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Spectrophotometric Methods for the Determination of Anti-diabetic Drug Glipizide in Pure and Pharmaceutical Formulations

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ABSTRACT
Four simple and precise spectrophotometric methods were developed and optimized for the assay of glipizide (GPZ) in pure form and in its pharmaceutical preparations. Method A was based on the reaction of drug with 1,2-naphthoquinone-4-sulfonate (NQS) in alkaline medium to form orange colored product (λ_{max} 456 nm). In the method B, drug reduced the reagent tetrazolium blue (TB) which lead to the formation of intense violet colored formazan (λ_{max} 515 nm). The method C employed Folin-Ciocalteu reagent (FC) that reacted with drug in alkaline medium to form blue colored complex (λ_{max} 760 nm). Method D was based on the reduction of iron(III) to iron(II) by the drug and their complexation with bathophenanthroline (BPT) to form pink colored complex (λ_{max} 547 nm). Linearity of Beer's plots was observed in the concentration range of 10.00 – 100.00 µg mL^{-1}, 2.00 – 22.00 µg mL^{-1}, 5.00 – 40.00 µg mL^{-1}, 0.50 – 7.50 µg mL^{-1} for method A, B, C and D respectively. The proposed methods were validated and values of analytical parameters like molar absorptivity, Sandell's sensitivity, limits of detection and quantification were also calculated. The methods were found to be selective for the quantitative determination of GPZ in commercially available formulations.
Keywords: folin's reagent, tetrazolium blue, folin c; bathophenanthroline, pharmaceuticals

INTRODUCTION

Glipizide (GPZ) is a second generation blood-glucose-lowering drug used in the management of diabetes mellitus [1]. It is one of the derivatives of sulfonylurea chemically known as *N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl]phenethyl)-5-methylpyrazine-2-carboxamide **(Figure 1)**. It is administered orally, well absorbed in the body and has greater efficacy in controlling hyperglycemia in diabetic patients [2]. Due to its potent hypoglycemic activity, it is considered as one of the therapeutic alternative for patients who do not respond to standard anti-diabetic drugs [3]. The enhanced pharmacological importance of GPZ has resulted in vast literature on its determination in commercial dosage forms.

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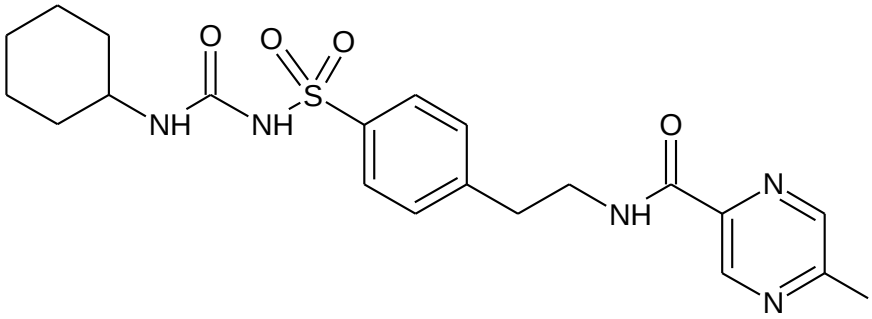

Figure 1. Chemical structure of glipizide

Several quantification methods of GPZ in pharmaceutical formulations have been reported in the literature which included high performance liquid chromatography [4-7], high performance thin layer chromatography [8], liquid chromatography-mass spectrometry [9] and derivative spectrophotometry [10-12]. The reported techniques are sophisticated, require complicated sample preparation, time consuming and are not of frequent choice in basic clinical laboratories. Literature survey reveals that only one visible spectrophotometric method [13] is known and there is a wide scope for the derivatization of GPZ by chromogenic reagents and the quantification of drug using spectrophotometry technique. The reported spectrophotometric method utilized the combination of acetaldehyde and bromanil for the oxidative condensation reaction with GPZ but unfortunately, it is a two step reaction, requires complicated experimental setup and meticulous control of reaction conditions. Thus, the present study described four new visible spectrophotometric methods for the quantitative determination of GPZ in pure and pharmaceutical preparations.

EXPERIMENTAL

Instrument

All spectrophotometric measurements were performed on SHIMADZU UV-2550 double beam ultraviolet-visible spectrophotometer (Shimadzu Corporation, Japan) with 1 cm matched quartz cells.

Materials and Reagents

Pharmaceutical grade glipizide drug was provided by CAD Pharma Inc., Bangalore, India. The following market samples were purchased from commercial source: Glide™ tablets batch No. M15002 (Franco-Indian Remedies Pvt. Ltd., India) labeled to contain 5 mg of glipizide per tablet, Glynase® tablets batch No. 32001281 (USV Ltd., India) labeled to contain 5 mg of glipizide per tablet. Glytop® 5 SR batch No. RSF081502 (Sidmak Laboratories Pvt. Ltd., India) labeled to contain 5 mg of glipizide per tablet. Dibizide® batch No. (DZIH0024) labeled to contain 5 mg of glipizide per tablet.

All reagents and solvents used in this study were of analytical grade. 1,2-naphthoquinone-4-sulfonate (NQS) and tetrazolium blue (TB) (Lobachemie, India), each of 0.01 mol L^{-1} were prepared in ethanol. Bathophenanthroline (BPT) 0.015 mol L^{-1} and ferric chloride 0.018 mol L^{-1} (Spectrochem, India) were prepared in 0.1 mol L^{-1} hydrochloric acid and distilled water, respectively. The solution of Folin-Ciocalteu (FC) reagent (SRL Pvt. Ltd., India) 1 mol L^{-1} was prepared by appropriately diluting the commercially available 2 mol L^{-1} reagent with distilled water.

Preparation of standard drug and sample solutions

A standard stock solution of GPZ (1000 µg mL^{-1}) was prepared by dissolving an accurately weighed 100 mg of pure drug in 50 mL ethanol. The solution was then transferred to 100 mL calibrated flask and diluted to the mark with ethanol. The working concentrations were prepared by appropriate dilution of the standard stock solution.

The tablet dosage forms were analyzed by pulverizing ten tablets into fine powder and an amount equivalent to 5 mg was weighed and dissolved in 25 mL of ethanol. The solution was filtered through Whatman No. 40 filter paper into 50 mL calibrated flasks and made up to the mark with ethanol. An appropriate aliquot was then subjected to analysis by the proposed methods.

Analytical procedures

Method A

Aliquots equivalent to 10.00–100.00 µg mL^{-1} of GPZ solution were transferred quantitatively into a series of 10 mL volumetric flasks. To each flask, 0.5 mL of 0.2 mol L^{-1} sodium hydroxide solution was added followed by the addition of 1 mL of prepared NQS solution. The reaction mixture was gently shaken and kept aside for 10 minutes at room temperature. The solutions were then diluted to the mark with distilled water and the absorbance of resulting solutions was measured at 456 nm against the corresponding reagent blank.

Method B

Aliquots equivalent to 2.00–22.00 µg mL^{-1} of GPZ solution were transferred quantitatively into a series of 10 mL volumetric flasks. Then, 1 mL of 0.2 mol L^{-1} ethanolic sodium hydroxide solution was added followed by the addition of 0.5 mL of prepared TB solution. The reaction was allowed to proceed at 80 °C for 10 minutes after which the solutions were cooled and diluted to volume with ethanol. The absorbance of the resulting solutions was measured at 515 nm against the corresponding reagent blank.

Method C

Aliquots equivalent to 5.00–40.00 µg mL^{-1} of GPZ solution were transferred quantitatively into a series of 10 mL volumetric flasks. To each flask, 1 mL of 2 mol L^{-1} sodium

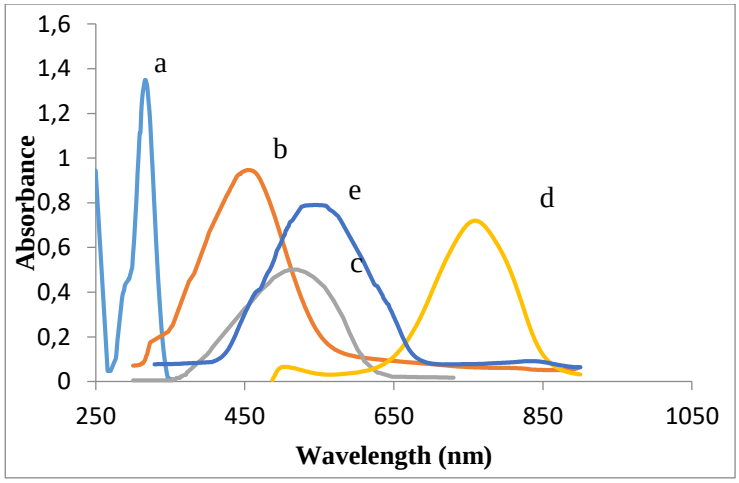

Figure 2. Absorption spectra of a) pure drug and reaction products b) GPZ-NQS c) GPZ-TB d) GPZ-FC e) GPZ-BPT

hydroxide solution was added followed by the addition of 2 mL of prepared FC solution and kept aside for 20 minutes at room temperature. The solutions were then swirled well, diluted to volume with distilled water and the absorbance of colored product was measured at 760 nm against the corresponding reagent blank.

Method D

Aliquots equivalent to 0.50–7.50 µg mL^{-1} of GPZ solution were transferred quantitatively into a series of 10 mL volumetric flasks. To each flask, 1 mL of prepared ferric chloride and BPT solution was added. The reaction mixture was heated on thermostatic water bath at 80 °C for 30 minutes to allow the reaction to proceed to completion. The solutions were then cooled to room temperature, diluted to mark with distilled water and absorbance of the resulting pink colored complex was measured at 547 nm against the corresponding reagent blank.

RESULTS AND DISCUSSION

Absorption spectra

The drug GPZ posseses secondary and tertiary amino groups that could be utilized for its derivatization with many chromogenic reagents. The absorption spectrum of the drug was recorded in ethanol and it exhibited a maximum absorption peak at 317 nm. The reaction of the drug with NQS, TB, FC and BPT resulted in the bathochromic shift and the reaction products GPZ-NQS, GPZ-TB, GPZ-FC and GPZ-BPT exhibited wavelength of maximum absorbance at 456 nm, 515 nm, 760 nm and 547 nm, respectively (**Figure 2**).

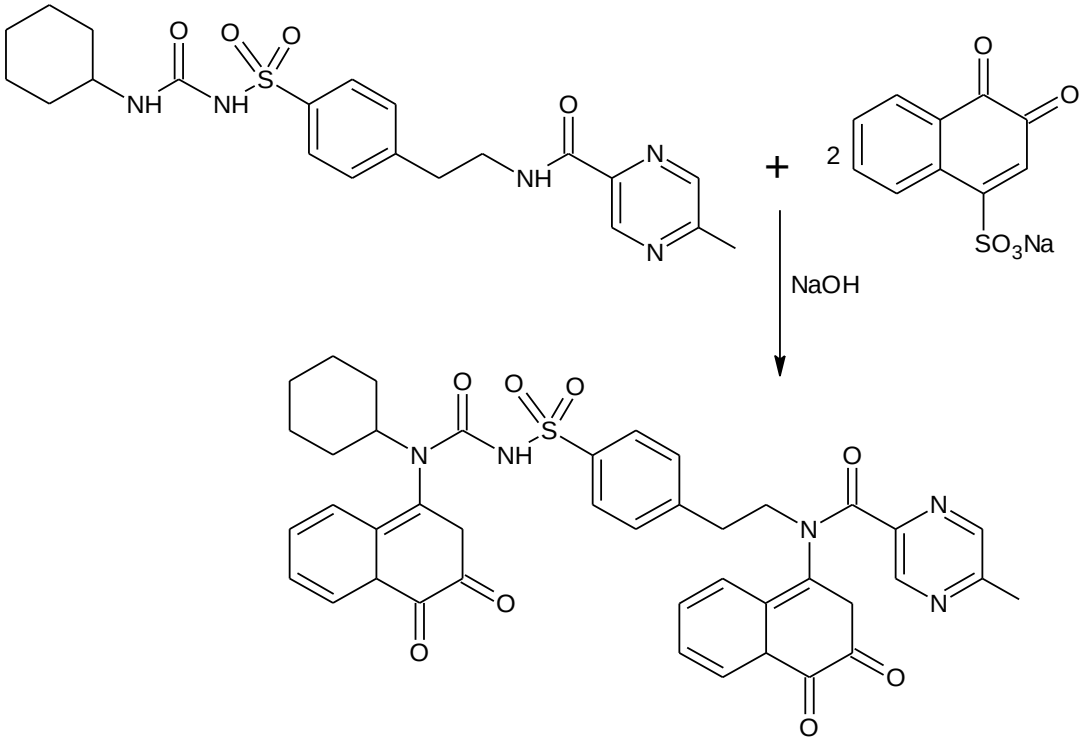

Figure 3. Probable reaction mechanism for method A

Method A

The reagent NQS has been a useful reagent in developing simple spectrophotometric method for the determination of pharmaceutically important drugs [14]. The reaction proceeded by the nucleophilic substitution of NQS at secondary amino group of the drug GPZ in slightly alkaline medium to yield a reddish-orange colored chromogen. The proposed mechanism is shown in **Figure 3**. The reaction conditions were optimized by studying the effect of reagent and alkali concentration. The highest absorbance value was attained at concentration of 0.01 mol L^{-1} NQS and 0.2 mol L^{-1} sodium hydroxide beyond which constant absorbance reading was not obtained. A standing time of 10 minutes was necessary for the reaction to go to completion. The effect of temperature on the reaction was also carried out and it was found that increasing the temperature did not accelerate the reaction and maximum color intensity was achieved at room temperature.

Method B

The reduction of tetrazolium salts which are colorless, water-soluble compounds yielded intensively colored water-insoluble formazan derivative [15]. The drug gets oxidized and in turn reduces TB to form red colored formazan. The reduction mechanism of tetrazolium by the drug GPZ is illustrated in **Figure 4**. The influence of different parameters such as alkalinity, volume of TB reagent, temperature and time were studied to achieve maximum

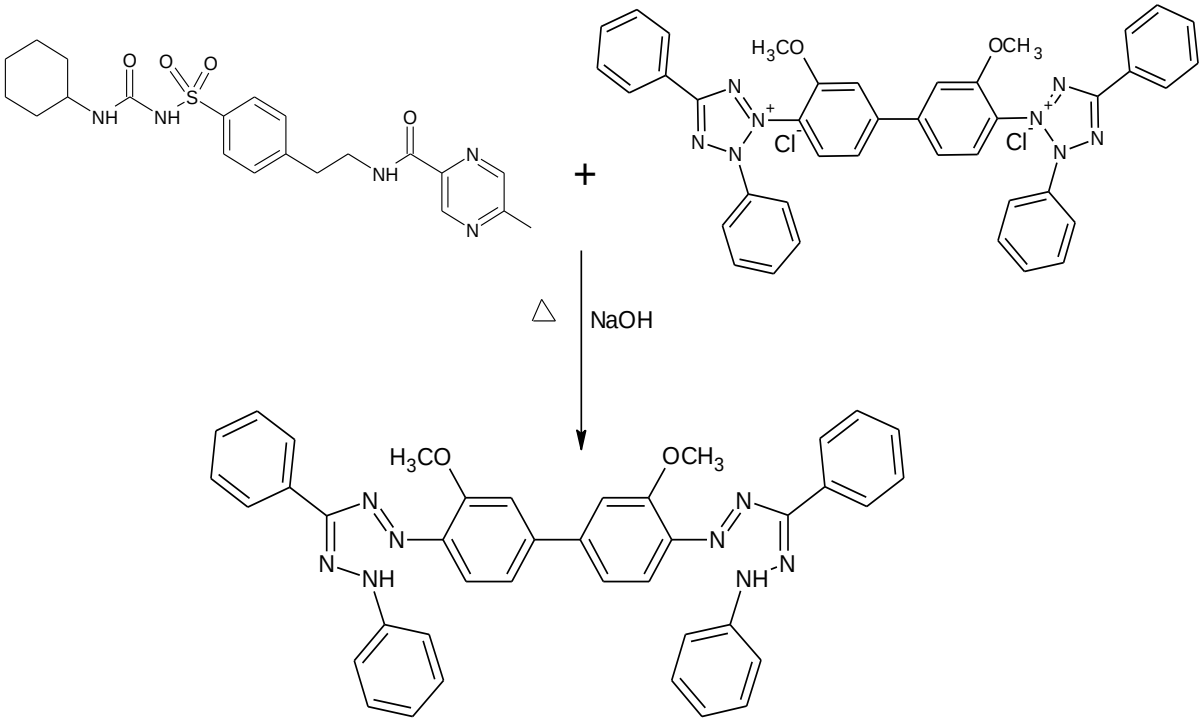

Figure 4. Probable reaction mechanism for method B

selectivity and sensitivity of the proposed method. It was found that absorbance increased with increasing reagent volume, but however, 0.5 mL of 0.01 mol L^{-1} TB and 1 mL of 0.2 mol L^{-1} sodium hydroxide solution was sufficient for producing maximum color intensity and constant absorbance values. The study of reaction time and temperature indicated that absorbance value reached a maximum at 80 °C for a heating time of 10 minutes. The developed color of the chromogen at optimized condition was stable for 45 minutes at room temperature.

Method C

The FC reagent has been used for the determination of many drugs of pharmaceutical interest. The present method was based on exploring the reducing nature of the GPZ drug and it involved the reduction of phosphomolybdic/phosphotungstic acid complexes of FC reagent to form blue colored complex. The stability of the complex was checked under various conditions of concentration of reagent and time. The optimum concentration and volume was selected on the basis of their ability to give maximum color intensity. It was found that 2 mL of 1 mol L^{-1} FC reagent and 1 mL of 2 mol L^{-1} sodium hydroxide were optimum for the development of color of maximum absorbance. The complex was stable at room temperature for 24 hours.

Method D

This method involved the reduction of ferric salts by the drug GPZ and the amount of ferrous ions thus produced corresponded to drug concentration. The amount of ferrous ions

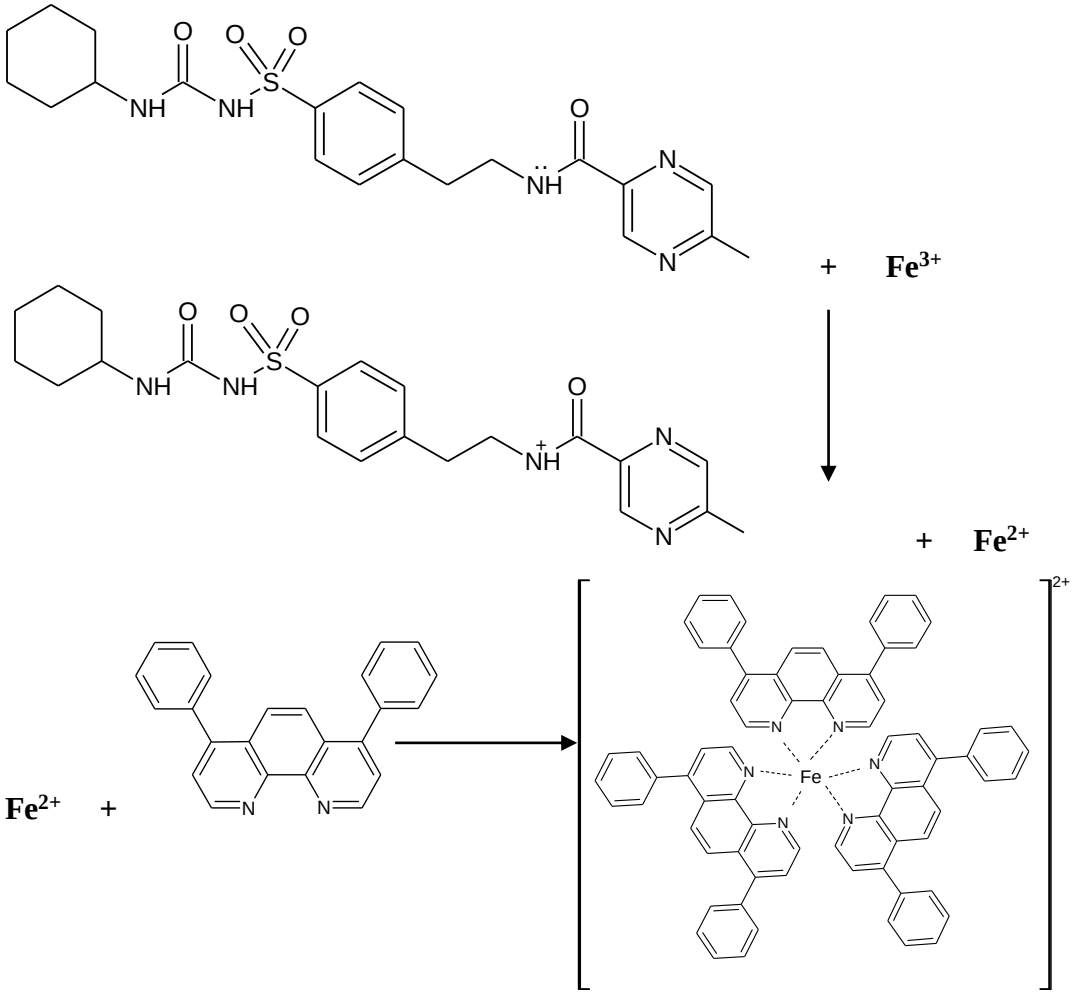

Figure 5. Probable reaction mechanism for method D

could be estimated by complexing with BPT which resulted in the formation of tris (BPT)-iron(II) complex [16]. The illustration of probable mechanism is given in **Figure 5**. The optimum experimental conditions were established by changing each parameter at a time and observing the effect on absorbance of pink colored product. It was found that 1 mL each of 0.018 mol L^{-1} ferric chloride and 0.015 mol L^{-1} BPT was sufficient for the complexation reaction. The study of the influence of reaction time and temperature suggested heating at 80 °C for 30 minutes for maximum and stable absorbance values.

Analytical Data

Under the optimized experimental conditions, calibration graphs were constructed by plotting the absorbance against the concentration of GPZ drug. Beer's law was obeyed in the concentration range 10.00 – 100.00, 2.00 – 22.00, 5.00 – 40.00 and 0.50 – 7.50 µg mL^{-1} with molar absorption coefficients of 0.5391×10^4, 0.1492×10^5, 0.9712×10^4, 1.0426×10^5 L mol^{-1} cm^{-1} for methods A, B, C and D respectively. **Table 1** summarizes the optical characteristics and the

Table 1. Validation parameters of the proposed methods

Parameters	Method A	Method B	Method C	Method D
λ_{max} (nm)	456	515	760	547
Beer's law limits (µg mL^{-1})	10 – 100	2 – 22	5 – 40	0.50 – 7.50
Molar absorptivity (L mol^{-1} cm^{-1})	0.5391×10^4	0.1492×10^5	0.9712×10^4	1.0426×10^5
Sandell sensitivity (µg cm^{-2})	0.0826	0.0298	0.0458	0.0042
Limit of detection[a] (µg mL^{-1})	1.0266	0.4692	0.2289	0.1427
Limit of quantification[a] (µg mL^{-1})	3.1111	1.4220	0.6936	0.4325
Regression equation[b]	Y = a + bX	Y = a + bX	Y = a + bX	Y = a + bX
Intercept (a)	0.0468	0.0347	0.0145	0.0717
Slope (b)	0.0090	0.0218	0.0173	0.0994
Correlation coefficient (r)	0.9995	0.9968	0.9976	0.9979

a Calculated according to ICH guidelines

b Y is the absorbance and X is the concentration of analyte in µg mL-1

Table 2. Validation parameters of the proposed methods

Amount taken (µg mL^{-1})	Amount found[a] (µg mL^{-1})	RE[b] (%)	SD[b] (µg mL^{-1})	RSD[b] (%)	Recovery (%)
Method A					
20	20.05	0.24	0.12	0.62	100.25
40	40.02	0.05	0.18	0.44	100.05
60	60.02	0.03	0.20	0.33	100.03
Method B					
6	6.01	0.23	0.08	1.31	100.17
14	14.04	0.30	0.09	0.66	100.29
22	22.07	0.31	0.15	0.67	100.31
Method C					
15	15.12	0.84	0.10	0.72	100.80
20	20.21	1.07	0.03	0.18	101.05
30	30.14	0.48	0.15	0.53	100.47
Method D					
1.5	1.51	0.85	0.02	1.44	100.67
3.5	3.53	0.95	0.03	0.79	100.85
4.5	4.51	0.41	0.02	0.49	100.22

aMean value of five determinations

bRE – Relative error, SD – Standard deviation, RSD – Relative standard deviation

results of statistical analysis of the experimental data such as Sandell's sensitivity, correlation coefficient, slope (b), intercept (a), limit of detection and quantification.

The accuracy and precision of the proposed methods were evaluated by performing five replicate determinations of GPZ in pure forms at three different concentrations and analyzing it by recommended procedures. The standard analytical errors, relative standard deviations obtained in the analysis of GPZ by the proposed methods were found to be acceptable and the calculated data is given in **Table 2**.

Table 3. Statistical results of the assay of formulation by proposed methods

Brand Name	Labeled Amount (mg)	Amount Found[a] ± SD			
		Method A	**Method B**	**Method C**	**Method D**
Glide	5	5.00 ± 0.08 t test[b] = 0.53 % Rec[c] = 100.00	5.04 ± 0.05 t test[b] = 0.70 % Rec[c] = 100.80	4.99 ± 0.15 t test[b] = 0.16 % Rec[c] = 99.80	4.97 ± 0.02 t test[b] = 1.84 % Rec[c] = 99.40
Glynase	5	5.02 ± 0.06 t test[b] = 1.39 % Rec[c] = 100.40	5.02 ± 0.12 t test[b] = 1.12 % Rec[c] = 100.40	5.03 ± 0.16 t test[b] = 1.93 % Rec[c] = 100.60	4.91 ± 0.04 t test[b] = 0.11 % Rec[c] = 98.20
Glytop	5	4.97 ± 0.29 t test[b] = 1.81 % Rec[c] = 99.40	5.00 ± 0.19 t test[b] = 0.09 % Rec[c] = 100.00	4.99 ± 0.15 t test[b] = 0.47 % Rec[c] = 99.80	4.99 ± 0.04 t test[b] = 0.18 % Rec[c] = 99.80
Dibizide	5	4.98 ± 0.24 t test[b] = 1.61 % Rec[c] = 99.60	5.02 ± 0.11 t test[b] = 1.16 % Rec[c] = 100.40	4.98 ± 0.09 t test[b] = 1.63 % Rec[c] = 99.60	4.99 ± 0.02 t test[b] = 0.19 % Rec[c] = 99.80

[a]Mean value of five determinations
[b]Theoretical *t*-value at 95% confidence level is 2.77
[c]Recovery

Interferences

The specificity of the proposed methods was assessed by performing recovery experiments through standard addition technique. For this study, a known amount of pure GPZ is added to pre-analyzed dosage forms and then determined by the recommended procedures. The results (**Table 3**) showed that the mean recovery and relative standard deviation were in the acceptable range. No interference from the common excipients of tablet formulation was observed.

Analytical applications

The proposed methodology was very adequate for the determination of GPZ in pure form and in pharmaceutical formulation. Moreover, the developed procedure was economical when compared to other methods. The performance of proposed method was assessed by calculation of Student's t-test at 95 % confidence limits for five degrees of freedom. The results given in **Table 3** revealed that the calculated *t*-values for the proposed methods are less than theoretical values and are equally accurate and precise.

CONCLUSIONS

A successful attempt was made to employ four new analytical reagents for the development of simple and rapid spectrophotometric method for the accurate determination of GPZ in its dosage forms. The analytical methods described in the present study have many advantages such as it does not need expensive sophisticated instrument, improved sensitivity, simple, rapid and reliable procedures. The proposed methods used affordable reagents with

excellent shelf life, and were available in any basic analytical laboratory. Therefore, the proposed methods were found to be practical, economical and convenient for its routine application in quality control laboratories for the analysis of selected drug.

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