UV-Visible spectrophotometric method development and validation for the estimation of glipizide in bulk and pharmaceutical formulation

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Abstract

The primary objective of the present research is to develop a simple, rapid, accurate, reproducible, and economical UV spectrophotometric method for measuring the quantity of glipizide (GLP) in pharmaceutical dosage form and bulk. Using a UV-visible double beam spectrophotometer, the amount of GLP was calculated at an absorption maximum of 273 nm using methanol as solvent. According to the specifications of the ICH guideline, the developed technique was validated for linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ. With a linear equation of y=0.0172x-0.0141 and a correlation coefficient of 0.9997, the linearity was determined to be 10-60 µg/ml. The accuracy was between 98% and 100.50%. The RSD percentages among samples was 0.19926 and 0.21806 for intra-day and inter-day precision, respectively. The limit of detection (LOD) and limit of quantification (LOQ)were discovered to be 4.77µg/ml and 14.47 µg/ml, respectively. According to ICH Q2(R1) requirements, the developed approach was validated. The innovative approach can be used to analyze drugs in their pharmaceutical dose form.

Keywords: UV-visible spectrophotometer, glipizide, ICH guidelines

Introduction

Glipizide (1-cyclohexyl-3-[[4-[[(5-methylpyrazine-2-yl) carbonyl] amino] ethyl] phenyl] sulphonyl]urea), is frequently administered to treat non-insulin-dependent diabetes (1). It functions by promoting the release of insulin by activating the SUR1 receptor on K/ATP-sensitive channels in pancreatic cells, which lowers blood glucose levels. GLP is classified as a weak acid (pKa = 5.9), nearly insoluble in water and acidic environments but it is easily soluble in methanol, dimethylformamide, and dimethyl sulfoxide (DMSO) (2). There are numerous test techniques available to determine glipizide for pharmacokinetic research (3–11).

The aim of the current research is to create a reliable, precise, repeatable, stability-indicating method for glipizide determination.

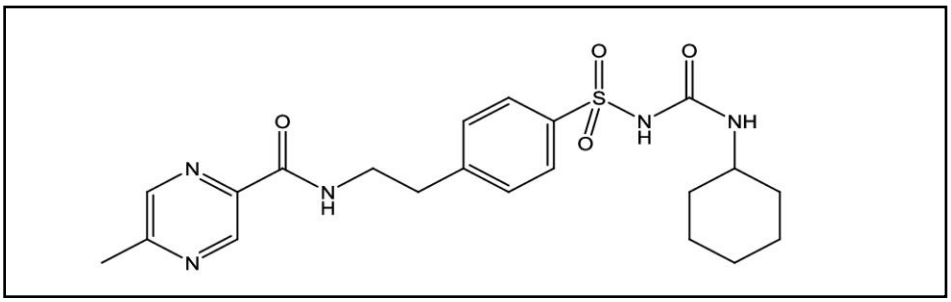

Figure 1: Chemical structure of glipizide

Materials and method

Materials

A free sample of pure standard Glipizide was received from Ranbaxy laboratories Ltd. In Gurugram. Methanol was used as the solvent of analytical grade, purchased from Merk Chemicals, India.

Instruments used

In the study, instruments including a sonicator (Oscar ultrasonic cleaner Microclean-103), an electronic balance (SHIMADZU-AY220), and a UV-Visible double beam spectrophotometer (Systronics-2201) with a quartz cell measuring 1 cm in length were employed.

Preparation of standard stock solution

Glipizide (GLP) standard stock solution was prepared by dissolving 10 mg of GLP in 10 ml of methanol to obtain a 1000 µg /ml concentration (Stock-1). Further the stock-1 was diluted using mobile phase (methanol) to obtained 100 µg/ml (stock-2) solution. This prepared stock-2 solution was used as a standard solution.

Determination of wavelength of maximum absorbance (λ max) of glipizide in methanol

The prepared standard solution was thereafter scanned in a UV-visible double-beam spectrophotometer ranging from 200 and 400 nm against mobile phase as a blank. The λ max was identified by obtaining the spectrum shown in figure 2 and the method was repeated thrice.

Preparation of working standard solution and construction of standard curve

A working standard solution with concentrations ranging from 10 µg/ml to 60 µg/ml was prepared by pipetting 1ml, 2ml, 3ml, 4ml, 5ml, and 6ml, from the stock solution into 6 separate 10ml volumetric flasks, the volume was then made up with methanol. These solutions were screened at λ max (273 nm), and the results are shown in table 1. Taking the obtained data into consideration a standard curve was constructed between concentration on X-axis and absorbance on Y-axis as shown in figure 3.

Assay

For analysis of commercial formulation, weigh 20 tablets and powdered them and powder equivalent to 10mg of glipizide was added to a 50 ml volumetric flask and dissolved in methanol. The resulting solution was sonicated, and the final volume was adjusted to the desired level to get 100 µg /ml solution by methanol. Then the 1 ml of the solution was transferred into a 10 ml volumetric flask and volume was adjusted with methanol up to the mark. The absorbance of the solution was measured at λ max (273 nm) and the average percent assay of glipizide tablet was calculated.

Method validation (12)

The developed UV method for estimation of glipizide was validated in terms of various validation parameters such as linearity and range, accuracy, precision, toughness, robustness, LOD, and LOQ in accordance with ICH Q2(R1) guidance.

Linearity and range

The term "linearity" was used to describe the analytical methodology's capacity to analyze data that are directly proportionate to analyte concentration. A standard solution of glipizide with a concentration of 10 – 60 µg/ml was made from the stock solution and examined to determine the linearity of the proposed approach. Three copies of each measurement were made (13).

Accuracy

The measure of closeness of experimental value to actual amount of substance in formulation is termed as accuracy. Recovery studies were used to determine the accuracy. The pure drug in three different concentrations (80%, 100%, and 120%) for the administration of the recovery tests. By creating solutions with varying concentrations accuracy was evaluated. Table 2 shows the % recovery calculation.

Precision

Precision expresses the measure of reproducibility of the measurement, it is a measure of degree of scatter. For the purpose of evaluating the dependability of the proposed analytical approach, precision studies were conducted. Six repetitions of the same sample at a concentration of 40 µg/ml proved the repeatability. As a result, the absorbance was monitored throughout the day, and a precision study was conducted by creating a drug resolution at a concentration of 40 µg/ml and analyzing it three times throughout the day. For three completely distinct days, the same process was used to produce work that was reportable as % RSD. A pair of outcomes from intraday and interday precision experiments were measured, although the precision result demonstrated an honest reliability.

Robustness

Robustness of an analytical method is the ability to resist the change in its performance in spite of small, delibrate change in method parameter. To assess the method's robustness, a 40 µg/ml solution of glipizide was examined six times at room temperature and 20°C.

Ruggedness

Ruggedness was determined by thoroughly analyzing 6 samples of 40 µg/ml concentration solutions in mobile phase at 273 nm and % RSD was used to represent the results.

LOQ and LOD

The smallest amount of analyte in the sample that may be identified is known as the limit of detection (LOD). The lowest amounts of analyte in the sample that can be quantitatively identified with sufficient precision and accuracy are known as the limit of quantification (LOQ). The following equation was used to determine LOQ and LOD:

LOD = 3.3xSD/S

Where, SD denotes the Y-intercepts' standard deviation.

S = Slope.

LOQ = 10xSD/S

Where, SD denotes the Y-intercepts' standard deviation.

S = Slope.

Result and Discussion

Method development and optimization

For quantitative UV analysis determination of the highest absorption wavelength is prerequisite. The maximum wavelength for glipizide solution (100 g/mL) was determined using the complete scan mode of a UV-Visible spectrophotometer taking into account the requirement and the suitability (Figure 2). The max was determined with the use of UV software after the full scan was analysed and it was found determined that glipizide have a 273 nm wavelength.

Table 1: Preparation of calibration curve

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.157
2	20	0.328
3	30	0.497
4	40	0.681
5	50	0.851
6	60	1.009
Regression equation y=0.0172x-0.0141		
R^2=0.9997		

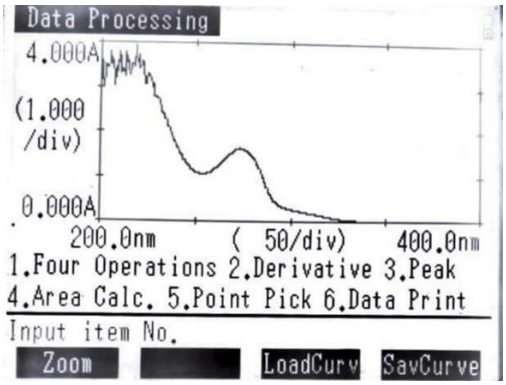

Figure 2: UV spectrum of glipizide at 273 nm

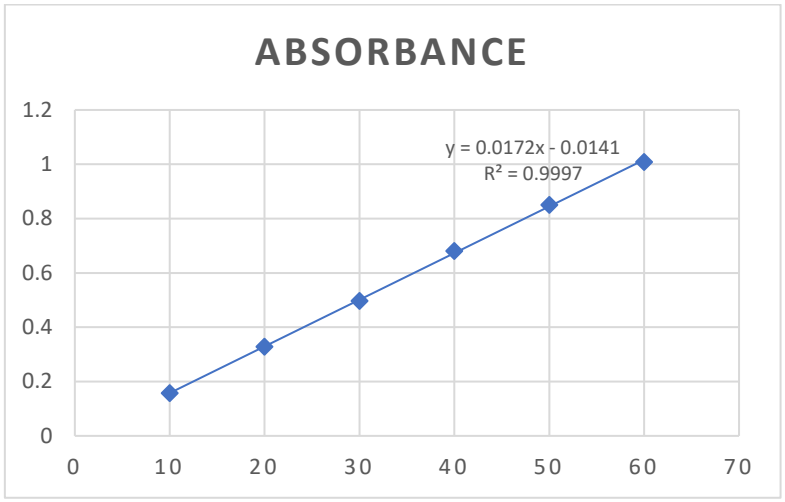

Figure 3: Calibration curve of glipizide at 273 nm

Linearity and range

The regression data for the calibration curves showed a good relationship within concentration range of 10–60 µg/ml and the correlation coefficients (r^2) value confirmed the calibration curve's linearity and yielded an equation; y = 0.0172x - 0.0141. According to figure 2, the correlation coefficient for glipizide was shown to be 0.997. the linearity study revealed that, developed method was linear in the pre-defined concentration range of calibration standards.

Accuracy

Regular addition was used to address the accuracy of proposed method, and it was hereby discovered that the recovery fell within the range of 99-100.593 % (Table 5). The % RSD was determined as less than 2. It was observed from the results of accuracy studies that the developed UV method is much accurate.

Table 2: accuracy data of UV method for glipizide

Concentration	Absorbance	Conc.	Accuracy
10	0.157	9.947674	99.47674
30	0.497	29.71512	99.05039
50	0.851	50.29651	100.593

Precision

There was little difference between the intraday and interday because they were performed on completely different days. The outcome demonstrates the consistency of the suggested methodology. The findings for precision are shown in (Tables 2, 3, 4). Additionally, the relative variance proportion was estimated.

Table 3: Intraday precision data of UV method for glipizide

Time	10:00	12:00	02:00
Conc.	Absorbance 1	Absorbance 2	Absorbance 3
30	0.497	0.497	0.498
30	0.496	0.497	0.498
30	0.496	0.496	0.495
30	0.497	0.498	0.498
30	0.495	0.497	0.497
30	0.498	0.498	0.497
Mean	0.4965	0.497167	0.497167
SD	0.001049	0.000753	0.001169
RSD	0.21124	0.151413	0.235142
%RSD	0.199265		

Table 4: Interday precision data of UV method for glipizide

Day	1 Day	2 Day	3 Day
Conc.	Absorbance 1	Absorbance 2	Absorbance 3
30	0.497	0.494	0.492
30	0.496	0.495	0.491
30	0.496	0.492	0.492
30	0.497	0.494	0.492
30	0.495	0.492	0.49
30	0.498	0.495	0.491
Mean	0.4965	0.493667	0.491333
SD	0.001049	0.001366	0.000816
RSD	0.21124	0.276758	0.16618
%RSD	0.218059		

Robustness

By securing the assay during the alteration wavelength, robustness was solidified. As stated in (Table 5), the sharp RSD was found to be no more than 2%, which was within the limit.

Table 5: robustness data of UV method for glipizide

Conc.	Absorbance AT 20°C	Absorbance at 30°c
30	0.497	0.496

30	0.496	0.496
30	0.496	0.496
30	0.497	0.498
30	0.495	0.497
30	0.498	0.498
Mean	0.4965	0.496833
sd	0.001049	0.000983
RSD	0.21124	0.197892
%RSD	0.204566	

Ruggedness

Ruggedness was determined by doing the test under constant conditions on totally different days, by completely different analysts, using a completely different apparatus, and at a completely different time. According to (Table 6) the check results were found to be in the region of 99-101%.

Table 6: ruggedness data of UV method for glipizide

Conc.	Absorbance 1	Absorbance2
30	0.497	0.498
30	0.496	0.496
30	0.496	0.495
30	0.497	0.497
30	0.495	0.497
30	0.498	0.498
Mean	0.4965	0.496833
sd	0.001049	0.001169
RSD	0.21124	0.235299
%RSD	0.22327	

LOQ and LOD

The values for the LOQ and LOD were discovered to be 4.775 and 14.468 µg/ml, respectively.

Assay of glipizide tablet

The developed UV visible method was successfully applied for the estimation of glipizide content in glipizide tablet and the average percent assay of glipizide tablet was found to be 98.5 %.

Conclusion

The proposed UV Spectrophotometric approach, which has been used in numerous research including [18], can be regarded as quick and affordable for sampling. The approach is appropriate for estimating glipizide with outstanding accuracy, precision, and linearity and is valid conformity with ICH criteria.

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