

Research Article

Stability-indicating UV-Spectrophotometric Determination of Glipizide in Pharmaceuticals

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Glipizide (GPZ) is an antidiabetic drug indicated for type-2 diabetes. Two uv-spectrophotometric methods, which are simple, sensitive and stability-indicating are presented for the determination of GPZ in pharmaceuticals. The methods are based on the measurement of absorbance of drug solution either in 0.1M NaOH at 260nm (NaOH method) or in 0.1M HCl at 255nm (HCl method). Under the optimum condition, absorbance-concentration plots were linear over 4-72 $\mu\text{g mL}^{-1}$ range in both methods. The calculated molar absorptivity values were 6.06×10^3 and $6.13 \times 10^3 \text{ l mol}^{-1}\text{cm}^{-1}$, respectively, with corresponding Sandell sensitivities of 0.0752 and 0.0743 $\mu\text{g cm}^{-2}$. The limits of detection (LOD) and quantification (LOQ), calculated as per the ICH guidelines, were 1.02 and 3.05 $\mu\text{g mL}^{-1}$ (NaOH method) and 0.85 and 2.55 $\mu\text{g mL}^{-1}$ (HCl method). The methods were validated for intra-day and inter-day accuracy and precision, and the percent relative error values (measure of accuracy) were ≤ 2 , and percent relative standard deviation (measure of precision) were < 1.5 . The methods were also validated for robustness, ruggedness and selectivity. When applied to tablets, the methods yielded results, which agreed with the label claim and those of a reference method. As part of forced degradation study, drug was subjected to acid-, base-, peroxide-, heat-, and light-induced stress conditions following the ICH guidelines, and the results showed that GPZ is prone to oxidative degradation, and remained intact under other stress conditions.

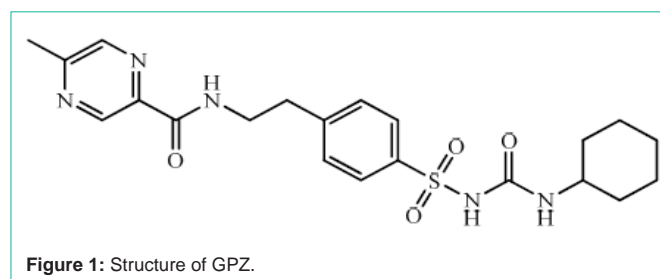
Keywords: Glipizide; Assay; UV-spectrophotometry; Pharmaceuticals; Stability-indicating.

Introduction

Glipizide (GPZ), chemically known as *N*-[2-[4-[[[(Cyclohexylamino)carbonyl]amino] sulfonyl] phenyl] ethyl]-5-methylpyrazinecarboxamide (Figure 1) is one of the sulphonyl urea derivatives that are widely used as oral anti-hyper glycaemic drugs for the treatment of non-insulin-dependent diabetes mellitus [1]. It functions by stimulating pancreatic beta-cell insulin production, which results in the reduction of glucose levels in blood.

The drug has an official monograph in European Pharmacopoeia [2] which describes a titrimetric assay for GPZ, in which drug solution in dimethylformamide is titrated with lithium methoxide using quinaldine red as indicator. Several other methods based on techniques such as high-performance liquid chromatography [3-17], ultra-performance liquid chromatography [18], thin layer chromatography [19,20] and high-performance thin layer chromatography [21] have been reported for the determination of GPZ in bulk and dosage forms. Though these techniques [3-18] often provide sensitive and selective means for the assay of GPZ, the required instrumental facilities are not available in many laboratories, and the other two techniques [19-21] are semi-quantitative.

Assay methods for routine analysis should be simple, rapid, easy to perform, sensitive and the instrument used should be easily available in most laboratories. UV-spectrophotometry meets these requirements, but little attention has been paid to the determination of GPZ in pharmaceuticals, using this facile technique. There is only



one direct method described by Mantri and Shanmukhappa [4], when the drug is present alone in the dosage form. Aruna and Nancey [22] have described the simultaneous determination of metformin (MET) and GPZ in solid dosage forms by two methods: solving simultaneous equations and second derivative mode. The methods are less sensitive with narrow linear ranges. Two more methods were developed for the simultaneous determination of GPZ and MET in tablet dosage forms by Chungath *et al.* [23]. Method A involved solving simultaneous equations, where two wavelengths: 238nm (for MET) and 275nm (for GPZ) were selected for the formation of simultaneous equations. Method B involved the formation of Q-absorbance equation at isobestic point (259.5nm). Linearity was observed in the range 1.2–6.0 $\mu\text{g mL}^{-1}$ for GPZ in both the methods. GPZ and MET in combined tablet formulation were assayed by Sarangi *et al.* [24] also. The authors used multi component mode at 276nm (for GPZ) and 237nm (for MET) for measurement in methanolic solution. Beer's law is obeyed in the concentration range 2-20 $\mu\text{g mL}^{-1}$ for GPZ. Adhikari

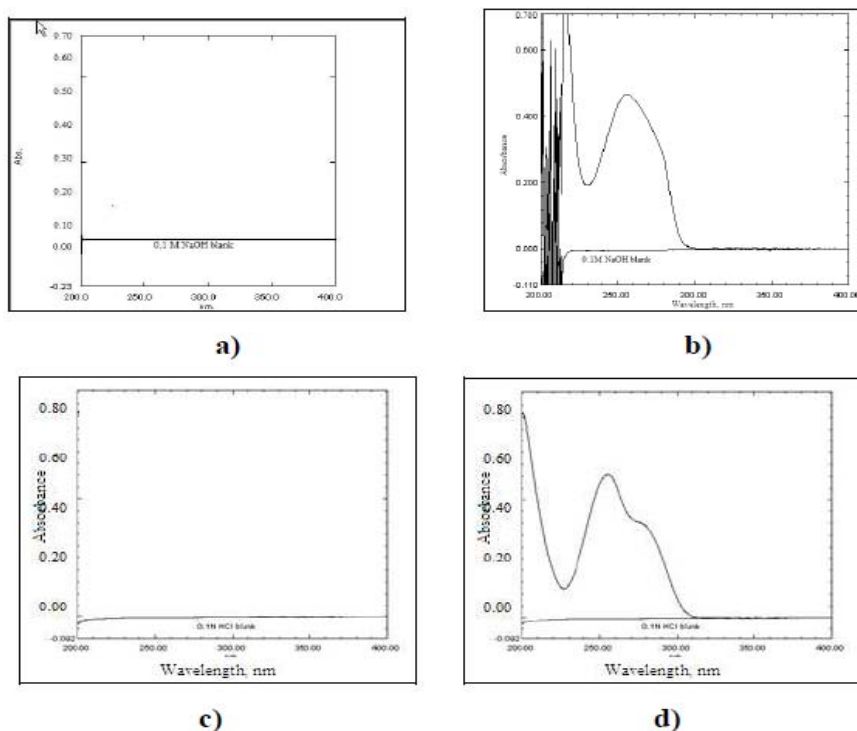


Figure 2: Absorption spectra of: **a)** 0.1M NaOH blank; **b)** GPZ in 0.1M NaOH ($40\mu\text{g mL}^{-1}$); **c)** 0.1M HCl blank; **d)** GPZ in 0.1M HCl ($40\mu\text{g mL}^{-1}$).

et al [25] used two methods for the simultaneous determination of pioglitazone (PGT), MET and GPZ in multi component formulation. The three-wavelength method used acetonitrile-methanol-water in the ratio (3:4:1) with λ_{max} at 236.5, 226.4 and 227.3 nm, for PGT, MET and GPZ, respectively. The isobestic point was found to be at 254nm. Method II was based multi wavelength spectroscopy. The Beer's law was obeyed over $5\text{--}55\mu\text{g mL}^{-1}$ range for GPZ. Except one [22], all other uv-spectrophotometric methods [22-25] are applicable to combined dosage forms. Since GPZ is found in many brands of single-component tablets, the need for a simple, rapid, convenient and inexpensive method is obvious. The aim of the present study was, therefore, to use uv-spectrophotometry-based methods to the determination of GPZ. The two developed methods are based on the measurement of absorbance of GPZ solution either in 0.1M NaOH at 260nm (NaOH method) or in 0.1M HCl at 255nm (HCl method). Stress testing of a drug substance is mandatory [26], but none of the methods reported for GPZ so far is stability-indicating. To determine the stability-indicating ability of the developed methods, GPZ was subjected to various stress conditions followed by assay after forced degradation. The methods were found to be both rapid and reliable.

Experimental

Apparatus

Shimadzu Pharmaspec 1700UV/Visible double beam spectrophotometer provided with matched 1-cm quartz cells (Hyderabad, India) was used for all absorbance measurements.

Reagents and materials

All chemicals and reagents used were of analytical reagent grade. Hydrochloric acid (HCl), sodium hydroxide (NaOH) and hydrogen

peroxide (H_2O_2) were purchased from S.D Fine Chem Ltd., Mumbai, India. HCl (2M) was prepared by diluting 17.6ml of concentrated acid (Sp. gr. 1.18) to 100ml with water and used in stress study. This was diluted to 0.1M concentration with water and standardised. NaOH (2M) was prepared by dissolving 8g of chemical in 100mL of water for use in stress study, and the same was diluted to 0.1M with water and standardised. H_2O_2 (5% v/v) was obtained by appropriate dilution of the commercial sample.

Preparation of standard GPZ solution

Pure sample of GPZ was kindly supplied by Bal Pharma, Bangalore, India, as gift. Stock standard GPZ solutions ($400\mu\text{g mL}^{-1}$) were prepared by dissolving 40mg of pure GPZ in 0.1M NaOH or 0.1M HCl separately, and diluted to 100mL with the respective solvent, in calibrated flasks. The solutions were diluted to obtain $80\mu\text{g mL}^{-1}$ each GPZ and used for assay. GPZ-containing tablets: Dibizide-5 (5mg) (Micro Labs Limited, Bangalore, India), Glynase-5 (5mg) (USV Limited, Aurangabad, India) were purchased from the local market.

Analytical procedures

Procedure for bulk drug

NaOH Method: Into a series of 10mL volumetric flasks, aliquots of standard solution equivalent to 40-720 μg GPZ were accurately transferred and volume made upto the mark with 0.1M NaOH. The absorbance of each solution was measured at 260nm vs 0.1M NaOH.

HCl method: Varying aliquots (0.5,1.0,...9.0mL) of $80\mu\text{g mL}^{-1}$ standard GPZ solutions were taken in a series of 10mL volumetric flasks and made upto the mark with 0.1M HCl. The absorbance of each solution was measured at 255nm vs 0.1M HCl.

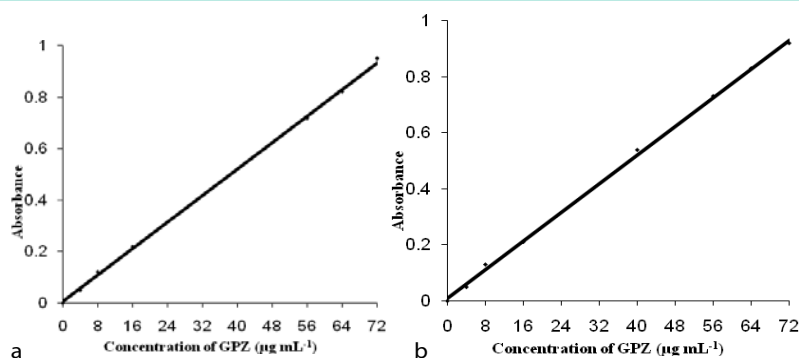


Figure 3: Calibration curve for GPZ: a) in 0.1M NaOH, b) in 0.1M HCl

Table 1: Sensitivity and regression parameters.

Parameter	NaOH method	HCl method
λ_{max} , nm	260	255
Linear range, $\mu\text{g mL}^{-1}$	4.0 – 72.0	4.0 – 72.0
Molar absorptivity(ϵ), $\text{L mol}^{-1} \text{cm}^{-1}$	6.06×10^3	6.13×10^3
Sandell sensitivity', $\mu\text{g cm}^{-2}$	0.0752	0.0743
Limit of detection (LOD), $\mu\text{g mL}^{-1}$	1.02	0.85
Limit of quantification (LOQ), $\mu\text{g mL}^{-1}$	3.05	2.55
Regression equation, y'		
Intercept (b)	0.0079	0.0142
Slope (m)	0.0129	0.0127
Standard deviation of intercept (S_b)	0.0003	0.0012
Standard deviation of slope (S_m)	0.0002	0.0002
Regression coefficient (r)	0.9996	0.9994

$y = mx + b$, Where y is the absorbance, x concentration in $\mu\text{g mL}^{-1}$, b intercept and m slope.

Calibration curves were prepared by plotting absorbance *versus* the concentration. Regression equation, derived using Beer's law data, was used to compute the concentration of unknown.

Procedure for tablets

Weighed amount of tablet powder equivalent to 40mg of GPZ was transferred into a 100mL volumetric flask. The content was shaken well with about 60mL of 0.1M NaOH or 0.1M HCl for 20min. The mixture was diluted to the mark with the respective solvent. It was filtered using Whatman No 42 filter paper. First 10mL portion of the filtrate was discarded and a subsequent portion was diluted to $40\mu\text{g mL}^{-1}$ and assayed in five replicates.

Procedure for placebo blank and synthetic mixture

A placebo blank of the composition: acacia (15mg), hydroxyl cellulose (10mg), magnesium Stearate (15mg), starch (10mg), sodium citrate (15mg), talc (15mg) and sodium alginate (10mg) was made and its solution was prepared by taking 20mg as described under 'procedure for tablets' and then subjected to analysis. A synthetic mixture was prepared by homogeneous mixing of 20mg of pure drug with placebo. Its solution was prepared as described under "procedure for tablets". The extract was subjected to assay following the general procedures and the percentage recovery of GPZ was calculated.

Procedure for forced degradation study

In both methods, a 1mL aliquot of $400\mu\text{g mL}^{-1}$ GPZ was taken in three separate 10mL volumetric flasks and mixed with 2mL of 2M HCl (acid hydrolysis), 2M NaOH (base hydrolysis) or 5% H_2O_2 (oxidative degradation) and boiled for 2h at 80°C in a hot water bath. Each solution was cooled to room temperature and diluted to the mark with 0.1M NaOH or HCl after neutralization with base/acid. For thermal and photo degradation, solid sample was kept in a Petri dish in oven at 100°C for 24h (thermal degradation) or exposed to UV radiation of wavelength 254nm and of 1200K lux intensity for 48h in a UV chamber (photo degradation). After cooling to room temperature, $40\mu\text{g mL}^{-1}$ GPZ solution in 0.1M HCl/NaOH was prepared separately and absorption spectrum recorded.

Results and Discussion

The absorption spectra of $40\mu\text{g mL}^{-1}$ GPZ solution in 0.1M NaOH and 0.1M HCl were recorded separately between 200 and 400nm, which showed maxima at 260 and 255nm, respectively. At these wavelengths, 0.1M NaOH and 0.1M HCl had insignificant absorbance as shown in Figure 2.

Method Validation

Analytical parameters

The regression parameters calculated from the calibration graphs (Figure 3), are presented in Table 1. Beer's law was obeyed upto $72\mu\text{g mL}^{-1}$ in both methods. Linearity of calibration graphs (Figure 3) was demonstrated by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The molar absorptivity, Sandell sensitivity values of both methods are also shown in Table 1. The limits of detection and quantification were calculated as per the current ICH guidelines [26] and are presented in Table 1 and speak of moderately high sensitivity of the methods.

Precision and accuracy

Precision and accuracy of the methods were evaluated in terms of intermediate precision and error (intra-day and inter-day). Three different concentration of GPZ (within the working limits) were analyzed in seven replicates during the same day (intra-day) and five consecutive days (inter-day). Percent RSD and RE values, which are the measures of precision and accuracy, are ≤ 2 and < 1.5 (Table 2), respectively, and indicate the excellent repeatability, reproducibility and accuracy of the proposed methods.

Table 2: Results of intra-day and inter-day accuracy and precision study.

Method	GPZ taken, $\mu\text{g mL}^{-1}$	Intra-day (n=5)			Inter-day (n=5)		
		GPZ Found ^a , $\mu\text{g mL}^{-1}$	%RSD ^b	%RE ^c	GPZ Found ^a , $\mu\text{g mL}^{-1}$	%RSD ^b	%RE ^c
NaOH method	20	20.3	1.07	1.50	20.4	0.95	2.00
	40	39.5	0.92	1.25	39.3	1.63	1.75
	60	58.9	0.63	1.83	61.0	1.12	1.67
HCl method	20	19.8	1.01	1.50	19.7	1.04	1.36
	40	39.5	1.09	1.25	40.7	1.25	1.75
	60	60.8	1.33	1.17	59.1	0.97	1.50

A Mean value of five determinations; ^bRelative standard deviation; ^cRelative error.

Table 3: Results of robustness and ruggedness, expressed as intermediate precision.

Method	GPZ taken, $\mu\text{g mL}^{-1}$	Method robustness ^a %RSD	Method ruggedness	
			Inter-analysts %RSD, (n=3)	Inter-cuvettes %RSD, (n=3)
NaOH method	20	1.05	1.05	1.03
	40	0.92	1.36	1.51
	60	1.47	0.91	0.81
HCl method	20	0.69	1.54	1.09
	40	1.15	0.75	1.54
	60	0.63	0.89	1.21

^aThe wavelengths were 259, 260 and 261nm (NaOH method) and 254, 255 and 256nm (HCl method).

Robustness and ruggedness

Robustness was determined by the analysis of standard solution at three concentration levels at three wavelengths (*viz* λ_{max} and $\lambda_{\text{max}} \pm 1\text{nm}$). To determine the method ruggedness, assays were performed by three analysts with the same cuvette and also by a single analyst with three different cuvettes in the same laboratory. Small deliberate alterations in the optimized and operational conditions did

Table 4: Results obtained by the analysis of tablets by the proposed methods and statistical comparison of results with the reference method.

Tablet brand name	Label claim mg/tablet	Found (Percent of label claim \pm SD) ^a		
		Official method	Proposed methods	
			NaOH method	HCl method
Dibizide-5	5.00	101.5 \pm 1.98	101.7 \pm 1.39	101.9 \pm 1.45
			t = 0.18 F = 2.03	t = 0.36 F = 1.86
Glynase-5	5.00	102.1 \pm 1.29	101.2 \pm 1.63	102.7 \pm 1.21
			t = 0.97 F = 1.60	t = 0.76 F = 1.14

^aMean value of five determinations.

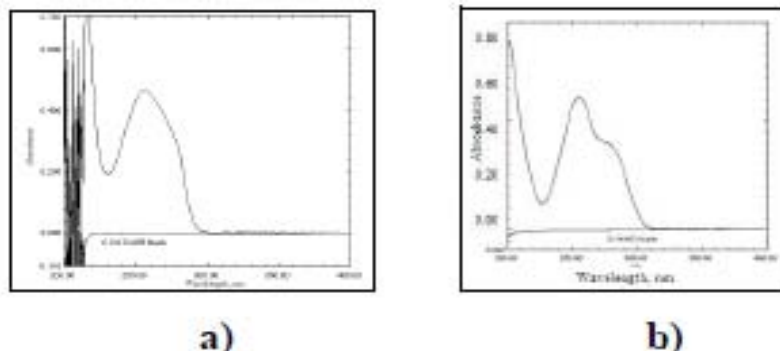
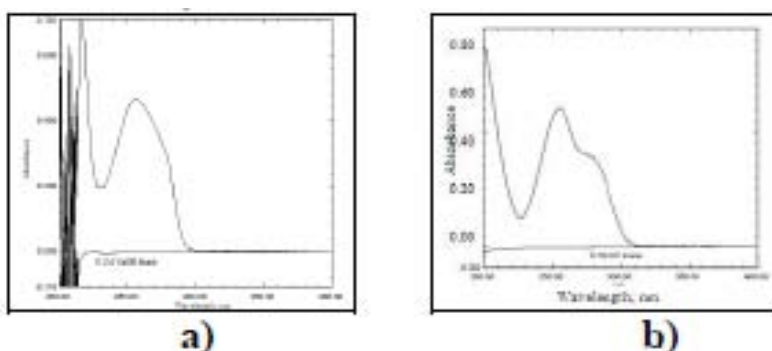
not significantly influence the results as indicated by small values of %RSD (Table 3).

Selectivity

The placebo blank when subjected to assays did not absorb at the wavelength of analysis. When the synthetic mixture solution was subjected to analyses at $40\mu\text{g mL}^{-1}$ concentration level by each method, the percent recoveries were 97.42 and 98.25 respectively, with %RSD being less than 1.9%, implying that the assays were not affected by inactive ingredients.

Application to tablets

The proposed methods were applied for the quantification of GPZ in two brands of commercial tablets. Same tablets were analysed by the official method [2] for comparison. The official method consisted of titration of drug in dimethylformamide with 0.1M lithium methoxide using quinaldine red indicator. Statistical analysis of the results did not detect any significant difference between the proposed methods

**Figure 4A:** UV-spectra of $40\mu\text{g mL}^{-1}$ GPZ after acid hydrolysis: a) NaOH method and b) HCl method**Figure 4B:** UV-spectra of $40\mu\text{g mL}^{-1}$ GPZ after base hydrolysis: a) NaOH method and b) HCl method.

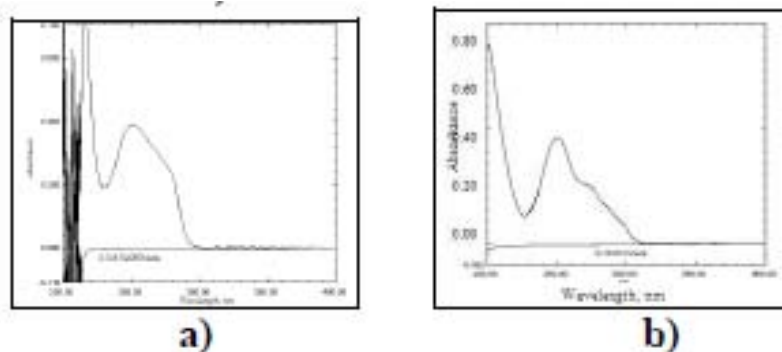


Figure 4C: UV-spectra of $40\mu\text{g mL}^{-1}$ GPZ after subjecting to oxidative condition: a) NaOH method and b) HCl method.

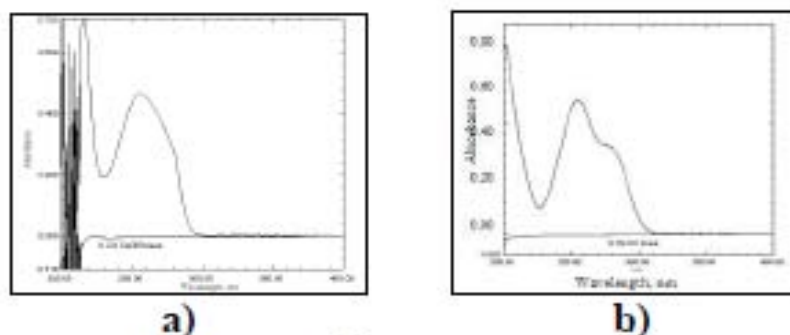


Figure 4D: UV-spectra of $40\mu\text{g mL}^{-1}$ GPZ after subjecting to thermal degradation: a) NaOH method and b) HCl method.

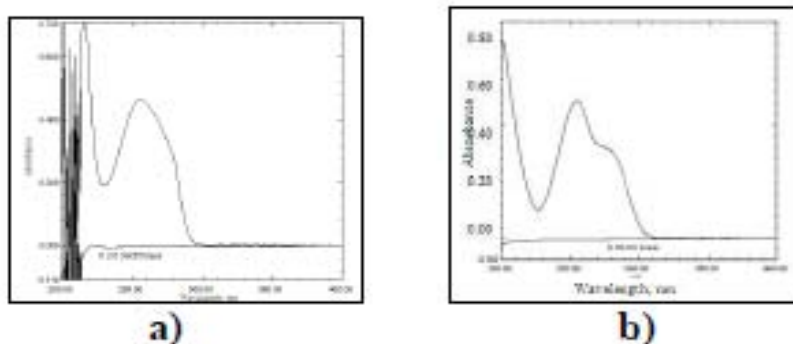


Figure 4E: UV-spectra of $40\mu\text{g mL}^{-1}$ GPZ after subjecting to photolytic condition: a) NaOH method and b) HCl method.

Table 5: Results of recovery study using standard addition method.

Tablet studied	NaOH method				HCl method			
	GPZ in tablet, $\mu\text{g mL}^{-1}$	Pure GPZ added, $\mu\text{g mL}^{-1}$	Total GPZ found, $\mu\text{g mL}^{-1}$	Pure GPZ recovered (Percent \pm SD*)	GPZ in tablet, $\mu\text{g mL}^{-1}$	Pure GPZ added, $\mu\text{g mL}^{-1}$	Total GPZ found, $\mu\text{g mL}^{-1}$	Pure GPZ recovered (Percent \pm SD*)
Dibizide-5	20.34	10.0	30.01	98.92 \pm 1.05	20.38	10.0	30.87	101.6 \pm 1.29
	20.34	20.0	40.98	101.6 \pm 1.43	20.38	20.0	40.23	99.63 \pm 1.63
	20.34	30.0	50.89	101.1 \pm 1.67	20.38	30.0	50.98	101.2 \pm 1.89
Glynase-5	20.24	10.0	30.90	102.2 \pm 1.36	20.54	10.0	31.27	102.4 \pm 0.54
	20.24	20.0	41.01	101.9 \pm 0.98	20.54	20.0	41.72	102.9 \pm 1.81
	20.24	30.0	49.16	97.86 \pm 0.83	20.54	30.0	49.97	98.87 \pm 1.45

*Mean value of three determinations.

and reference method with respect to accuracy and precision as revealed by the Student's t-value and variance ratio F-value [27]. The results of this study are presented in Table 4.

Accuracy by recovery test

The test was done by spiking pre-analyzed tablet powder with pure GPZ at three different levels (50, 100 and 150% of the content

Table 6: Results of stability indicating study by forced degradation study.

Stress condition	% Degradation*	
	NaOH method	HCl method
Acid hydrolysis	No degradation	No degradation
Alkali hydrolysis	No degradation	No degradation
Oxidation	57.2	59.8
Thermal (105°C, 3 hours)	No degradation	No degradation
Photolytic (1.2 million lux hours)	No degradation	No degradation

*Mean value of three determinations.

present in the tablet powder (taken) and the total was determined by the proposed methods. Each test was repeated three times. In both the cases, the recovery percentage values ranged between 97.86 and 102.9% with standard deviation in the range, 0.85-1.89%. Closeness of the results to 100% indicates good accuracy as well as selectivity of the methods, as shown in Table 5.

Results of forced degradation study

The UV-spectra of 40µg mL⁻¹ GPZ each in 0.1M NaOH and 0.1M HCl after forced degradation are shown in Figure 4A to Figure 4E. The drug was found to undergo substantial degradation under oxidative stress condition and remained intact under other conditions (Table 6).

Conclusions

This is the first report dealing with stability-indicating method for glipizide. The present uv-spectrophotometric methods allow determination of glipizide over a wide concentration range (4-72µg mL⁻¹) unlike many reported methods. Each method employs a single aqueous base or acid medium compared to organic solvent or mixed organic solvent media used in the reported methods. The results of validation are satisfactory and the methods can be used in routine analysis.

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