IJPSR (2014), Vol. 5, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 April, 2014; received in revised form, 27 August, 2014; accepted, 17 October, 2014; published 01 November, 2014

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF GLIBENCLAMIDE IN PURE FORM AND IN DOSAGE FORM

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Keywords:

Glibenclamide, Spectrophotometry, Calibration curve, Pharmaceutical dosage form.

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ABSTRACT: Two simple, sensitive and rapid spectrophotometric methods was developed for estimation of glibenclamide in pure and in pharmaceutical dosage forms. Method-A is a uv spectroscopic method with the absorption maxima at 276nm.Method-B is based on the oxidation/reduction of glibenclamide with iron (III) and chelation of iron with 3-methyl 2-benzothiozoline hydrazone(MBTH) to form green colored complex with absorption maxima at 630nm.Calibration curve was plotted. The assay was validated for the parameters like precision, accuracy, robustness, ruggedness, LOD and LOQ. The proposed methods can be useful for the routine analysis for the determination of glibenclamide in pharmaceutical dosage form.

INTRODUCTION: Chemically, glibenclamide is 5-chloro-N (4-(N-(cyclohexyl carbomoyl) sulfamoyl) phenethyl) – 2 - methoxy benzamide. Glibenclamide is used for the treatment of insulin dependent type – II diabetes mellitus which acts by stimulating release of insulin from pancreas. Several UV-Visible methods have been reported for estimation of glibenclamide in dosage forms. Development and validation of analytical method for simultaneous estimation of glibenclamide and metformin HCL in bulk and tablets using UV- visible spectroscopy has also been reported. Drugs are available as tablet dosage forms in the market.

EXPERIMENTAL:

Reagents and chemicals:

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(11).4925-28

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).4925-28

Glibenclamide pure form, all chemicals were of analytical grade and all the solutions were prepared with double distilled water. Ethanol used was spectrograde for method-A. Solutions of 0.5%w/v $FeCl_3$, MBTH 0.2%w/v,0.1N HCL were prepared separately for method-B.

Instrument:

A systronics UV-Vis double beam spectrophotometer (model: 2202) with 1cm matched quartz cell was used for all spectral measurements.

Standard Preparation:

Standard stock solution of glibenclamide (1mg / ml) was prepared with ethanol in a 50 ml volumetric flask. The working standard solutions of glibenclamide (1 to 5 were prepared by diluting the stock solution suitably with water.

Sample Preparation:

About 10 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and powder equivalent to 50 mg

glibenclamide was dissolved in quantity sufficient 50ml ethanol.

Procedure:

Method A: 1 ml of the working standard solution of glibenclamide was transferred to 10 ml volumetric flask, diluted up to mark with distilled water. The resulting solution was scanned in range of 200 - 400 nm for maximum absorbance. The absorption maximum was found to be at 276 nm.

Method B: In this method one ml of working standard solutions was taken in a 10 ml volumetric flask. To this, one ml of 0.5 % ferric chloride solution was added, followed by one ml of 0.2 % MBTH reagent solution and 0.1 N normal HCl solutions. The volume was made up to 10 ml with water, and they were heated in a water bath for 20 minutes at 60 degree centigrade. A bluish green color complex was formed having maximum absorbance at 630 nm.

Validation of Method:

The method developed here was validated as per ICH guidelines for its accuracy, linearity, precision, and limit of detection, limit of quantification, robustness and ruggedness by using the following procedure.

Linearity:

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of glibenclamide at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance and concentration of the drug. The response was found to be linear in the range 1-5µg/ml for glibenclamide. The data was given in **Table-1**and **2** and following the **Figure-1**and **2**.

TABLE 1: ABSORBANCE VALUES IN METHOD - A

S.No.	Concentration(µg/ml)	Absorbance
1.	1	0.159
2.	2	0.353
3.	3	0.535
4.	4	0.708
5.	5	0.922

TABLE 2: ABSORBANCE VALUES OF THE METHOD- B

S. No.	Concentration(µg/ml)	Absorbance
1.	1	0.190
2.	2	0.360
3.	3	0.445
4.	4	0.543
5.	5	0.665

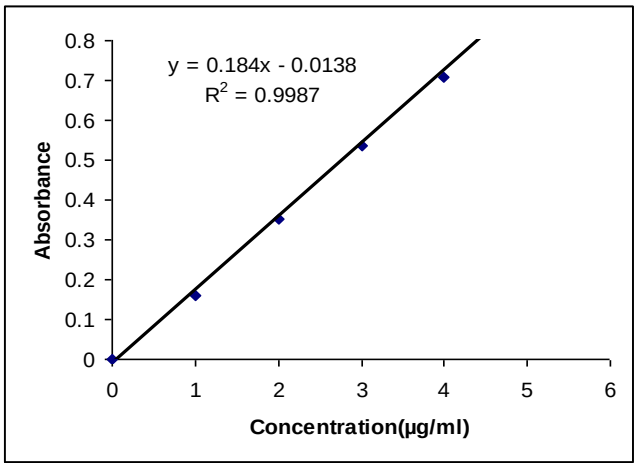

FIGURE 1: CALIBRATION CURVE IN METHOD - A

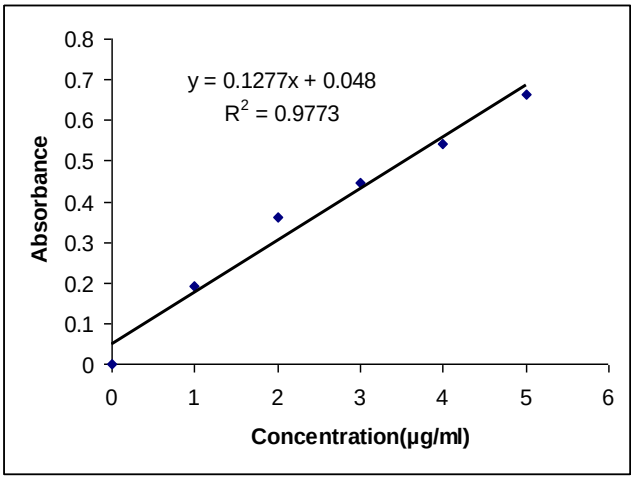

FIGURE 2: CALIBRATION CURVE IN METHOD - B

Accuracy:

Accuracy was performed by spiking 0.5 ml of standard stock solution (1mg / ml) with 0.5ml, 1ml, and 1.5 ml of working standard solution (10 µg / ml). They were made up to 10 ml and were analyzed by method A and B. These studies were carried out in triplicate. The data is given in **Table - 3** and **4.**

TABLE 3: ACCURACY OF METHOD - A

S.NO.	Spiked level	Amount added (µg/ml)	% Recovery
1.(n=3)	50 %	0.5 µg	98 %
2.(n=3)	100 %	1.0 µg	99 %
3.(n=3)	150 %	1.5 µg	100.5 %

TABLE 4: ACCURACY FOR METHOD – B

S.NO.	Spiked level	Amount added	%Recovery
1.(n=3)	50%	0.5 µg	99 %
2.(n=3)	100%	1.0 µg	100.7 %
3.(n=3)	150%	1.5 µg	98.5 %

Precision:

Six samples solutions of the same concentration were prepared and calculated as per test procedure. The data was given in **Table-5**and **6**.

TABLE 5: PRECISION OF METHOD - A

S.No.	Concentration(µg/ml)	Absorbance
1.	2	0.351
2.	2	0.353
3.	2	0.356
4.	2	0.356
5.	2	0.356
6.	2	0.353
Mean		0.354
S.D		2.071
%RSD		1.07

TABLE 6: PRECISION OF METHOD - B

S.No.	Concentration(µg/ml)	Absorbance
1.	2	0.376
2.	2	0.374
3.	2	0.374
4.	2	0.378
5.	2	0.378
6.	2	0.374
Mean		0.375
S.D		2.062
%RSD		1.06

LOD and LOQ:

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines.LOD value was 0.67µg/ml and 0.41µg/ml for Method A and B. LOQ value was 2.26µg/ml and 1.38µg/ml for Method A and B respectively.

Robustness:

To evaluate robustness, Small deliberate changes in the spectrophotometric conditions were introduced and the effects on the results were examined. Small variation in wavelength i.e., -2nm and +2nm.The data was given in **Table-7**and **8**.

TABLE 7: ROBUSTNESS OF METHOD - A

S.NO	Concentration (µg/ml)	Absorbance at 274 nm	Absorbance at 276 nm	Absorbance at 278 nm	Mean
1	10	0.153	0.159	0.159	0.157
2	20	0.334	0.353	0.372	0.353
3	30	0.437	0.535	0.541	0.504

TABLE 8: ROBUSTNESS OF METHOD - B

S.NO	Concentration (µg/ml)	Absorbance at 628 nm	Absorbance at 630 nm	Absorbance at 632 nm	Mean
1	10	0.180	0.190	0.181	0.183
2	20	0.327	0.360	0.335	0.340
3	30	0.446	0.445	0.465	0.452

Ruggedness:

To evaluate ruggedness, the test is carried by change in the instrument. The effects on the results were examined. The data was given in the **Table-9and10**.

TABLE 9: RUGGEDNESS OF METHOD - A

S.NO.	Concentration (µg/ml)	Absorbance in Method - A Instrument - I	Absorbance in Method - A Instrument - II	Mean
1	10	0.159	0.140	0.149
2	20	0.353	0.330	0.341
3	30	0.535	0.516	0.525

TABLE 10: RUGGEDNESS OF METHOD – B

S.NO.	Concentration (µg/ml)	Absorbance in Method - B Instrument - I	Absorbance in Method - B Instrument - II	Mean
1	10	0.190	0.179	0.184
2	20	0.360	0.354	0.357
3	30	0.445	0.433	0.439

TABLE 11: SYSTEM SUITABILITY PARAMETERS

		Method	
S.NO.	Optical character	A	B
1.	λ max	276nm	630nm
2.	Beer's law(µg/ml) limits(µg/ml)	1-5	1-5
3.	Molar Absorbtivity	7.854×10^{4}	$1.254*10^{5}$
4.	Sendell's sensitivity	0.062×10^{-1}	$0.039*10^{-1}$
5.	Regression equation(y)	Y=7.43x+1.56	Y=12.27x-8.35
6.	RSD	1.22	1.23
7.	%Range of error	±1.020	±1.028

TABLE 12: ASSAY METHOD

S. No.	Labelled Amount	Assay Amount(mg)		% Recovery	
		A	B	A	B
1.	Daonil (5mg)	4.98 mg	4.94 mg	99.6%	98.8%

RESULTS: A UV method and visible method was proposed for the suitable determination of glibenclamide in its dosage form. All the optical parameters were optimized by changing various parameters like wavelength, instrument and temperature. Finally, the optimized method was selected. The optimized method of UV was at wavelength of 276 nm as the drug showed optimized absorption maxima at this wavelength. The optimized wavelength for visible method was at 630 nm as the drug showed the optimized absorption maxima at this wavelength.

DISCUSSIONS: The statistical analysis of data showed that the method was simple, rapid, economical, sensitive, precise and accurate and therefore can be easily adopted for routine quality control analysis. Hence; the proposed methods can be successfully applied in the estimation of glibenclamide in marketed formulation.

CONCLUSION: The proposed methods were rapid, accurate and sensitive. The reagents, chemicals were used in this method is very less which makes it inexpensive. This method does not suffer any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

ACKNOWLEDGEMENTS: The authors are thankful to the management SIR C.R. Reddy College of pharmaceutical sciences, Eluru for providing all the facilities to complete the work.

REFERENCES:

1. Cicy Eapen: Development of UV Spectrometric Method of Glibenclamide in Bulk and Pharmaceutical Formulations. Int.J. Chem Tech Res.2012, vol.4, no.1, pp 356-360.
2. Anandkimar R. Tengli: Method development and validation metformin, pioglitazone nd glibenclamide in tablet dosage form by RP-HPLC. Biochem Anal Biochem 2013, 2:130.
3. Subhashini Edla and B. Syama Sundhar: New method development and validation for the simultaneous estimation of glibenclamide and metformin in bulk and tablet dosage form by using RP-HPLC. Rasyan J. chem 2014, vol 7, no.1,55-63.
4. Bandarkar FS, Khattab IS: Simultaneous estimation of glibenclamide, gliclazide and metformin hydrochloride from bulk and commercial products using a validated ultra fast liquid chromatographic technique. J Liq Chromatogr Relat Technol.2010, 33: 1814-1830.
5. Patil Sudarshan S: Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV – visible spectroscopy. 2009, Vol.1, No.4, pp 905-909.
6. Lakshmi KS, Rajesh T: Separation and quantification of eight antidiabetic drugs on a high-performance liquid chromatography: its application to human plasma assay. ISRN Pharm 2011: 521353.
7. Nalini Shastri: Development and Validation of Glibenclamide in Nanoemulsion Formulation by using RP-HPLC. JPBMS, 2011, vol. 8, issue 8.
8. Modi DK, Patel BH: Simultaneous determination of metformine hydrochloride and glipizide in tablet formulation by HPTLC. J Liq Chromatogr Relat Technol 2010, 35: 28-39.
9. Uday KR, Anna PN: Determination of glipizide, glibenclamide and gimepride in a tablet dosage form in the presence of metformine hydrochloride by ion pair reversed phase liquid chromatography technique.J anal bioanal techniques 2011, 1:105.
10. Ketan P. Dadhania: Development and validation of spectrophotometric method for simultaneous estimation of gliclazide and metformin hydrochloride in bulk and tablet dosage form by simultaneous equation method. IJPSR, 2011; vol. 2, issue 6, 1559-1563.

How to cite this article:
Umadevi N, Mounika CH and Babu IS: Spectrophotometric Method Development and Validation for the Estimation of Glibenclamide in Pure Form and In Dosage Form. Int J Pharm Sci Res 2014; 5(11): 4925-28.doi: 10.13040/IJPSR.0975-8232.5 (11).4925-28.