>
</tr>
</tbody>
</table>

GC-MS
GC connected with MS with various interfaces. Effluent from the column of GC enters into the MS through the interface. Identification of drug molecules and their degradants, characterization of impurities, bioassay, and separation of components can be analyzed using GC-MS method, for example, metformin [74].

DIAGNOSIS
Diagnosis of diabetes can be done by determining the blood glucose levels. In fasting conditions, the blood glucose level could be >6.7 mmol/L or random glucose levels will be more than 10 mmol/L considered as diabetes. If there occur any doubts in the diagnosis, glucose tolerance test have to be conducted to measure the blood glucose level. Before the test, the patient needs to be on fast at least for 10–12 h. During the test, the patient is advised to take 75 mg glucose orally and the test will be repeated after 2 h. Thus, from the results observed, one can determine the glucose tolerance of the patient [5].

In the diagnosis process, glucose levels can be determined using various analytical methods.

UV spectroscopy
The glucose level of the blood can be determined by comparison of the UV spectrum of normal serum and diabetic blood serum. Diabetes leads to changes in the metabolism of fats, carbohydrates, and proteins. Blood samples are usually collected from normal and diabetic people, and the serum is separated by centrifugation of the blood samples. The separated serum will further be diluted with deionized water and absorbance of the spectrum will be analyzed [75].

Fourier-transform (FT)-IR
Diabetic conditions induce alterations in the content of macromolecules and their concentrations, changes in the structure and size of tissues. FT-IR is used to determine these changes. Renal plasma membrane was separated, and fats were removed from tissue at 0 to 4°C. In general, the tissue will be placed in mannitol and buffer for homogenization. After homogenization, the sample will be mixed with calcium chloride solution (10 mM) and diluted with buffer and centrifuged for 12 min. The sediment separated will be dissolved in buffer solution and recentrifuged again. The sediment, thus, will be dissolved in the supernatant, and this sample solution will be analyzed using FT-IR windows [76].

NMR
Identification of metabolite is usually done using NMR spectroscopy in diabetes. The collected urine samples are stored in frozen condition. The frozen urine sample is maintained in ice for 30 s, and 500 μL of the sample will be mixed with buffer. The NMR spectrum for this sample solution will be taken and the spectrum thus obtained will be compared with the spectra of normal urine and diabetic patient’s urine [77].

Gel electrophoresis
Type-I diabetes affects the function of the salivary gland. Protein modification in the saliva indicates Type-I diabetes. After 2 h of breakfast make the subject to rinse mouth with water and paraffin has to be chewed. The saliva was collected for 5 min in a tube containing protease inhibitor, and it is stored at ~20°C. The sample solution was applied to the gel electrophoresis strips were applied with the sample solution and allow to equilibrate in buffer solution for 15 min. A constant voltage is applied to the strips, and the proteins are allowed proteins to separate. Gel images are digitalized. The comparison of healthy subject saliva protein with diseased subject saliva can be helpful in the determination of Type-I diabetes [78].

LC-MS and GC-MS
For carrying out the analysis, a 20 mL of the antecubital venous blood sample is collected and processed at 80°C for 6 h to collect the serum and is allowed to stand overnight at 4°C to achieve metabolic profiling. These samples are diluted to get 30 μg/mL. The samples were added with internal standard and centrifuged for 10 min. The supernatant was used for metabolite profiling using LC-MS or GC-MS method. The presence of amino acids such as leucine, isoleucine, and valine, free fatty acids such as palmitic acid and stearic acid and lysophosphatidylcholine confirms the occurrence of the diabetic condition [79].

In recent times HbA1c, the glycated hemoglobin has been considered as an important marker for the diagnosis of diabetes. Usually, HbA1c analytical methods are in general based on the differences in charge or structure. The different analytical techniques include ion-exchange chromatography, CE, affinity chromatography, and immunoassay. Other analytical methods include immunoturbidimetry and ion-exchange HPLC. Among these methods, HbA1c measured using HPLC was significantly higher than compared to other immunoturbidimetric methods [80].

CONCLUSION
The present review discusses the types of diabetes and various analytical methods used for the diagnosis of diabetes and to study the different antioxidant drugs in a comprehensive manner. This will help the future researchers about the different analytical methods that are available for the diagnosis of diabetes and the assay of antidiabetic drugs and thus helps in the development of novel methods for the study of antidiabetic newer drugs molecules.

ACKNOWLEDGMENTS
The authors are thankful to the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS), for providing the necessary library facilities, infrastructure, and equipment for carrying out the research work.

AUTHORS’ CONTRIBUTIONS
The authors SV and MM had contributed equally toward the collection of literature and preparation of the manuscript.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES


