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Research Article

Development and Validation of Spectrophotometric Methods for the Determination of Glibenclamide

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Abstract

Glibenclamide (Glyburide) is an oral hypoglycemic drug. It acts by stimulating the pancreatic beta cells and secretes insulin. Often it is used to treat diabetes mellitus type 2. Three new spectrophotometric methods were developed for the assay of Glibenclamide tablet dosage forms using borate buffer pH 9.0, phosphate buffer pH 8.0 and pH 2.0 and the methods were validated. Shimadzu UV-1800 Model UV-VIS double beam spectrophotometer was employed for the study. Glibenclamide Linearity was observed over the concentration range 1-20, 1-100 and 1-100 µg/ml respectively in borate buffer pH 9.0 (Method I), phosphate buffer pH 8.0 (Method II) and phosphate buffer pH 2.0 (Method III) respectively. The linear regression equations are found to be y = 0.0261x - 0.0014 (R^2 = 0.9996), y = 0.0225x + 0.0088 (R^2 = 0.9994) and y = 0.0249x + 0.0163 (R^2 = 0.999) in method I, II and III respectively. The methods were validated by precision and accuracy studies and are suitable for the determining the assay of Glibenclamide tablets.

Keywords: Glibenclamide; Phosphate Buffers; Borate Buffer; Validation; ICH Guidelines

Introduction

Glibenclamide (Figure 1) is (5-chloro-N-(4-[N-(cyclo hexyl carbamoyl) sulphamoyl] phenethyl)-2-methoxy-benzamide) It is an antidiabetic drug administered orally. Glibenclamide is a sulphonylurea containing two moietiesa benzamide moiety and a sulphonylurea moiety. Glibenclamide is very poorly soluble in water and have a very good biomembrane permeability [1,2]. Glibenclamide was assayed by using different techniques such as spectrophotometry [3-7], spectrofluorimetry [8], LC-MS [9] and HPLC [10-14] methods for pharmaceutical dosage forms, nano emulsions [15,16] and biological fluids [17-19].

Figure 1: Chemical structure of Glibenclamide.

The present article describes three new validated [20]. UV spectrophotometric methods for the determination of Glibenclamide in their tablet dosage forms.

Materials and Methods

- **Preparation of borate buffer (pH 9.0):** 6.2 grams of boric acid was dissolved in 500 ml water, pH was adjusted to 9.0 with 1M sodium hydroxide (about 41.5 ml) and diluted with water to make up to 1000 ml in a volumetric flask
- **Preparation of phosphate buffer (pH 8.0):** 50.0 ml of 0.2 M potassium di hydrogen phosphate was mixed with 46.8 ml of 0.2 M sodium hydroxide and sufficient water was added to make up the volume to 500 ml in a volumetric flask.
- **Preparation of phosphate buffer pH 2.0:** Dissolve 0.136g of potassium dihydrogen phosphate in 800ml of water, adjust the pH to 2.0 with hydrochloric acid and add sufficient water to produce 1000ml.
- **Instrumentation:** Model No. UV-1800 double beam UV-VIS spectrophotometer (Shimadzu) was used for the present study. All the solutions were scanned 200-400 nm keeping samples in quartz cuvettes. Glibenclamide is available as tables with brand names Afidex (Label claim: 2.5 mg) (Cadila pharmaceuticals Ltd) and Giclamide (Label claim: 5.0 mg) (Leben laboratories Pvt. Ltd).

Method validation

Linearity, Precision and accuracy studies

A series of Glibenclamide solutions 1-20, 1-100 and 1-100 µg/ml were prepared in borate buffer pH 9.0 (Method I), phosphate buffer pH 8.0 (Method II) and phosphate buffer pH 2.0 (Method III) from the stock solution respectively and scanned (200-400 nm) against their reagent blank. The absorption spectrum has shown λmax at 227 nm in all the three reagents for Method I, II and III. The absorbance of all these drug solutions were recorded at λmax and calibration curves were plotted taking concentration on the x-axis and the corresponding absorbance on the y-axis for Method I, II and III respectively.

Precision studies were performed on the same day and different days (Inter day and Intraday) (n=6) and accuracy studies were carried out by spiking the solutions (50%, 100%, and 150%) and there by calculating the % recovery in their respective buffers solutions for Method I, II and III respectively.

Assay of Glibenclamide tablets

Glibenclamide stock solution was prepared by dissolving 25 mg of accurately weighed Glibenclamide in a 25 ml volumetric flask in methanol (1000 µg/ml) and dilutions were made from the stock solution with borate buffer pH 9.0, phosphate buffer pH 8.0 and phosphate buffer pH 2.0 respectively for Method I, II and III respectively. Two different brands of available Glibenclamide tablets were procured and the API was extracted with methanol. Dilutions were made from this solution and assay was performed.

Results and Discussion

Three new UV spectrophotometric methods were developed for the determination of Glibenclamide API and the three methods were applied for the Glibenclamide tablets. Literature survey was thoroughly done and the proposed three methods were compared with the previously published spectrophotometric methods and Table 1 describes the details. The characteristic UV absorption spectra of Glibenclamide obtained in three methods I, II and III has shown λmax at 227 nm in borate buffer (Figure 2A), phosphate pH 8.0 (Figure 2B) and phosphate buffer pH 2.0 (Figure 2C) respectively.

Reagent	Linearity (µg/ml)	λ_{max} (nm)	References
Distilled water	1-5	276	3
Ethanol and Water (1:5)	2-14	230	4
Ethanol	3-15	229.5	5
Chloroform	5-30	242	6
Methanol and Water (50:50, v/v)	10.0–70	308	7
Ethanol (Spectrofluorimetry)	1.4- 10	354	8
Borate buffer pH- 9	0.5-20	227	Present work
Phosphate buffer pH -8	0.5-100	227	
Phosphate buffer pH-2	0.5-100	227	

Table 1: Comparison of spectrophotometric methods published with the present.

Figure 2A: Absorption spectra of Glibenclamide (60 µg/ml) in Borate buffer (pH 9.0).

Figure 2B: Absorption spectra of Glibenclamide (100 µg/ml) in Phosphate buffer (pH 8.0).

Figure 2C: Absorption spectra of Glibenclamide (40 µg/ml) in Phosphate buffer (pH 2.0).

Method validation

Beer-Lambert's law was obeyed over the concentration range 1-20, 1-100 and 1-100 µg/ml in borate buffer pH 9.0, phosphate buffer pH 8.0 and phosphate buffer pH 2.0 (Table 2) respectively. The linear regression equations are found to be y = 0.0261x - 0.0014 (R^2 = 0.9996), y = 0.0225x + 0.0088 (R^2 = 0.9994) and y = 0.0249x + 0.0163 (R^2 = 0.999) in borate buffer (Figure 3A), phosphate pH 8.0 (Figure 3B) and phosphate buffer pH 2.0 (Figure 3C) respectively.

Conc (µg/ml)	Method I	Method II	Method III
0.5	0.018	0.0128	0.0123
1	0.0261	0.0225	0.0262
1.5	0.032	0.0342	0.0482
2	0.048	0.0452	0.050
2.5	0.065	0.056	0.0622
5	0.128	0.128	0.1250
10	0.258	0.263	0.31
20	0.523	0.5	0.544
40	-	0.9	1.061
60	-	1.33	1.531
80	-	1.836	2.007
100	-	2.255	2.470

Table 2: Linearity of Glibenclamide.

The percentage RSD in intra-day precision was found to be 0.60-0.77, 0.56-0.76 and 0.48-1.30 whereas for inter-day precision it was 0.38-0.78, 0.22-0.76 and 0.36-1.30 for Method I, II and III respectively which is less than 2.0 proving that the methods are precise. The % RSD in accuracy studies was found to be less than 2.0 and the % recovery was found to be 99.49-99.71, 99.26-99.5 and 99.34-99.78 indicating that the methods are accurate. The optical characteristics observed for all the three methods was shown in Table 3.

Parameters		Method I	Method II	Method III
Reagent		Borate buffer pH 9	Phosphate buffer pH 8	Phosphate buffer pH 2
Linearity range (µg /ml)		0.5-20	0.5-100	0.5-100
λmax (nm)		227	227	227
Molar extinction coefficient (Litre/mole/cm)		1.27453 × 104	1.29923 × 104	1.5314 × 104
Sandell's sensitivity (µg/cm2 /0.001 absorbance unit)		0.0387	0.0380	0.0322
Slope		0.0261	0.0225	0.0249
Intercept		0.0014	0.0088	0.0163
Correlation coefficient		0.9996	0.9994	0.999
Precision (% RSD)	Interday	0.38-0.78	0.22-0.76	0.36-1.3
	Intraday	0.60-0.77	0.56-0.76	0.48-1.3
Accuracy (% Recovery)		99.49-99.71	99.26-99.5	99.34-99.78
Assay (%)		99.66-99.84	99.68-99.82	99.58-99.92

Table 3: Optical characteristics of Glibenclamide.

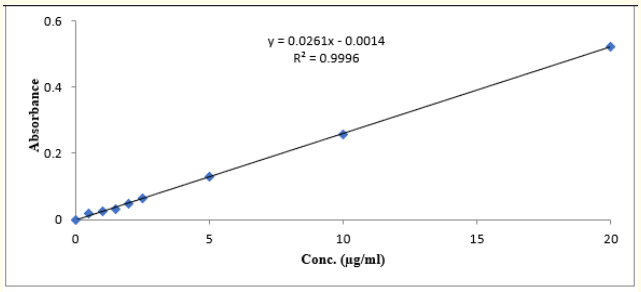

Figure 3A: Calibration curve of Glibenclamide in Borate buffer (pH 9.0).

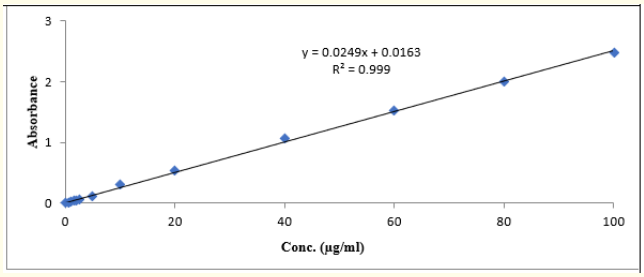

Figure 3C: Calibration curve of Glibenclamide in Phosphate buffer (pH 2.0).

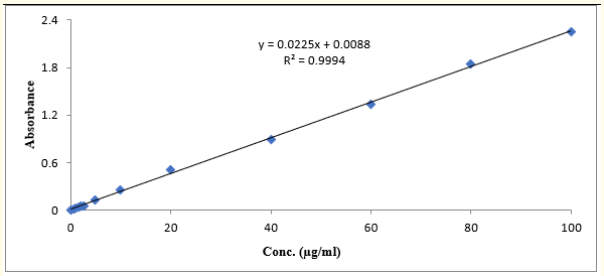

Figure 3B: Calibration curve of Glibenclamide in Phosphate buffer (pH 8.0).

Assay of Glibenclamide tablets

Tablet formulations of two different brands were extracted with the three reagents and separately and the percentage of purity was determined with the help of linear regression equations. The assay was found to be 99.66-99.84, 99.68-99.82 and 99.58-99.92 in borate buffer, phosphate pH 8.0 and phosphate buffer pH 2.0 for Method I, II and III respectively (Table 4).

Brand name	Label claim (mg)	Amount obtained			% Recovery		
		Method I	Method II	Method III	Method I	Method II	Method III
I	2.5	2.496	2.492	2.498	99.84	99.68	99.92
II	5	4.983	4.991	4.979	99.66	99.82	99.58

Table 4: Assay of Glibenclamide tablets.

Conclusion

The three validated UV spectrophotometric methods were found to be very simple and economical for the regular analysis of Glibenclamide tablets. The percentage RSD in precision and accuracy studies was found to be within the acceptable criteria and the methods are prcise and accurate.

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