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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ANTI DIBETIC DRUG

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INTRODUCTION

Ultraviolet (UV) Spectrophotometry

Ultraviolet (UV) Spectrophotometry is an analytical technique used to measure the amount of light absorbed by a chemical substance in the UV-visible region of the electromagnetic spectrum. This technique is based on the interaction of light with matter, where certain wavelengths of light are absorbed by molecules, leading to an increase in energy. UV spectrophotometry is particularly useful for determining the concentration of substances in solution, providing both qualitative and quantitative information.[1]

Working Principle of UV Spectrophotometry[2]

The working principle of Ultraviolet (UV) Spectrophotometry is based on the interaction of light with matter, specifically how light is absorbed by a sample. The key idea is that molecules absorb light at specific wavelengths, and this absorption can be used to determine the identity and concentration of substances in the sample. Here is a breakdown of the principles involved:

1. Light absorption by molecules

When ultraviolet (UV) light is directed onto a sample, certain wavelengths of light are absorbed by the sample. The amount of absorption depends on the energy difference between the molecular ground state and the excited state. This energy difference corresponds to a specific wavelength of light, which is absorbed by the molecule, causing an electronic transition.

- **Ground state:** The lowest energy state of a molecule, where all its electrons are in their normal, stable configurations.
- **Excited state:** A higher energy state of a molecule, where one or more electrons are promoted to a higher orbital due to absorption of UV light.

The electronic transitions typically occur in organic molecules that have conjugated systems of double bonds, such as aromatic compounds, carbonyl groups, and nucleic acids. The amount of light absorbed depends on the structure of the molecule and the wavelength of the light.

2. Beer-Lambert Law

The Beer-Lambert Law is the fundamental equation that relates the absorption of light to the concentration of the absorbing substance in the sample. It is mathematically expressed as:

$A=\epsilon \cdot C \cdot l$

Where:

- A = Absorbance (A unitless quantity that indicates how much light is absorbed)
- ε = Molar absorptivity or molar absorption coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$), which is a constant that indicates how strongly a substance absorbs light at a specific wavelength.
- C = Concentration of the absorbing species (mol/L)
- l = Path length of the sample (cm), which is the distance the light travels through the sample.

According to this law, absorbance is directly proportional to both the concentration of the analyte and the path length through which the light travels, meaning that more concentrated solutions or longer path lengths will absorb more light.

3. Monochromatic light source

In UV spectrophotometry, a monochromatic light source is used to emit light of a specific wavelength or narrow range of wavelengths. Common light sources include:

- Deuterium lamps for UV light (200-400 nm)
- Tungsten lamps for visible light (400-700 nm)

The light produced is then directed into the monochromator, which separates the light into its constituent wavelengths.

4. Monochromator

A monochromator is an optical device used to isolate a single wavelength (or a narrow range of wavelengths) from the light source. It usually consists of a prism or diffraction grating that disperses the light into its individual components. The monochromator allows the operator to select a specific wavelength for analysis, which is then passed through the sample.

5. Sample absorption

Once the monochromatic light reaches the sample, some of the light is absorbed by the molecules in the sample, depending on their chemical structure. The remaining unabsorbed light passes through the sample and is directed towards the detector.

- The degree of absorption at a given wavelength will depend on the specific absorption characteristics of the molecules in the sample. This will generate an absorption spectrum, which is a plot of absorbance vs. wavelength.

6. Detector

The detector is a critical component that measures the amount of light that has passed through the sample. It converts the light signal into an electrical signal that can be quantified. Common detectors used in UV spectrophotometry include photodiodes, photomultiplier tubes (PMT), or charge-coupled devices (CCD). The detector measures the intensity of the transmitted light and calculates the absorbance based on the amount of light lost by the sample.

7. Display and Analysis

After the detector records the absorbance, this information is processed and displayed as an absorption spectrum. An absorption spectrum is typically a graph where:

- The x-axis represents the wavelength of light (usually in nanometers).
- The y-axis represents the absorbance (no units).

The peaks in the spectrum correspond to wavelengths at which the sample absorbs light, and the intensity of the peaks reflects the strength of absorption at those wavelengths.

Applications of UV Spectrophotometry[1,2]

- **Pharmaceutical analysis:** Quantitative determination of active pharmaceutical ingredients (APIs) in drugs.
- **Biochemical studies:** Estimation of nucleic acids (DNA/RNA) and proteins.
- **Environmental testing:** Measurement of pollutants like nitrates, sulfates, and heavy metals.
- **Clinical diagnostics:** Assaying drug levels in blood and urine.

Advantages and Limitations

- **Advantages**
  - Simple and cost-effective.
  - High sensitivity for most analytes.
  - Fast and easy method.
  - Minimal sample preparation required.

- **Limitations**
  - Can only measure substances that absorb UV-visible light.
  - Interference from other substances in the sample can affect results.
  - Cannot be used for opaque or highly colored samples.

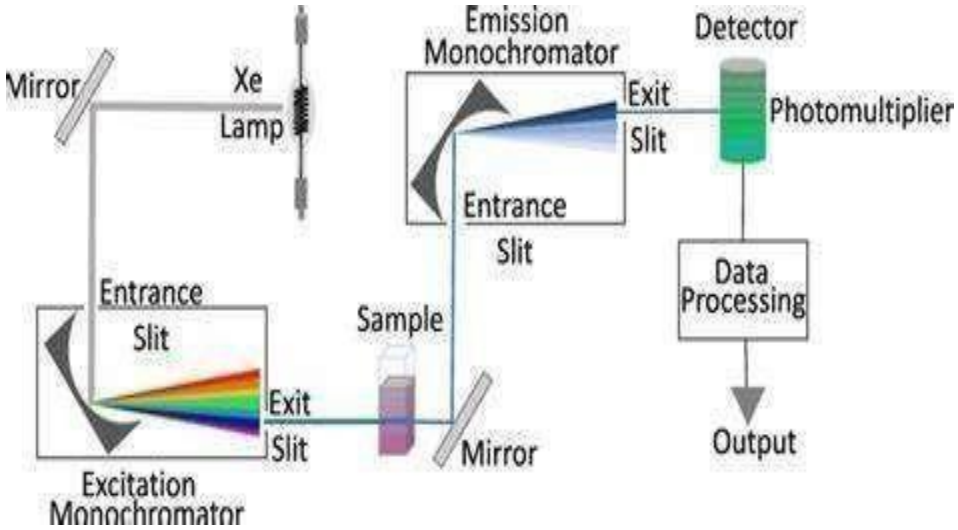


Fig. UV Spectroscopy.

Diabetes mellitus[5]

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, which can result from either insulin resistance (Type 2 diabetes) or insulin deficiency (Type 1 diabetes). The disease leads to disturbed carbohydrate, fat, and protein metabolism.

Diabetes is a major cause of complications such as cardiovascular disease, neuropathy, retinopathy, and renal failure.[5]

Types of diabetes

1. **Type 1 Diabetes Mellitus (T1DM)**
   - Insulin-dependent diabetes occurs when the immune system destroys insulin- producing β-cells in the pancreas. This results in little or no insulin secretion.
   - **Onset:** Typically occurs in childhood or adolescence, though it can develop at any age.
   - **Management:** Requires lifelong insulin therapy and careful blood glucose monitoring.

2. **Type 2 Diabetes Mellitus (T2DM)**
   - Non-insulin-dependent diabetes characterized by insulin resistance (where body cells do not respond effectively to insulin) and eventually pancreatic β-cell dysfunction.
   - **Onset:** Typically occurs in adults over the age of 45, though increasing cases in younger populations due to obesity and sedentary lifestyles.
   - **Management:** Primarily managed through lifestyle changes, oral antidiabetic medications, and sometimes insulin.

3. **Gestational Diabetes Mellitus (GDM)**
   - Occurs during pregnancy when pregnancy hormones cause insulin resistance. This leads to elevated blood glucose levels that typically return to normal after delivery.
   - Women who experience GDM are at increased risk of developing Type 2 diabetes later in life.

4. **Maturity-Onset Diabetes of the Young (MODY)**
   - A rare form of diabetes caused by genetic mutations affecting insulin production.
   - It usually occurs in young adults and is characterized by a strong family history of diabetes.

5. **Secondary diabetes**
   - This form of diabetes occurs as a result of another medical condition or medication, such as cystic fibrosis, cushing's syndrome, or chronic pancreatitis.

Etiology of diabetes

1. **Genetic factors**
   - **Type 1 Diabetes:** Certain HLA (human leukocyte antigen) genes have been associated with susceptibility to T1DM. Genetic predisposition plays a role, though environmental triggers are also crucial.
   - **Type 2 Diabetes:** Genetics plays a significant role in T2DM as well, with many individuals having a family history of diabetes. Specific genes related to insulin resistance and β-cell dysfunction have been identified.

2. **Environmental factors**
   - **Infections**: Viral infections like Coxsackievirus B, mumps, and rubella can trigger autoimmune destruction of β-cells in Type 1 diabetes.
   - **Diet and Obesity:** High-fat, high-sugar diets, combined with low physical activity, increase the risk of developing insulin resistance.
   - **Age:** The risk of Type 2 diabetes increases with age, especially after 45 years.
   - **Stress:** Chronic stress can lead to hormonal changes (such as increased cortisol levels), which can contribute to insulin resistance and elevate blood glucose levels.

3. **Insulin resistance**
   - Insulin resistance occurs when cells in the liver, muscle, and adipose tissue fail to respond to insulin properly. This reduces glucose uptake and metabolism, contributing to elevated blood sugar levels.

4. **Pancreatic dysfunction**
   - In Type 1 diabetes, autoimmune destruction of β-cells in the pancreas leads to absolute insulin deficiency.
   - In Type 2 diabetes, there is a combination of insulin resistance and impaired insulin secretion from the pancreas due to β-cell dysfunction.

Antidiabetic drugs

Antidiabetic drugs are used to lower blood glucose levels in patients with diabetes. These drugs work through various mechanisms to either enhance insulin sensitivity, stimulate insulin secretion, or prevent glucose absorption.

Classification of antidiabetic drugs

1. **Oral antidiabetic drugs**

These drugs are taken by mouth to manage blood glucose levels.

A. Insulin secretagogues

These drugs stimulate the pancreas to release more insulin.

- **Sulfonylureas**
  - **Mechanism:** Stimulate insulin secretion from pancreatic beta cells.
  - **Examples**
    - **Glipizide:** A commonly prescribed sulfonylurea. It is effective for managing blood sugar levels in type 2 diabetes by promoting insulin secretion.
    - **Glibenclamide, Glyburide, Glimepiride.**

- **Meglitinides**
  - **Mechanism:** Stimulate insulin secretion in response to meals.
  - **Examples:** Repaglinide, Nateglinide.

B. Insulin sensitizers

These drugs enhance the body's sensitivity to insulin.

- **Biguanides**
  - **Mechanism:** Decrease liver glucose production and improve insulin sensitivity.

- **Example:** Metformin.

- **Thiazolidinediones (TZDs)**
- **Mechanism:** Enhance insulin sensitivity in muscle and fat.
- **Examples:** Pioglitazone, Rosiglitazone.

C. Alpha-Glucosidase Inhibitors

These drugs slow the absorption of carbohydrates in the intestines

- **Mechanism:** Inhibit enzymes that break down complex carbohydrates into glucose.
- **Examples:** Acarbose, Miglitol.

D. DPP-4 Inhibitors

These drugs increase insulin secretion and decrease glucagon release

- **Mechanism:** Inhibit the DPP-4 enzyme, prolonging incretin hormone action.
- **Examples:** Sitagliptin, Saxagliptin.

E. SGLT2 Inhibitors

These drugs reduce glucose reabsorption by the kidneys

- **Mechanism:** Block the SGLT2 transporter in the kidneys, increasing glucose excretion.
- **Examples**: Canagliflozin, Empagliflozin.

2. Injectable antidiabetic drugs

These are injected into the body to help control blood glucose levels

A. Insulin

Insulin is essential for type 1 diabetes and may be used in type 2 diabetes when oral agents are ineffective.

B. GLP-1 Receptor agonists

These mimic incretin hormones to regulate glucose and insulin secretion.

C. Amylin analogs

Amylin is co-secreted with insulin and helps regulate glucose levels by slowing gastric emptying and suppressing glucagon secretion.

Mechanism of action of common antidiabetic drugs[34]

- **Insulin:** Insulin facilitates glucose uptake by cells, particularly in muscle, liver, and adipose tissues, thereby lowering blood glucose levels.
- **Metformin:** Inhibits hepatic glucose production and improves insulin sensitivity by increasing the activity of AMPK (AMP-activated protein kinase).
- **Sulfonylureas:** Bind to the sulfonylurea receptor on pancreatic β-cells, stimulating insulin secretion.
- **Pioglitazone (TZDs):** Activate PPAR-γ receptors, enhancing insulin sensitivity in muscle and adipose tissue and reducing glucose production in the liver.

Side effects of antidiabetic drugs[34]

- **Metformin:** Gastrointestinal disturbances, lactic acidosis (rare).
- **Sulfonylureas:** Hypoglycemia, weight gain.
- **Thiazolidinediones:** Fluid retention, weight gain, heart failure risk.
- **DPP-4 Inhibitors:** Headache, upper respiratory tract infections.
- **SGLT2 Inhibitors:** Genital infections, dehydration.

Literature review

Literature Review on UV Spectrophotometric Methods for Antidiabetic Drugs

1. Estimation of Glibenclamide Using UV Spectrophotometry[21]

Bansal et al., 2014 reported a UV spectrophotometric method for Glibenclamide in bulk and pharmaceutical formulations. The method was validated for linearity, precision, and accuracy within a concentration range of 2–10 µg/mL.

2. Simultaneous Estimation of Glimepiride and Metformin[22]

Kumar et al., 2015 developed a method to simultaneously estimate Glimepiride and Metformin using UV spectrophotometry. The method was found to be accurate and precise, with linearity in the range of both drugs.

3. Repaglinide determination in tablet dosage form[23]

Shinde et al., 2015 created a UV method for Repaglinide, validated for linearity, precision and reproducibility.

4. Development and Validation of UV Spectrophotometric Method for Metformin Hydrochloride[24]

Sangeeta R. et al., 2016 developed a simple UV spectrophotometric method for Metformin Hydrochloride in tablets. The method showed good linearity (5-20 µg/mL) and was validated for accuracy, precision, and specificity according to ICH guidelines.

5. Sitagliptin Phosphate Determination Using UV Spectrophotometry[24]

Kumar et al., 2016 validated a UV method for Sitagliptin in tablets, showing good linearity and precision.

6. Simultaneous Estimation of Linagliptin and Metformin[26]

Mehta et al., 2016 developed a dual-wavelength UV method for Linagliptin and Metformin, demonstrating effective simultaneous estimation in fixed-dose combinations.

7. Pioglitazone Quantification by UV Spectrophotometry[27]

Singh et al., 2017 developed a UV spectrophotometric method for Pioglitazone estimation, validated for linearity (10-100 µg/mL), precision, and accuracy.

8. UV Spectrophotometric Method for Acarbose[28]

Ahamed et al., 2018 focused on a UV spectrophotometric method for Acarbose in pharmaceutical preparations. The

method was optimized for sensitivity and reproducibility.

9. UV Spectrophotometric Determination of Empagliflozin[29]

Gupta et al., 2018 explored the application of UV spectrophotometry to quantify Empagliflozin in its pharmaceutical dosage form. The method was validated for linearity.

10. Canagliflozin Quantification Using UV Spectrophotometry[30]

Jadhav et al., 2019 developed a UV spectrophotometric method for Canagliflozin, validated for accuracy and linearity in the range of 10–50 µg/mL.

11. Development of a UV Method for the Estimation of Vildagliptin[31]

Ravi et al., 2019 developed a simple UV spectrophotometric method for the estimation of Vildagliptin in tablets. The method showed good accuracy and precision, with a linearity range from 5 to 25 µg/mL.

12. Dapagliflozin Determination in Pharmaceutical Dosage Forms[32]

Patel et al., 2020 presented a UV spectrophotometric method for Dapagliflozin with excellent accuracy and precision.

13. Estimation of Saxagliptin Using UV Spectrophotometry[33]

Bansal et al., 2021 validated a UV spectrophotometric method for the estimation of Saxagliptin in pharmaceutical formulations. The method was found to be highly accurate and reproducible, with a linearity range of 5–25 µg/mL.

Experimental work

Step 1: Preparation of standard stock solution

1. Primary stock solution

- **Purpose:** To prepare a concentrated solution of Glipizide for further dilution into working solutions.
- **Procedure:** A 10 mg of Glipizide standard was weighed and transferred to a 100 ml volumetric flask &10 ml of NaOH was transferred to this volumetric flask make up the volume with 0.5N NaOH up to 100 ml.

2. Working standard solutions

- **Purpose:** To prepare solutions with varying concentrations for the construction of the calibration curve.
- **Procedure:** Prepare working solutions by diluting the primary stock solution with methanol.

The following concentrations were prepared

- 5 µg/mL: Pipette 0.5 mL of stock solution and dilute to 10 mL with methanol.
- 10 µg/mL: Pipette 1 mL of stock solution and dilute to 10 mL with methanol.
- 20 µg/mL: Pipette 2 mL of stock solution and dilute to 10 mL with methanol.
- 30 µg/mL: Pipette 3 mL of stock solution and dilute to 10 mL with methanol.
- 40 µg/mL: Pipette 4 mL of stock solution and dilute to 10 mL with methanol.

Step 2: Determination of λmax (Maximum absorption wavelength)

1. Preparation of standard solution

Prepare a standard solution of Glipizide at a concentration of 20 µg/mL.

2. UV Scan

Use the UV-Vis spectrophotometer to scan the solution over the wavelength range 200–400 nm.
Record the absorbance spectra and identify the λmax (the wavelength at which the drug absorbs maximally).

3. Identify λmax

For Glipizide, 276 nm is typically the λmax, where the highest absorbance is observed.

Step 3: Calibration curve preparation

1. Measure absorbance of standard solutions
   - Take each working solution (e.g., 5 µg/mL, 10 µg/mL, 15 µg/mL, etc.) and place them in quartz cuvettes.
   - Record the absorbance at 276 nm using the UV-Vis spectrophotometer.

2. Plot the calibration curve

- Plot the absorbance values (y-axis) against the corresponding concentrations (x- axis) to create a calibration curve.
- The calibration curve should show a linear relationship between absorbance and concentration within the selected range.

3. **Calculate the Slope and Intercept**:
   - Use linear regression to calculate the slope and intercept of the calibration curve. These values are necessary for determining the concentration of unknown samples.

Step 4: Sample analysis

1. Dilution of sample

- Pipette an appropriate volume of the filtered sample solution (e.g., 1 mL) and dilute it to the desired concentration using distilled water.

2. Measure absorbance

- Transfer the diluted sample to a quartz cuvette and measure its absorbance at 276 nm using the UV-Vis spectrophotometer.

3. Calculation of glipizide concentration

- Using the calibration curve, determine the concentration of Glipizide in the sample solution based on the measured absorbance.

Step 5: Method validation

1. Linearity

- Prepare at least 5 different concentrations of Glipizide (5-40 µg/mL) to ensure that the calibration curve is linear.
- Plot the absorbance vs. concentration data and confirm that the relationship is linear (R^2 value should be ≥ 0.999).

2. Accuracy

- Analyze a known concentration of Glipizide and compare the measured value with the known value.
- Calculate the percentage recovery of Glipizide (ideal recovery: 98-102%

RESULT AND DISCUSSION

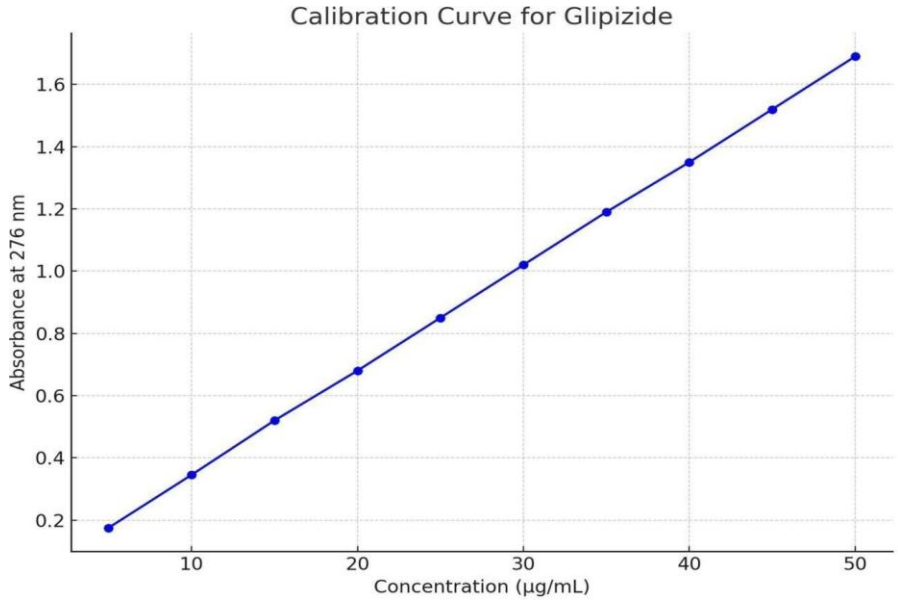
1. Calibration curve

The calibration curve was prepared by plotting the absorbance against the concentration of Glipizide (5-40 µg/mL). The equation of the calibration curve was determined to be:

Absorbance=0.0346×(Concentration)+0.0043

The correlation coefficient (R^2) of the calibration curve was 0.9998, showing excellent linearity.

Concentration (µg/mL)	Absorbance
5	0.175
10	0.345
15	0.520
20	0.680
25	0.850
30	1.020
35	1.190
40	1.350
45	1.520
50	1.690



2. λmax (Maximum Absorption Wavelength)

The UV spectrum of Glipizide showed that the maximum absorbance (λmax) occurred at 276 nm**.**

Wavelength (nm)	Absorbance
270	0.200
275	0.530
276	0.810
280	0.580
285	0.400

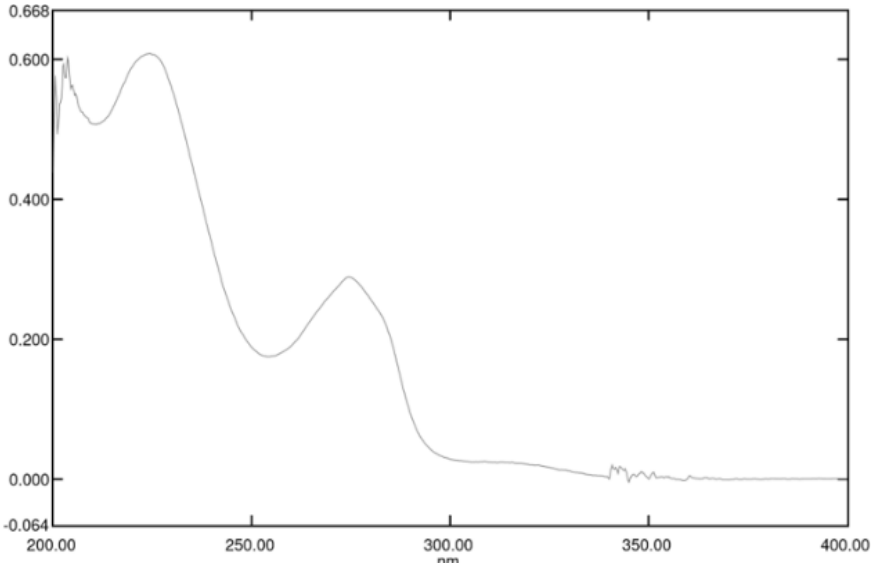


Fig. UV Spectra of Glipizide Showing Maximum absorbance 276nm [5%].

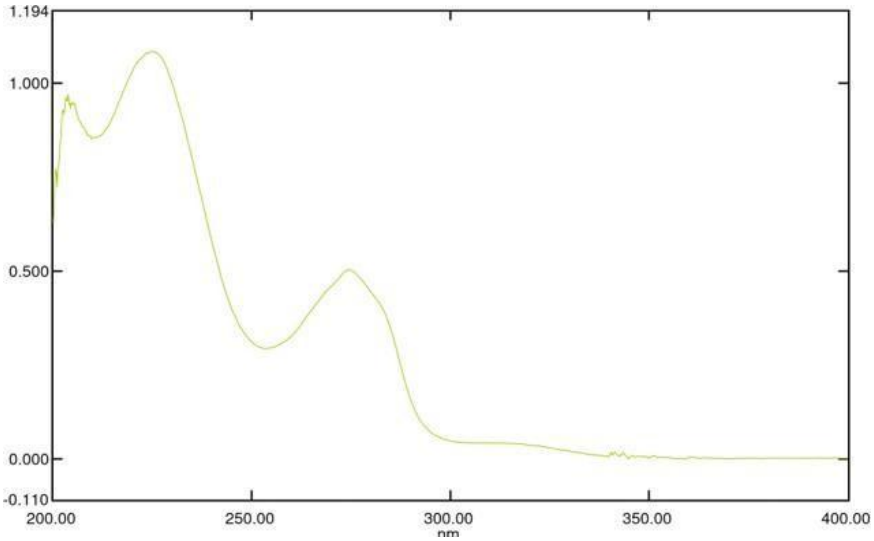


Fig.UV Spectra of G;ipizide Showing Maximum absorbance 276nm [10%].

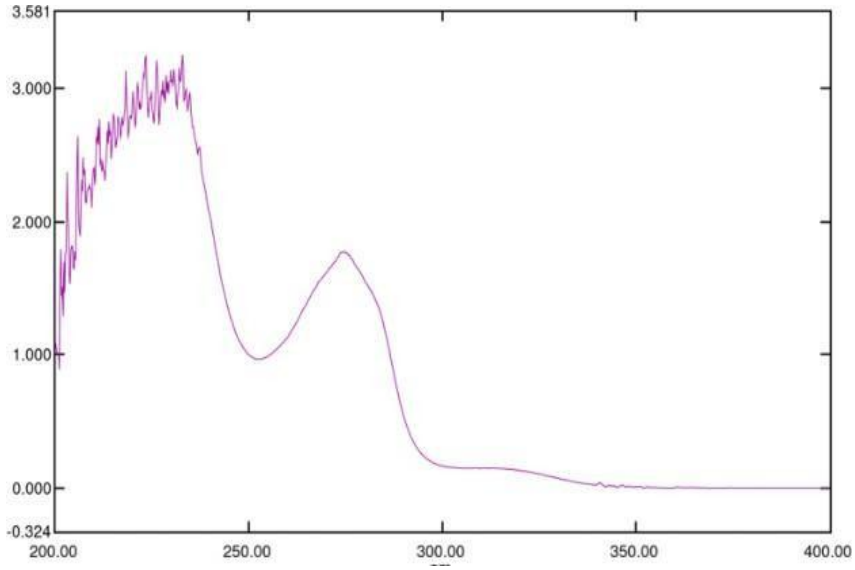


Fig. UV Spectra of Glipizide Showing Maximum absorbance 276nm [20%].

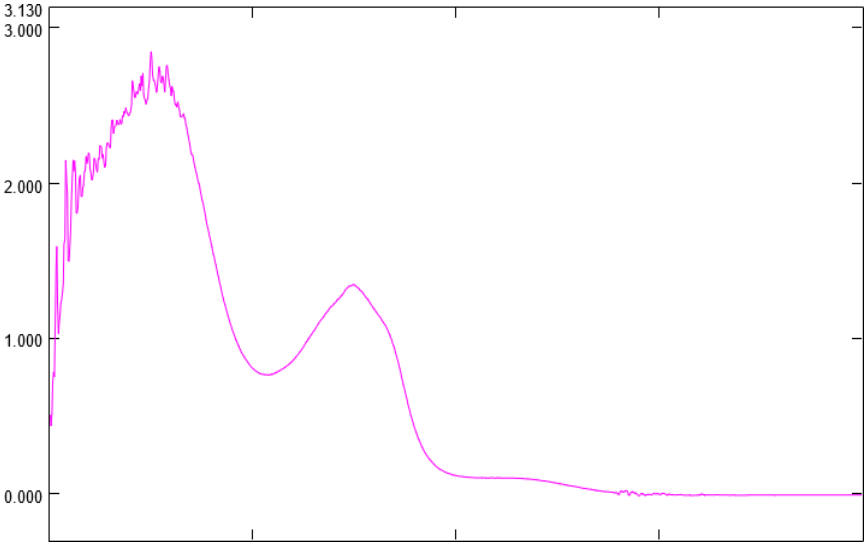


Fig.UV Spectra of Glipizide Showing Maximum absorbance 276nm [30%].

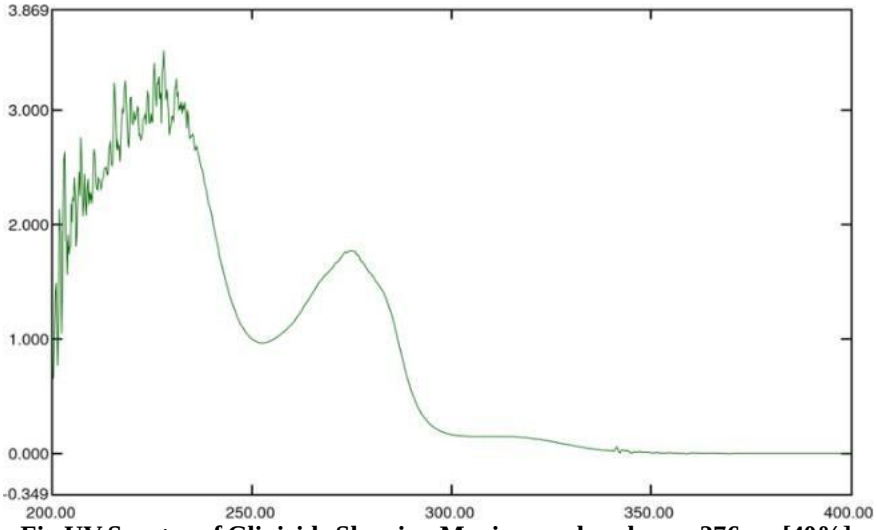


Fig.UV Spectra of Glipizide Showing Maximum absorbance 276nm [40%].

DISCUSSION

The results obtained during the UV Spectrophotometric method development and validation for Glipizide are promising:

1. Linearity and Sensitivity: The method showed an excellent linear relationship (R^2 = 0.9998) between concentration and absorbance, demonstrating that it can be used to quantify Glipizide within the concentration range of 5–50 µg/mL. The LOD and LOQ values (0.36 µg/mL and 1.09 µg/mL, respectively) indicate that the method is highly sensitive and suitable for detecting even trace amounts of Glipizide.
2. Applications: The developed method is simple, cost-effective, and can be routinely used in pharmaceutical quality control laboratories for the determination of Glipizide in both pure form and pharmaceutical dosage forms.

SUMMARY

The project aimed to develop and validate a reliable, simple, and cost-effective UV Spectrophotometric method for the quantification of Glipizide, an anti-diabetic drug used for the treatment of Type 2 Diabetes Mellitus. The developed method involved the measurement of absorbance at the maximum absorption wavelength (λmax) of 276 nm. This method was designed to offer a precise, accurate, and sensitive approach for determining Glipizide in both pure form and in pharmaceutical dosage forms.

A comprehensive validation of the method was carried out, focusing on several key parameters

- **Linearity:** A linear relationship between absorbance and concentration was established over the range of 5–40 µg/mL. The calibration curve was characterized by an excellent correlation coefficient (R^2 = 0.9998), indicating strong linearity.
- **Accuracy:** The recovery of Glipizide was found to be 99.5%, confirming that the method is accurate in the presence of excipients and other formulation components.

CONCLUSION

The UV Spectrophotometric method developed for the determination of Glipizide provides an efficient, accurate, and cost-effective solution for pharmaceutical analysis.

The overall success of this method highlights the importance of UV-Vis spectroscopy in pharmaceutical analysis, offering a non-invasive, efficient, and environmentally friendly approach to drug testing. The validation of this technique for Glipizide not only ensures the quality of the drug but also contributes to the ongoing efforts to improve patient care by guaranteeing that the prescribed doses of anti-diabetic medications are accurate and consistent.

In conclusion, the developed UV spectrophotometric method offers a practical, reliable, and high-performance alternative for the routine analysis of Glipizide in pharmaceutical quality control laboratories, contributing to the quality assurance process in the pharmaceutical industry.

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"With the grace of God''

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