ORIGINAL ARTICLE

SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF GLIQUIDONE IN BULK DRUG, PHARMACEUTICAL FORMULATIONS AND HUMAN SERUM

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ABSTRACT

A quick and sensitive spectrophotometric method for quantitative determination of gliquidone in bulk drug, pharmaceutical formulations and human serum has been developed and validated. The reported method is fourteen times more sensitive than British Pharmacopoeial method. Gliquidone is a second generation sulfonyleurea derivative, indicated to improve glycemic control in NIDDM patients. The method was validated as per ICH guidelines and found advantageous for simplicity, sensitivity, reproducibility, linearity, precision and accuracy. The

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developed method has also been applied to the analysis of GLURENOR® tablet and evaluated statistically to assess the application of the method. The limit of detection and quantification of gliquiodone at 225 nm was 68.31ng mL^{-1}. The, serum did not interfere with the estimation and the drug spiked in serum was totally recovered. The present method has further advantage in estimating the drug in concentrations even lower than the amount usually present in blood after oral intake. Calibration was linear in the range of 0.207–20µg mL^{-1} ($r^2 = 0.9972$). In addition, the proposed method is simple, easy to apply, low cost, requires relatively inexpensive instrument and used as an alternate to the existing spectrophotometric and non-spectrophotometric methods for the routine analysis of the drug in raw material, pharmaceutical formulations and from human serum.

Keywords: Ultraviolet spectrophotometry; gliquidone; pharmaceutical analysis; sulfonylurea derivative; gliquidone in serum.

INTRODUCTION

Gliquidone is a second generation sulfonyleurea derivative [1-cyclohexyl-3-[[p-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo2(1H)isoquinolyl)ethyl]phenyl]sulfonyl] urea] (Martindale, 1999,The Merck Index 1999, Clark's,1986) (figure. 1), is an oral medication indicated to improve glycemic control in NIDDM patients. It works by stimulating the production and release of insulin from the pancreas and also promotes the movement of sugar from blood into the required cells (Martindale, 1999). It has both sugar reducing and nephroprotective effects (Mazurov VI *et al.*, 1998). It is suitable for combined treatment (Podrouzkova B and Krusova D, 1992) and also has a protective effect on damage of liver of streptozotocin-induced diabetes in rats (Yanardag R *et al*., 2005).

Analytical methods appeared in literature for the determination and quantitation of gliquidone employ techniques such as spectrophotometry (British Pharmacopoeia 2005), atmospheric pressure chemical ionization liquid chromatographic-mass spectrometric (APCI-LC-MS) LC-MS (Maurer HH *et al*., 2002) and HPLC. HPLC assay methods were reported in biological fluids including serum (Guo P *et al*., 1992, Sener A *et al*., 1995, Von Nicolai H *et al.,* 1997). LC methods most often require relatively expensive reagents, are time-consuming and require expertise. Spectrophotometric techniques provide practical and significant economic advantages over other methods; therefore, they are a frequent choice for pharmaceutical analyses. As an alternative to existing methods, we developed and validated a new spectrophotometric method to determine gliquidone in tablets based on UV detection. The UV scan (figure 2) of gliquidone shows absorption maxima at 225 and 311 nm (table 2). The absorptivity at 225 nm was found to be higher as compared to at 311 nm, with very low LOD and LOQ values. Hence, this wavelength was chosen as the analytical wavelength. The aim of this work was to develop a simple and reliable ultraviolet spectrophotometric method, for its determination in bulk drug, dosage formulations and human serum. The developed method was validated as per ICH guidelines.

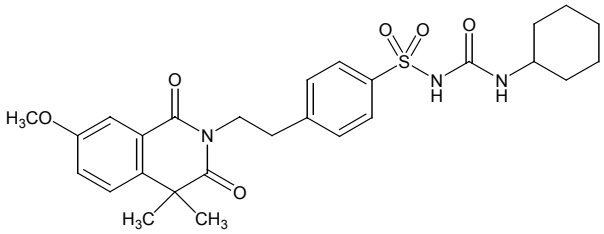

Fig. 1

EXPERIMENTAL

Apparatus and Regents

UV visible spectrophotometer (Model 1601, Shimadzu, Japan) with 10-mm path length connected to a P-IV computer loaded with Shimadzu UVPC version 3.9 software was used in these studies. Gliquidone reference standard was a kind gift from Pharmatec Pakistan (Pvt) Limited. GLURENOR® tablet (30 mg), manufactured by Pharmatec Pakistan (Pvt) Limited were purchased from the market, which had an expiry of not less than 365 days at the time of study. Methanol (Merck, Darmstadt, Germany) and other solvents were of analytical grade, while deionized water was freshly prepared every day from double distilled water in our laboratory.

Preparation of standard and sample solution

Stock solution of gliquidone reference standard (100 µg ml^{-1}) was prepared by dissolving 10 mg of gliquidone in 100 ml of methanol. Twenty commercially available 30 mg gliquidone tablets GLURENOR® were crushed and an amount equivalent of 10 mg of drug was dissolved in small amount of methanol, sonicated in an ultrasonic bath for 10 min at 25°C, filtered and diluted to 100 ml in a volumetric flask.

Spectrophotometric measurement

Spectral scans of each sample and standard against methanol were recorded in the range 200 to 360 nm where maxima was observed at 225 nm. Absorbance of each solution was recorded and apparent molar absorptivities of reference standard and tablets were calculated (table 2).

Table 1: Mean absorbance values and statistical data of the calibration curve for the estimation of gliquidone

Concentration (µg mL^{-1})	Mean ABS[a]
2	0.2474
4	0.3527
6	0.4803
8	0.6230
10	0.7029
12	0.8372
14	0.9755
16	1.1206
18	1.2578
20	1.4050

[a]Mean of three values.
Regression equation[++]statistical data. Intercept (a) = 0.0953; slope (b) =0.0641; correlation coefficient=0.9972; [++], n=30.

Table 2: Optical characteristics for gliquidone in reference standard and tablets

Characteristic	Value in reference standard	Value in tablets
Absorption maxima (nm)	225[a], 311	225[a], 311
Beer's law limits[b] (µg mL^{-1})	02-20	02-20
Apparent molar absorptivity[b] (l mol^{-1} cm^{-1})	3.69x10^7	3.90x10^7

[a]Analytical wavelength for proposed method.
[b]At analytical wavelength.

Preparation of calibration curve
Suitable aliquots of the stock solutions were pipetted into 50 ml volumetric flasks and the volume was made up to the mark with methanol, shaken well for proper mixing and the absorbance measured at 225 nm. The above procedure was repeated three times. Mean absorbance values along with the statistical data for the method are shown in table 1. The optical characteristics for the solution of gliquidone in methanol are given in table 2.

Determination of analytical performance parameters
Linearity
To establish linearity of the proposed method, ten separate series of solutions of the drug (2–20 µg ml^{-1}) in methanol were prepared from the stock solutions and analyzed. Least square regression analysis was used to obtain data (table 1).

Precision
The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample (United States Pharmacopeia, 2000). In order to determine precision of the method, solutions containing known amounts of pure drug were prepared and analyzed in three replicates and absorbance was recorded in six replicates to get the mean. The low %RSD values obtained from the analyses of pharmaceutical formulations indicated that the method has high precision. The analytical results obtained from these investigations for the methods are summarized in table 3.

Table 3: Precision of the proposed method

Drug added (mg)	Drug found (mg)	Mean (S.D)	%RSD	Confidence limits
27.05	26.99			
27.11	27.00			
26.91	27.05	27.01(0.032)	0.119	26.93-27.09
30.01	29.93			
30.05	29.98			
30.05	30.00	29.97(0.036)	0.365	29.88-30.05
33.09	32.97			
32.92	32.85			
32.96	32.87	32.90(0.064)	0.355	32.78-33.09

Accuracy
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (United States Pharmacopeia, 2000). For accuracy aliquots of gliquidone standard solution plus excipients obtained after filtration were transferred to 100 ml volumetric flasks, was measured at 225 nm. The absorbance reading of above procedure was carried out and was recorded six times to get the mean. Results of these determinations are included in table 4.

Table 4: Accuracy of the proposed method

Drug added (mg)	Drug found (mg)	Mean (S.D)	%Bias*	R.M.E.**
27.10	27.01			
27.00	26.90			
27.11	26.95	26.95(0.055)	-0.43	0.035
30.00	29.94			
30.12	30.01			
30.20	30.04	30.00(0.051)	-0.37	0.029
33.00	32.90			
32.90	32.84			
33.10	33.01	32.92(0.086)	-0.25	0.049

* = [{found-added}/added] x100
** = Relative Mean Error

Limit of detection and quantitation
Limit of detection (LOD) and limit of quantitation (LOQ) revealing the sensitivity of the method was calculated by the equations given in ICH guidelines (ICH guideline Q2B, 2003). The detection limit (LOD) may be expressed as:

$$LOD = \frac{3.3\ \sigma}{S}$$

Where σ is the standard deviation of the response, S is the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. A specific calibration curve may be studied using samples containing an analyte in the range of LOD. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines was used as the standard deviation. The Limit of detection of the proposed method was 68.31ng mL^{-1}. LOQ may be expressed as:

$$LOQ = \frac{10\ \sigma}{S}$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. A specific calibration curve should be studied using samples, containing an analyte in the range of LOQ. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines were used as the standard deviation. The limit of quantitation of gliquidone was 207 ng mL^{-1}.

Application of the method in human serum

Samples of pure drug were also estimated in human serum using the proposed method. Serum was coagulated by the acetonitrile and filtered through Whatman 41 filter paper circles of diameter 125 mm (Whatman, Maidstone, England). Stock solution of gliquidone (300 µg ml^{-1}) prepared by dissolving 30 mg of gliquidone in 100 ml of methanol using a bath sonicator. 1 ml of the stock solution was pipetted to a 10 ml volumetric flask and diluted with 4 ml of methanol and 5 ml of serum. The solution was shaken well for proper mixing and absorbance measured at 225 nm. The above procedure was carried out in triplicate and absorbance readings were recorded three times to get the mean. Results of these determinations are included in table 5.

RESULTS AND DISCUSSION

Gliquidone in methanol yields a characteristic curve when scanned in the ultraviolet wavelength range between 200 and 360 nm. The scan (figure 2) shows absorption maxima at 225 and 311 nm (table 2). British Pharmacopoeia refers the estimation of gliquidone at 311 nm. (British Pharmacopoeia 2005) The peak response of gliquidone is relatively very small at 311 nm as compared to peak at 225 nm. The absorptivity at 225 nm was found to be 3.69×10^7 l mol^{-1} cm^{-1} and this wavelength was chosen as the analytical wavelength. Regression analysis was performed on the experimental data. The raw data along with the results of regression analysis (method of least squares) is shown in table 1. Regression equation was y=0.0641X + 0.0953. Correlation coefficient was found to be 0.9972, signifying that a linear relation existed between absorbance and concentration of the drug. The limit of detection of gliquidone at 225 nm was 68.31ng mL^{-1} and limit of quantitation was 207ng mL^{-1}. The precision of the method was carried out using known amounts of pure drug that were subjected to recovery studies in triplicate and evaluated using the S.D. of the results and %RSD. Table 3 summarizes the results of these investigations. The lower S.D. of the results and the lower %RSD make the method more precise. In order to determine the accuracy of method, estimation of gliquidone was carried out in the presence of various commonly used excipients like magnesium stearate, cellulose microcrystalline, talc and lactose at the levels they are normally used in tablets. From table 4, it can be seen that there is no significant difference between the amount of drug added to the placebo and the amount recovered. Thus, excipients did not interfere with the estimation. Also, the filtration medium did not absorb the drug to any extent. To establish the accuracy of method the relative mean error (RME) of method was calculated and is shown in table 4. The relative mean error is less at each level signifying that the method is accurate.

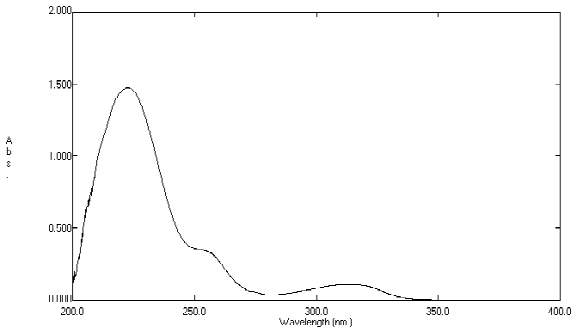

Fig. 2: A typical spectrum showing absorption of gliquidone showing higher absorption at 225 nm as compared to 311 nm.

Table 5: Results of the proposed method for estimation of gliquidone in human serum

Amount of drug	Individual amounts	Coefficient of variation	Confidence limits [a]
added (mg)	found (mg) mean (S.D.)	(CV)	-
30.20	30.28	0.477	29.79 - 30.47
30.10	30.01		
30.10	30.06		
	30.12 (0.144)		

[a] Confidence limits at *P*=0.95

Estimation of gliquidone was also carried out in the human serum. The data given in table 6 shows that there is no significant difference between the amount of drug spiked in serum and the amount recovered. Thus, serum did not interfere with the estimation. In addition, the filtration medium did not absorb the drug to any extent. The present method is advantageous in estimating drug concentrations lower than the amount of drug usually present in blood after oral intake. The peak plasma concentration achieved after oral in take of 15 mg tablets to 10 subjects were in the range of 0.7–0.37 µg/ml (Kopitar 1975), whereas in our analytical method, even lower concentrations up to 0.207 µg/ml can be quantitated while the detection limits are even lower (0.068 µg/ml).

CONCLUSION

The method reported for the estimation of gliquidone has the advantages of simplicity, sensitivity, accuracy and is associated with higher sensitivity and precision. The quantitation of drug by measuring absorbance at 225 nm is approximately fourteen times more senitive than at 311 nm. The non-interference of tablet excipients makes the method suitable for the estimation of the drug in tablets and hence can be used for routine quality control of gliquidone formulations of all potencies. Results of the above study indicate the suitability of the method to estimate gliquidone in bulk drug, dosage formulations as well as in human serum. Gliquidone can conveniently be estimated to much lower limits then usually present in human serum after therapeutic dosages. The developed UV spectrophotometric method is cheaper than the reported LC methods for analysis of gliquidone in the pharmaceutical preparations. These advantages encourage the application of this method in routine analysis of gliquidone.

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