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REVIEW ARTICLE

Analysis of Anti-Diabetic Drugs using Different Analytical Techniques

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ABSTRACT
Anti-diabetic drugs are used to cure diabetes mellitus. It is a type of metabolic disorder caused by increased sugar level in the blood. There are two types of diabetes mellitus i.e. Type 1 diabetes mellitus in which the body does not produce insulin and Type 2 diabetes mellitus in which the body is unable to use insulin properly for transfer of glucose. For the analysis of such anti-diabetic drugs, different analytical techniques have been used such as UV visible spectroscopy, High Performance Liquid Chromatography (HPLC), High Pressure Thin Layer Chromatography (HPTLC) and Infrared spectroscopy (IR). In this review, analysis of some anti-diabetic drugs like Metformin Hydrochloride, Voglibose, Pioglitazone, Repaglinide and Rosiglitazone using the above analytical techniques has been discussed.
Keywords *:anti-diabetic drugs, analysis, techniques*

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INTRODUCTION

Analytical Chemistry finds significant application in pharmaceuticals industries. In pharmaceutical field, analytical techniques have been used since a long time. The commonly used analytical techniques in pharma are Infrared spectroscopy, UV-visible spectroscopy, High performance liquid chromatography, thin layer chromatography etc. Nowdays, analytical techniques are used with more advancement and produces more accurate results in short time. HPTLC (High Performance Thin Layer Chromatography) is an important tool used in analysis of drugs with advanced techniques. HPTCL has the ability of fast separation and is also less time consuming. HPLC (High Performance Liquid Chromatography) is the advanced version of liquid chromatography. It is used to separate complex mixture of molecules and is the most widely use analytical tool. Various anti-diabetic drugs are determined by HPLC in pharmaceutical industries [1]. IR (Infrared spectroscopy) is a rapid and simple process. It has widespread importance in pharmaceutical industry where it is used for the testing of raw materials. Product quality control and process monitoring with the help of infrared spectroscopy analysis is done without any sample preparation.

Anti-Diabetic Drugs

Anti-diabetic drugs are used for controlling sugar levels in the blood. Diabetes is a disease in which a patient's blood sugar is lower than the normal. It is due to the low insulin secretion or no insulin secretion by the pancreas. If proper care is not provided to the patient, it causes complications and requires hospitalization and also increases the risk of cardiovascular diseases (CVDs) [2]. Insulin is used for transferring the glucose from blood to cells but when insulin is not secreted or secreted in lower amount than normal, it causes blood sugar to increase in the blood because low amounts of insulin cannot transfer glucose from the blood to cells. The most common types of diabetes are:

Type 1 Diabetes: In type 1 diabetes, the human body is not able to produce insulin. This is because the immune system attacks and kills the pancreatic cells by which insulin is secreted. This type of diabetes is

usually found in children and young adult. People suffering from diabetes has to mandatorily take insulin injections every day.

Type 2 Diabetes: In type 2 diabetes, the human body cannot use insulin properly for transfer of glucose. This type of diabetes is usually found in middle age and old aged people. It is the most common form of diabetes detected in humans [3]. Gestational Diabetes: Gestational diabetes is usually found in pregnant ladies. Mostly this type of diabetes goes away after delivery of the baby.

Classification of Anti-diabetic Drugs

Anti-diabetic drugs are classified into following classes:

1. Biguanide: It enhances the effect of insulin hormone secreted by pancreas. The side effects are Lactic acidosis, weight loss and gastrointestinal problems (e.g. Metformin).
2. Sulfonyl Urea: It increases insulin secretion from pancreatic beta cell. The side effects are tendency to be hypoglycemic, weight loss, hematologic agranulocyto, hemolysis. (e.g.Glyberide, Glimepride).
3. Alpha glucose: With the help of Alpha glucose, intestinal glucose absorption can be reduced. The side effects are gastrointestinal complaints, feeling of satiety (e.g.Voglibose).
4. Thiazolidinediones: It reduces insulin resistance through the stimulation of PPARS (Peroxisome Proliferator activated receptors) and also increases transcription of adipokinase. The side effects are weight gain, edema, cardiac failure and increased risk of bone fractures (osteoporosis) (e.g. Rosiglitazone, Pioglitazone).

Analytical processes play an important role for producing good quality and reliable analytical spectral data. Diabetes as a disease is responsible for increment in the rate of illnesses such as heart disease, obesity and is also responsible for deaths due to certain specific condition. Due to diabetes, patients can face harmful complications like hypertension, cardiovascular disease, diabetic retinopathy, renal nephropathy etc. The diabetes mellitus is prevented by controlling blood glucose, blood pressure, lipid concentration, body weight etc. By prescribing oral anti-diabetic drugs and healthy diet, these factors can be controlled. Several techniques have been used for rapid determination of various anti-diabetic drugs such as Metformin, Biguanides, Linagliptin, *Empagliflozin*,Dipeptidye peptidase-4 (DPP-4) inhibitor Sodium glucose Co- Transporter 2 Inhibitors, Repaglinide, Gliclazide, Rosiglitazone. The commonly used analytical techniques are HPLC (High Performance Liquid Chromatography), LC-MS (Liquid Chromatography with Mass Spectroscopy) HPTLC (High Performance Thin Layer Chromatography) have been used.

DIFFERENT ANALYTICAL TECHNIQUES USED FOR ANTI-DIABETIC DRUGS

There are different analytical techniques used to determine the purity or property of chemical compounds like Ultraviolet spectroscopy and Infrared spectroscopy. Chromatography is the technique which determines the mixture of compounds by separating them. Different types of chromatographic techniques are High Pressure Liquid Chromatographic, High Pressure Thin Layer Chromatography, Liquid Chromatography- Mass Spectroscopy.

ANALYSIS BY HPLC TECHNIQUE

HPLC (High Performance liquid Chromatography) is used to separate, identify and quantify components in a mixture. HPLC technique is based on the principle of distribution of analyte between mobile phase and stationary phase. The efficiency of purification is dependent on the pumps used to pass a pressured liquid solvent containing the sample mixture through a column filled with a solid adsorbent material causing different flow rates for different components and leading to the separation of the components (separation due to different of interaction with adsorbent particles). The commonly used stationary phases are organochlorosilane and silica gel and the mobile phase contains methanol, acetonitrile etc. The separation with the help of chromatography got initiated in 1900s. TSWETT was the botanical scientist who worked on the separation of pigments of leaf. HPLC (High Pressure Liquid Chromatography) was introduced in the 1970s. It is very strong technique in the area of analytical techniques. Recently, Ultra-Performance Liquid Chromatography was introduced in the year 2004. To attain increased speed, the approach employs tiny particles and high pressure. UHPLC has a similar sensitivity and resolution to HPLC. HPLC uses high pressure to force the mobile phase and analyte through a tight column packed with micron-sized particles. There are nine basic components in commercial HPLC instruments:

i. Mobile phase
ii. Sample introduction device column
iii. Solvent delivery system
iv. Post column apparatus
v. Data collection and output system

vi. Detector
vii. Data collection and output system
viii. Post detection processing of eluent
ix. Connective fittings and tubing

Mobile phase usually contains upto 2.0 L of solvent and is preferably capped to allow an inlet tube. This cap helps to prevent the system from dust particles and evaporation of solvent releases excess pressure in the bottle. Gas bubble is removed with the help of line degassers and reduces dissolved air. Nowdays, these advanced techniques of HPLC are commonly used.

1. Ultra-Performance Liquid Chromatography
2. Rapid Resolution Liquid Chromatography
3. Nano Liquid Chromatography
4. Ultrafast Liquid Chromatography

Advantages of Advancement in HPLC Techniques

- Reverse phase HPLC is a rapid and effective method and has been formulated for the analysis of paeoniflorin extracted from Paeonia 'Singing in the Rain' with lesser run time.
- In UPLC the operation cost is reduced in the techniques.
- It also provides selectivity, sensitivity and dynamic ranges of LC analysis.
- It consumes less solvent.
- UPLC (Ultra Pressure Liquid Chromatography) improves the drugs discovery process by means of high throughput screening.
- High quantitative analysis.
- UPLC provides high speed, accuracy and reproducible result for isocratic and gradient analysis of drugs and their related substance.
- It is widely used for the analysis of natural product and traditional herbal medicine.

Analysis of Metformin by RP-HPLC

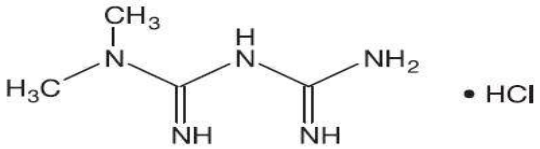

Figure 1: Metformin Hydrochloride

Metformin (Figure 1) is an oral anti- diabetic drug and belongs to the class of Biguanides. Metformin has the ability to put down the glucose level in the blood as well as elongates life expectancy. To study the different action of metformin and its use in the treatment of diabetes, analysis of these drugs is important. For the determination of these drugs, highly sensitive and selective methods are used. The (RP-HPLC) method using C18 analytical reverse-phase column has been developed for determination of metformin hydrochloridein pharmaceutical formulations. using C18 analytical reverse-phase column. The analysis is done on a OD-5-100, C_{18} µ - bond pack column (04 x 25 cm) at ambient temperature with particle size 0.5 µm. For the method, mobile phase used was gradient elution water- methanol (70:30) with 0.5 mi/min. As a result, optimum retention time of metformin was 4.4 min [4].

Analysis of Glimepiride by HPLC

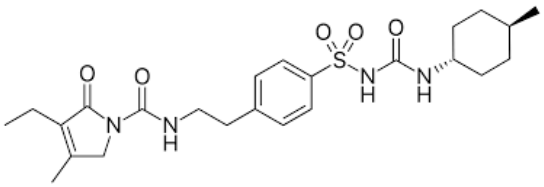

Figure 2: Glimepiride

Type 2 Diabetes is mainly controlled by the Glimepiride (Figure 2) which is an oral anti-diabetic drug and belongs to the class Sulfonylurea. The determination of glimepiride was done by HPLC with UV or array-diode (DAD) Detection Liquid Chromatography-Atmospheric pressure chemical ionization – mass spectroscopy (LC-APCI-MC). According to European and US Pharmaceutical, Glimepiride determination was done by high performance liquid chromatography with C-18 (25 mm x 4 mm) column using a buffer solution of sodium phosphate having pH in the range of 2.1 to 2.7 and acetonitrile and water (4:1) as eluent with a flow rate at 0.1 ml per minutes and detection at 228 nm. The temperature for this analysis does not exceed 120 ˚C and sample is stored for less than 15 hours [5].

ANALYSIS BY HPTLC TECHNIQUE

HPTLC is a widely used technique and also used in quantitative & qualitative analysis. HPLC has a high retention time due to smaller particles dimension while in TLC it is less. In HPTLC sample spotting is done by auto sampler while in TLC manual spotting is involved. It is only the method which has option for presenting the results in image form. More developments in HPTLC are less expensive and has operational simplicity. Simultaneous sample analysis, fast result determination and capability of detecting multiple samples make it an important analytical tool. The principle of HPTLC is based on adsorption and the flow of mobile phase is based on the capillary action. In HPTLC the movement of components is according to their affinities for the adsorbant. The compound which has higher affinity towards the stationary phase moves slower and the compound which has less affinity towards the stationary phase moves faster and these compounds are separated on the chromatographic plate.

- The chromatographic plate used in TLC is hand-made or pre-coated but in HPTLC the chromatographic plate is always pre-coated
- Sorbent layer thickness in TLC is 250 mm while in HPTLC it is 100-200
- The particle size range should be 5-20µm in TLC and 4-8 µm in HPTLC
- In TLC pre-washing of the plate is not followed while in HPTLC pre-washing of plate is mandatory.
- Sample application is manual or semi-automatic in TLC. In HPTLC, sample application is through an auto-sampler
- In TLC the spot size is 2-4 mm and shape volume is 1-10µl, while in HPTLC it is 0.5-1 mm and shape volume is 0.2-5 µl
- The development time in TLC depends on mobile phase and in HPTLC it is 40% less then TLC.

In HPTLC pre-coated plates are used with different support material and sorbent layers with different format thickness are used. For qualitative and quantitative analysis, the sorbent thickness of plate is 100-250 µm are used. Usually, plate size in HPTLC used are:

- 20 x20cm
- 10 x 20 cm
- 5 x 10 cm
- 5 x 7.5 cm
- To get accurate value of RF, good cut edge is required

To remove impurities such as water vapour and other undesirable substances, pre-washing is compulsory. The most widely used sorbent is Silica gel 60 F and major disadvantage is that it consists iron impurity which is removed with the help of methanol: water (9:1). Some other pre-washing solvent used are: methanol, chloroform: methanol(1:1), chlorofom: methanol: ammonia (10:10:1), methylene chloride: methanol (1:1) and ammonia solution. Freshly opened HPTLC does not need activation. The plate needs activation when kept in hand for long time or if it absorbs moisture when kept under high humidity. For activation of these plates, they are placed in oven for 30 min at 110– 120°C temperature. If the plates are activated at high temperature, then there can be a high risk of decomposition of sample. Volatile and non-polar solvents are used for normal chromatography and polar solvent is used for dissolving sample in reverse phase chromatography. When working on the HPLC mobile phase optimization is necessary and it should be of high grade [6].

Advantages of HPTLC Technique

- With coloured compounds the separation process is easy.
- Simultaneously various samples can be differentiated on the same plate resulting in the high through-put and fast analysis at less cost.
- It is easier to carry out two dimensional separations.
- For the detection of separation spot, certain sensitive and specific coloured reagents are used.
- In TLC there is no clean-up and no regeneration is required because these are disposable [6]

Analysis of Pioglitazone by HPTLC

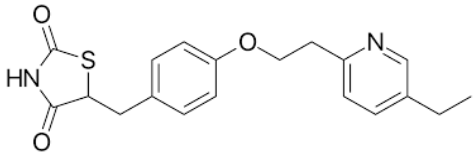

Figure 3: Pioglitazone

Pioglitazone or PGZ (Figure 3) is an oral anti-diabetic drug which is the member of class Thiozolidinedione which decreases insulin resistance. Pioglitazone is used to cure diabetes mellitus 2. It

enhances the sensitivity of insulin and adipose tissue in muscles. Inhibitor hepatic Gluconeogenesis enhances glycemic control while reducing circulating insulin level [7]. 10 mg PGZ hydrochloride was weighed and a solution in methanol was prepared. The final solution contained 1000 mg of pioglitazone per ml of solution. Added 10 ml of same solvent in the solution (0.5 l) for further dilution and the final solution contained 50 mg of PGZ/ml of the solution. Ten tabletswere equivalent to 10 mgpioglitazone which were weighed and finely powdered to prepare a 5 ml solution in methanol. The solution was then filtered. Methanol was used for washing the residue. The chromatography was done by using stationary phase as pre-coated Silica gel 60 F_{354}aluminium sheets (20 x10cm) (washed previously with methanol and dried in air). Mobile phase was toluene: methanol: ammonium (7:3:1) v/v chamber saturation time 30 min, Temperature 24 ˚C, distance of migration was 45 mm at a wavelength of 268nm, slit dimension 3 x 0.3 mm, scanning speed 5 mm/s. Following spotting parameter were used with band width, 4mm i.e. interband spacing was 4 mm and rate of spraying was10 sec/µl. This method was developed for analysis of pioglitazone hydrochloride pure powder. HPTLC method is time consuming but cost effective [8].

Analysis of Repaglinide by HPTLC

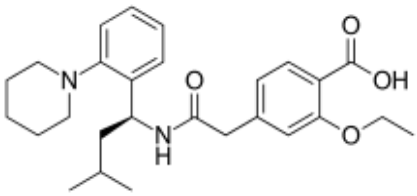

Figure 4: Repaglinide

Repaglinide (Figure 4) belongs to the Meglitinide class of drugs. Repaglinide controls the release of insulin from pancreatic beta cells by inhibiting ATP-dependent potassium channels. Repaglinide regulates these channels by attaching to a different binding location on beta cells than sulfonylurea. In the absence of glucose, however, it does not release insulin [9].Chromatography was performed on a 10 cm × 20 cm TLC plate covered with RP-8F2545 octyl silica gel. Using a dose AS30 HPTCL applicator, sample solution was placed on TLC plates as sharp 5 mm spots 10 mm apart from the plate's lower side. At a maximum wavelength of 225 nm and a slit dimension of 0.1 mm x 2.0 mm, repaglinide spots were scanned in reflectance and transmittance mode with reference wavelength as 360 nm [10].

ANALYSIS BY UV-VISIBLE SPECTROSCOPY TECHNIQUE

UV-Visible spectroscopy deals with the interaction between electromagnetic radiation and organic molecules [11]. In this technique, either absorption emission, or scattering of incident electromagnetic radiation takes place [12]. Because electrons shift from a low-energy state to a high-energy state when a material is bombarded with light, spectroscopy in the UV and IR regions of the electromagnetic spectrum is also known as electronic spectroscopy. UV visible spectroscopy is an absorption spectroscopy technique that uses visible and nearby light to determine the absorption of radiation. When this light passes through the chemical compound then electronic transition takes place between atoms and molecules present in that chemical compound. When the radiation passes through the molecules, bonding and anti-bonding orbital show the transition from ground state level to excited state level by absorption.

The possible transitions are:

$\sigma - \pi^*$ Transition: High energy is required for this type of transition. e.g. methane shows absorption at 125 nm.

$\pi - \pi^*$ Transition: This transition takes place in chemical compounds which contain multiple bonds like alkenes, alkynes, carbonyl. e.g. alkenes.

$n - \sigma*$ Transition: This transition usually requires less energy than $\sigma - \sigma*$ transition. e.g. halogens

$n - \pi*$ Transition: This transition takes place in the compound which have double bond involving heteroatoms e.g. C=O, C=N.

$\pi*$ and $\pi - \sigma*$ Transition: This type of transition is theoretically possible and electronic transition is forbidden.

Lambert-law Beer's is the underlying principle of UV visible spectroscopy. When monochromatic light is delivered through an absorbent solution, Lambert-law Beer's states that the rate of decrease in beam intensity throughout the thickness of the solution is directly proportional to the concentration of the absorbant and is proportional to the strength of the incident monochromatic radiation. Source, filter, and monochromator, sample chamber, detector, and recorder are the components of UV visible spectroscopy.

Applications of UV-Visible Spectroscopy

- Detection of functional groups

- Extent of conjugation determination
- Distinction in conjugation and non-conjugation compounds
- Identification of an unknown compound
- Examination of polynuclear hydrocarbons
- Elucidation of the structure of vitamins A and K
- Preference over two tautomeric forms of compounds
- Identification of compound in different solvent
- Determination of configurations of geometrical isomers
- Distinguishing between equatorial and axial conformation
- Determination of strength of hydrogen bonding
- Hindered rotation and conformational analysis determination

Tungsten lamp, mercury vapour lamp and carbon one lamps are used as light source in visible radiation and in UV radiation deuterium lamp, hydrogen lamp, tungsten lamp etc. are used as light source. Derivative spectrophotometry, an advanced form of UV-spectroscopy is an analytical technique which is used in differentiation of normal spectrum by mathematical transformation of spectra curve into a derivative. This technique helps in the modification of resolution band and also removes the influence of background as a result gives more accurate fingerprints. This technique is widely used for the quantitative analysis, characterization and quality control in agriculture, pharmaceutical, and biomedical field.

Analysis of Voglibose by UV-Visible Spectroscopy

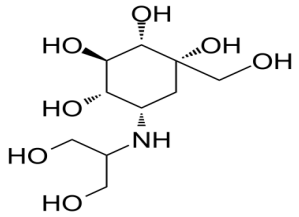

Figure 5: Voglibose

Voglibose (Figure 5) is an anti-diabetic drug which belongs to the family alpha glucose inhibitor. It is used to decrease the post prandial blood glucose level in patients. The main concern of this analysis is to develop and validate UV spectroscopic method as per ICH guideline. The instrument used for this analysis was JASCO V- 630 double beam UV visible spectrophotometer.

To prepare a solution having a concentration of 100 µg/ml, 10 mg of the Voglibose tablets were mixed in methanol (100 ml). This sample was scanned between 200-400 nm and the maximum absorption showed at 282 nm followed by blank.

The proposed method for blank solution preparation was applied to commercially available Voglibose tablets. Thirty tablets were taken where each tablet was weighed which consisted of 0.2 mg of volglibose followed by calculating the average weight. Weighed accurately 5 mg of voglibose and poured into 100 ml of volumetric flask followed by extraction then made up with methanol and then filtered with the help of Whatman filter paper. From the above prepared solution, a range of suitable diluted solutions were prepared and then recorded absorbance with the help of UV region and absorbance were recorded at 282 nm[13].

Analysis of Glimepiride by UV-Visible Spectroscopy

UV spectroscopy method has been developed for the analysis of Glimepiride. For Glimepiride standard solution, weighed accurately 10 mg of glimepiride then poured into a volumetric flask having 100 ml capacity containing chloroform then sonicated for 5 min and made up with chloroform. Pipetted out different volumes from 5-30 µg/ml solutions and they were diluted by using chloroform for final concentration from 5-30 µg/ml. Then absorption was taken at 200-400 nm wavelength range as a result sharp peak was observed at 249 nm[14].

ANALYSIS BY INFRARED SPECTROSCOPY TECHNIQUE

FTIR (Fourier Transform Infrared Spectroscopy) is the advanced version of infrared spectroscopy. The first infrared spectrometer was developed in the late 1950s. It worked with the help of optical prism splitting system which is made up of NACL. The scan range is however narrow and therefore 1st generation infrared spectrometry is not in use. The 2nd type of infrared spectroscopy was developed in 1960s. It utilizes monochromator with a harsh sound. Second generation IR is best in comparison to 1st generation infrared but it has some disadvantages like less sensitivity, low scanning process and sub-

standard wavelength accuracy.The 3rd type of infrared spectrometer is also called Fourier Transform Infrared Spectrometer.FTIR replaced the monochromator to interferometer and become exceptionally powerful. Infrared spectroscopy is also called vibrational spectroscopy and is used as an analytical method in pharmaceutical industry. It is most commonly and widely used for the determination and identification of the various compounds in organic and inorganic fields[15]. The basic components of FTIR are source, interferometer, sample compartment, detector, amplifier A/D convertor and computer. The main difference between Fourier Transform Infrared Spectroscopy & infrared spectroscopy is the replacement of monochromator with Michelson Interferometer. This technique was developed to reduce the limitations present in IR. All IR frequencies can now be simultaneously determined using FTIR. In FTIR a very simple optical device is present known as Michelson interferometer which produces different types of signals. Interferometer is a beam splitter. It splits incoming IR beam into two paths. There is a flat mirror which is fixed in place and one of the beams gets reflected on it. Another beam is reflected on a flat mirror with a mechanism that permits it to move a very little distance away from the beam splitter. Due to this reason, one of the beams is reflected on fixed mirror and another moves constantly as a result of interfacing of two beam and the signal so formed is called interferogram. There are three areas in IR region. Near IR region: 400- 10 cm^{-1}, mid IR region: 4000 – 400 cm^{-1}, far IR region: 4000-14000 cm^{-1}.

Analysis of Metformin and Glibenclamide by FT-IR

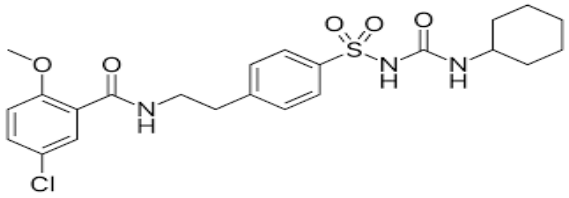

Figure 6: Glibenclamide

In recent years, FTIR has been widely used for the analysis of various drugs. It is simple, fast and a green method for quantification of various active constituents and determination of purity of the drugs [16-25].This technique is use for the determination of functional groups since different functional groups absorb different IR radiation frequencies. Analysis of anti-diabetic drugs like Metformin and Glibenclamide was done by taking five tablets of Metformin hydrochloride and Glibenclamide (Figure 6). These five tablets were crushed by motor and pestle and then dissolved the powder in 25 ml distilled water and sonicated for five minutes. After that 25 ml CH_3Cl also added into the above solution and shaken for 5 to 10 minutes followed by centrifugation at 2500 rpm for 5 to 10 minutes. The layer of CH_3Cl was separated by centrifugation and was evaporated by using rotavapour. The remaining extract was dried at 60 ˚C to 70 ˚C under vacuum. After that 2 mg was taken from the mixture which was crushed under vaccum at a pressure of 80 mpa and placed on KBr pressure disk for IR identification. The result was obtained by using the test sample along with standard sample of the compound [26].

Analysis of Repaglinide [RPG], Rosiglitazone [RGZ] and Pioglitazone [PGZ] by FT-IR

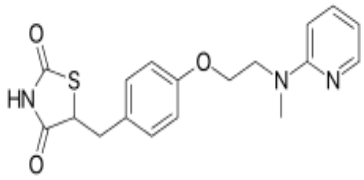

Figure 7: Rosiglitazone

The FTIR model Nicolet 380 FT-IR spectrometer with OMNIC TM software was used to analyse these medicines [27]. RPG, RGZ, and PGZ samples were obtained from their respective manufacturers. The purity of these medications ranged between 99.9% and 100%. For sample disc preparation, KBr was employed, whereas chloroform was used for repaglinide extraction from tablets. The mixture of Repaglinide, Rosiglitazone (Figure 7), Pioglitazone was prepared in potassium bromide and quantity was about 2.0 g % (w/w). This mixture was grounded into a fine powder. Tablet sample was taken from RPG from Novonorm 2 mg, RGZ from Rosozone 4 mg, Diabetic 30 mg. The IR spectra were obtained for fused discs followed by blank with 100 % KBr. The range of spectra was taken from 400 – 4000 cm^{-1}. There were 32 scans between this range and took 15 seconds to record a spectrum. For the analysis of RPG, the absorbance was shown between 2811.7 cm^{-1} and 2796.28 cm^{-1} for the C-H stretching baseline was one point at 2832.92 cm^{-1}. The absorbance of RGZ was measured in two areas: area I was between 1587.13 and 1577.49 cm^{-1}, with a baseline one point at 1590.99 cm^{-1}, which represents the stretching of the double bond of the benzene ring. The ketone group's Area II was between 1697.0 and 1689.34 cm^{-1}, with

no need for a baseline. The absorbance for PGZ was between 1336.43 and 1330.64 cm^{-1} for C-S stretching, with the base line altered at two points at 1346.07 and 1324.86 cm^{-1}[27]

ANALYSIS BY LC-MS

LC-MS is the combination of techniques like High Performance Liquid Chromatography and Mass Spectroscopy. Combination of these two techniques lead to decrement in error and improved accuracy. The advanced version of Liquid Chromatography is HPLC (High Performance Liquid Chromatography) which is widely used for the analysis of drugs in pharmaceutical and food industry. Generally, HPLC is used for the non-volatile organic compounds which are not analysed by Gas Chromatography. In mass spectroscopy atoms and molecules ionize for better separation. The separation in Mass Spectroscopy is based on molecular masses and mass to charge ratio. The separation is first done by the High Performance Liquid Chromatography where the separation is dependent on hydrophobic interaction, ion exchange, ion pair surface localization etc. After that, separated samples are sprayed on to an Atmospheric Pressure Ion source (API). It is the ion source which changes ions into the gas phase then differentiating ions according to their mass to charge ratio with the help of mass analysis. These ions are counted with the help of detector. The basic components of Liquid Chromatogram unit consist of a pump which delivers the mobile phase at a required flow rate. Second one is an auto sampler through which sample is injected. And then sample is separated by column. At last, the detector is used for the analysis of the compounds separated in a sample. The basic components of LC - MS (Liquid chromatography-Mass chromatography) are the LC unit and interface between liquid chromatography and Mass spectroscopy, ion source which ionizes the sample such as APPI, API. Based on their mass to charge ratio, ions are separated in mass analyzer and these ions are detected by detector. Electron multiplier, dynode, photodiode and multi-channel plate are commonly used detector units. Mass analysis and detection takes place under high vacuum established using a combination of fore line and turbo-molecular pumps. Determination of impurity, degradant identification, separation of compounds is done by LC-MS techniques[28].

There are three most common ionization methods used in LC-MS

- Electrospray Ionisation (ESI) – Ionization in the condensed phase [29-30]
- Atmospheric Pressure Chemical Ionization (APCI) – Ionization in the gas phase [31-32]
- Matrix-assisted Laser Ionization (MALDI) [33-35]

Some anti-diabetic drugs such as Vildagliptin, *Alogliptin* benzoate, Sitagliptin linaglipein, Glibenclamide, Pioglitazone Hydrochloride, Mitiglinide Calcium Hydrate, Glimepiride are analyzed by liquid chromatography – mass spectroscopy technique [36].

CONCLUSION

Various techniques have been developed for the analysis of different types of drugs. One of them is HPLC which is one of the most common separation techniques. HPLC works on the basis of distribution of analyte between mobile phase (also called as eluent) and a stationary phase (also known as packing material of column). The separation of compounds by HPLC is dependent on the interaction with adsorbent particles. The advanced versions of HPLC such as RPLC, UPLC, ULC, NLC have come into prominence in recent times. These versions have advanced application as compared to normal HPLC technique such as low cost, more accurate result and less time consumption. HPTLC is the advance version of TLC (Thin layer chromatography) which is a strong analytical method as it has various advantages like low cost, simplicity, high sample capacity and possibility of multiple detections. On the other hand, for quantitative analysis, characterization and quality control in agriculture, pharmaceutical and biomedical field FTIR is widely used. In FTIR due to the replacement of monochromator by interferometer it has become more exponentially powerful.

REFERENCES

1. S. Baokar, K. Mane, A. Bhujbal; S. Pharande; G. Patil; R. Patil; P. Jain and A. Pandey (2020). Journal of Pharmaceutical Research International, 32; 34: 67-83.
2. Internal Clinical Guidelines Team. (2015). Type 2 Diabetes in Adults: Management. London: National Institute for Health and Care Excellence; 28.
3. Santos-Longhurst; G. Krucik; Type 2 Diabetes Statistics and Facts. Health line (2014). Available from: www.healthline.com/health/type-2-diabetes/statistics
4. M. Saeed Arayne; Najma sultana and M. Hashim Zuberi. (2006).Pakistan Pak. J. Pharm. Sci, 19; 3: 231-235.
5. European Pharmacopoeia (2008), 6th ed. Strasbourg: Council of Europe.
6. D.H. Shewiyoa; E. Kaale; P.G. Risha; B. Dejaegher; J. Smeyers-Verbekec; Y. Vander Heydenc; (2009). Tanzania Food and Drugs Authority, P.O. Box 77150, Dar es Salaam, Tanzania b School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65013, Dar es Salaam, Tanzania c Department of Analytical Chemistry and

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7. http:// rxlist.com/actos-drug.htm
8. Meeta A Jiladia; SS Pandya; and Viidyasagar G. (2009). A Simple and Sensitive HPTLC Method for Estimation of Pioglitazone In Bulk and Tablet Dosage Forms. Asian Journal of Research in Chemistry, 2; 2: 207-209.
9. M. Natras; C.J Bailey C.J. Baillieres. (1999).Clin. Endocrinol. Metab, 13: 309–329.
10. Anna Gumieniczek; Anna Berecka and Hanna Hopkała. (2005).Journal of Planar Chromatography, 18: 155–159.
11. Claßen Jens; Aupert Florian; F. Reardon Kenneth; SolleDörte; Scheper Thomas. (2017). Anal. Bioanal. Chem, 409; 3: 1–16.
12. E. Dufour. (2011). Int. J. Dairy Technol, 64; 2: 153–165 https://doi.org/10.1111/j.1471-0307.2010.00665.x.
13. N. Mallikarjuna Rao; J. Bagyalakshmi and T. K. Ravi. (2010). RP-HPLC method development and validation for estimation of Voglibose in bulk and tablet dosage forms. Int. J. Res. Pharm. Sci.,1; 2: 190-194.
14. Sakala Bhargavi; Gopisetty Suryasagar; Dantu Krishna Sowmya; Kota Ashok. (2013). UV Spectrophotometric Method for Determination of Glimepiride in Pharmaceutical Dosage Forms. Int. J. Pharm. Sci. Rev. Res,21; 2: 131-133.
15. Manjusha ND, Priyanka AP, Sanjay DS, Priyanka SS. Review on Advance applications of FTIR Spectroscopy. Intl. J. Pharm. Sci. Rev. & Res. 2011;7(2): 29.
16. C Ayala; M. R Brunetto; F. Y Ovalles; M Gallignani. (2009). Transmission FTIR derivative spectroscopy for estimation of furosemide in raw material and tablet dosage form. Rev. Téc. Ing. Univ. Zulia, 32: 238.
17. Faiza Fahim; Ayesha Naseer; Shakil Ahmed; Syed Tufail; H. Sherazib and Muhammad Bhanger. (2014).Journal of the Brazilian Chemical Society,25; 11: 2032-2038. https://dx.doi.org/10.5935/0103-5053.20140185
18. M. A Mallah; S. T. H Sherazi; S. A Mahesar; A Rauf. (2011). Pak. J. Anal. Environ. Chem, 12: 61.
19. M. A Mallah; S. T. H Sherazi; S. A Mahesar; A Rauf. (2012). J. Chem. Soc. Pak, 34: 556.
20. Y Zuo; L Zhang; J Wu; W Johnathan; F. M Suzanne; R Christopher. (2005). Abstracts of Papers of the American Chemical Society, 229: 130.
21. P. Prasad; K. B Murarilal; K Rajani. (2012). Quantitative determination of Domperidone and Paracetamol in combined dosage form by FT-IR spectroscopy J. Chem. Pharm. Res, 4: 180.
22. C Kogawa; H. R. N Salgado. (2013). Development and Validation of Infrared Spectroscopy Method for the Determination of Darunavir in Tablets. Phys. Chem, 3: 1. doi:10.5923/j.pc.20130301.01
23. H Moreno; H. R. N Salgado. (2012). Development and Validation of the Quantitative Analysis of Ceftazidime in Powder for Injection by Infrared Spectroscopy. Phys. Chem, 2: 6. doi: 10.5923/j.pc.20120201.02
24. E. G Tótoli; H. R. N Salgado. (2012). Phys. Chem, 2: 103. DOI:10.5923/j.pc.20120206.04
25. D. C. M Vieira; P. C Ricarte; H. R. N Salgado. (2012). Adv. Anal. Chem, 2: 80. doi: 10.5923/j.aac.20120206.02
26. Elhamili; J. Bergquist; M. El-Attug; S. Saad; F. Saad; G. Hemiss; T. Almog. (2014). International Journal of Pharma Research & Review, 3; 6: 1-9.
27. Faten Farouk; Bahia Abbas Moussa and Hassan Mohamed El-Said Azzazy. (2011). Journal of Spectroscopy, 26: 297–309.
28. I Khan; K Mulpuri; B Das; MD Mohiuddin; MH Rahman. (2015). Int J Res Pharm Nano Sci, 4: 19-22.
29. CS Ho; CW Lam; MH Chan; RC Cheung; LK Law; LC Lit et al. (2003).Clin Biochem Rev, 24; 1: 3-12.
30. JB Fenn; M Mann; CK Meng; SF Wong; CM Whitehouse. (1989). American Association for the Advancement of Science, 246; 4926: 64-71.
31. AG Marshall; CL Hendrickson; GS Jackson. (1998). Mass Spectrom Rev., 17; 1: 1-35.
32. AG Marshall; CL Hendrickso, (2005). Int. J. Mass Spectrom.,215; 1-3: 59-75.
33. A Calderaro; MC Arcangeletti; Rodighiero; M Buttrini; C Gorrini; F Motta et al. (2014).Sci Rep, 30; 4: 6803.
34. L.F Marvin; M.A Roberts; L.B Fay. (2003). Clin Chim Acta, 337; 1-2: 11-21.
35. J. Ragoussis; Elvidge GP; Kaur K; Colella S. (2006).Ploss Genetics, 2; 7: 100.
36. ShigemiKai; Ken Ishikawa; (2015). Hajime Ito; Tatsuya Ogawa; Hidetaka Yamashita;Yoshiko Nagata; Japan, 0087-253 Chigasaki, Shimomachiya 1-3-1, Health Public of Institute Prefectural Kanagawa, Japan, 8512-105 Tokyo, ku-Minato, Shibakoen 30-5-1, University Keio, Pharmacy of Facul, 36; 19-24.