METHOD DEVELOPMENT AND VALIDATION OF GLIBENCLAMIDE BY UV-SPECTROSCOPY

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Abstract

This research is based on utilization of Electromagnetic radiation of Ultraviolet region (200-400nm). The study shows an easy and cheap UV-spectroscopic method for the quantitative determination of Glibenclamide tablet dosage form as well as Glibenclamide API. Glibenclamide shows the maximum absorbance at 300 nm in absorption maxima method. Drug followed the linearity in the range of 10-140 µg/ml for this method with correlation coefficient (R2) of 0.999. Further the method developed was validated including precision, accuracy, specificity, assay parameters as per the International Conference on Harmonization (ICH) guidelines. This method is simple, cost effective and so efficient that can be suitable for regular testing of the pharmaceuticals.

Keywords: Method Development, Validation, Glibenclamide, Ethanol.

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1. INTRODUCTION

Glibenclamide, also known as glyburide, is a medication used to treat diabetes mellitus type 2, has $C_{23}H_{28}CIN_3O_5S$ molecular formula. Its chemical name is 5-chloro-N-{2-[4-(cyclohexylcarbamoylsulfamoyl)phenyl]ethyl} -2-methoxybenzamide. Glibenclamide has 90% bioavailability and it will 93% bind with protein. It metabolizes in Liver and has upto 10 hours elimination half-life. It is in the sulfonylureas class of medications and works by increasing the release of insulin from the pancreas. The medication works by binding and inhibiting the ATP-sensitive potassium channels (K_{ATP}) inhibitory regulatory subunit sulfonylurea receptor 1 (SUR1)[9] in pancreatic beta cells. This inhibition causes cell membrane depolarization, opening voltage-dependent calcium channels. This results in an increase in intracellular calcium in the pancreatic beta cell and subsequent stimulation of insulin release. It is practically insoluble in water, slightly soluble in alcohol and in methyl alcohol and sparingly soluble in dichloromethane. It is taken by mouth.

Literatures tells that, a number of methods for determining Glibenclamide have been published, utilising various instrumental approaches such as spectroscopic, HPLC, RPLC and HPTLC in both API and pharmaceutical dosage form, and even in combination of Glibenclamide with other medications like metformin hydrochloride[14],[18],[19]. In the present study the authors have been developed a simple, efficient and cost effective UV spectrophotometric method for the quantitative determination of Glibenclamide tablets as well as API. Further the method developed were validated as per ICH guidelines.

2. MATERIALS AND METHODS

Materials
A UV-visible spectrometer of Cary-60 UV-VIS model were used for the Spectroscopic analysis. Calibrated Digital weighing balance of TX323L model was used for the accurate weighing of the sample. Glibenclamide API was obtained as complimentary from Nitin Pharmaceuticals. As well as Glibenclamide tablet dosage form (DAONIL 5mg) was used for the study. The calibrated instruments & analytical grade chemicals were used for this study as shown in Table 1.

Table 1: Instruments and Chemicals Used

S.NO	NAME	MODEL	SUPPLIER/MANUFACTURER
INSTRUMENTS			
1	Single Beam UV Spectrophotometer	Cary-60 UV-VIS	Agilent Tech.
2	Digital Weight Balance	TX323L	Shimadzu Instrument Pvt, Ltd
CHEMICALS			
1	Ethanol		Central Drug House Pvt, Ltd
API & TABLET			
1	Glibenclamide API		Nitin Pharmaceuticals
2	DAONIL TABLET (5MG)		SANOFI

Method Development
Preparation of standard stock solution:
Stock solutions of Glibenclamide was prepared by transferring 50 mg of the drug in 50 ml volumetric flask and dissolved in 30 ml of ethanol and the volume was made up to the mark with ethanol. 5 ml of this solution was transferred to additional 25ml volumetric flask and further diluted up to 25ml mark with ethanol. This standard solution contained 200μg of drug per ml.

Selection Of Wavelength Maxima (λmax):
Pipette out 1 ml of working standard solution and transfer into 10 ml volumetric flask and the volume was made up to the mark with solvent to get the concentration 10μg/ml. The resulted 10μg/ml solution was scanned in UV-Spectrophotometer between 200-400 nm using ethanol as blank. The wavelength maxima were establish at 300 nm and result was shown in fig no. 1.

Preparation of Calibration Curve

Pipette out 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0, 6.0 and 7.0 working standard solution and was transfered into eight separate 10 ml volumetric flasks and made the volume all of them to 10 ml with ethanol to get the concentrations 10, 20, 40, 60, 80, and 100, 120 and 140μg/ml respectively. Absorbance of the resultant solution was measured at 300nm using ethanol as blank. A graph was plotted between the concentrations and their respective absorbance. The response of the drug was found linear in the entire investigational range of 10 - 140μg/ml. The calibration equation was obtained y = 0.0069x + 0.0123 with 0.999 correlation coefficient as shown in the figure no. 2. Result was shown in table 3.

Determination of optical parameters

The molar absorptivity and Sandell's Sensitivity were calculated as:

Molecular Absorptivity (ε) = AM/CT
A = Absorbance
M = Molecular weight
C = Concentration
T = Path length

Sendall's Sensitivity = M/ε
M = Molecular weight
E = Molecular absorptivity

Other optical parameters that is Beer's limit, slope, intercept and correlation coefficient were calculated from calibration curve.

Table 2: Optical Parameters of Glibenclamide

PARAMETERS	OBSERVATION
Beer's law limit(μg/ml)	10-140
Molar absorbtivity (L mol^{-1} cm^{-1})	3542.745
Sandell's Sensitivity (μg/cm2/0.001 absorbance unit)	0.139440
Regression equation (y=mx+c)	(Y=0.0069x+0.0123)
Slope (m)	0.0069
Intercept (c)	0.0123
Correlation Coefficient (R2)	0.999

The table shows the optical and regression characteristics of Glibenclamide. This shows that the method is linear and obeys Beer's law in the concentration range from 10-140 μg/ml, with molar absorptivity L mol^{-1} cm^{-1} of 3542.745 L mol^{-1} cm^{-1} and sandell's sensitivity of 0.139440 μg/cm2/0.001 absorbance unit with correlation coefficient 0.999.
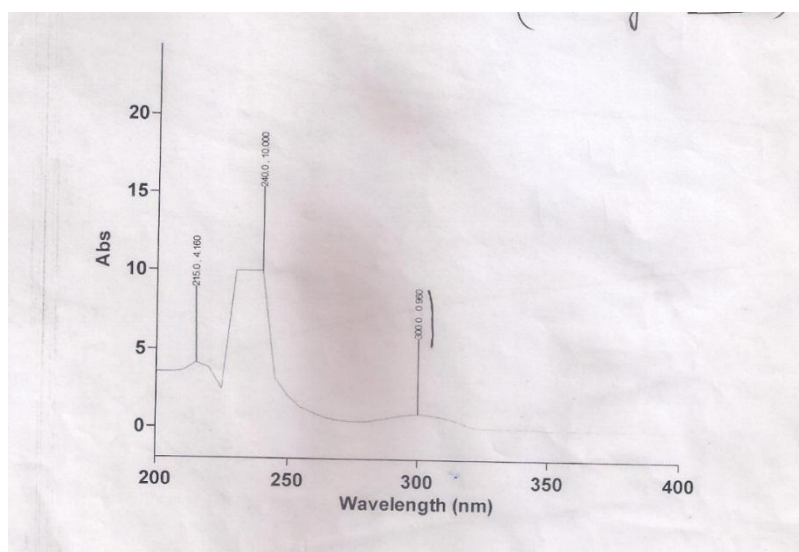
3. RESULT AND DISCISSION

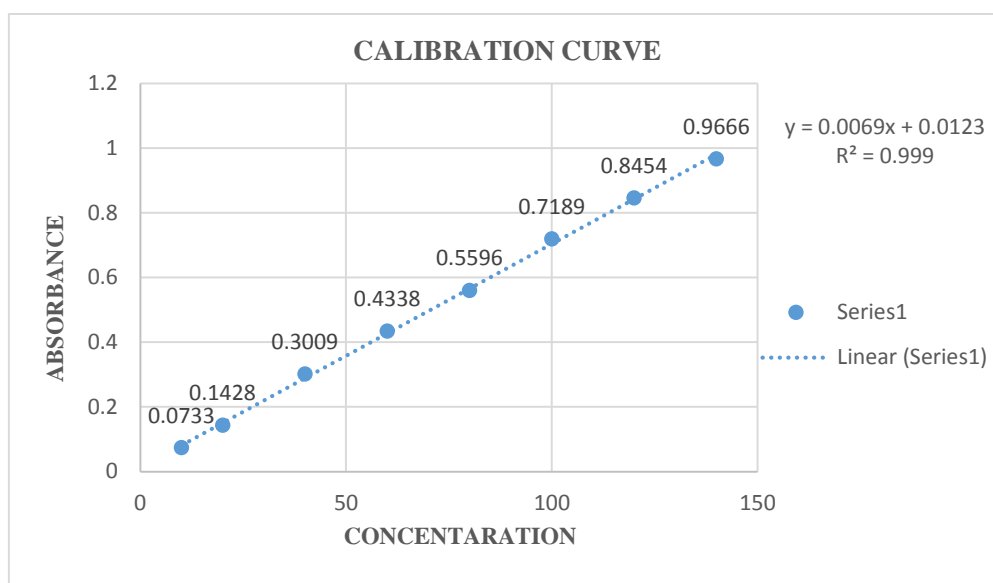**Fig no. 1 Scan of Glibenclamide at 200-400nm**

Fig no. 2 Calibration Curve of Glibenclamide

Table 3: Data for calibration curve of Glibenclamide

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE
1	10	0.0733
2	20	0.1428
3	40	0.3009
4	60	0.4338
5	80	0.5596
6	100	0.7189
7	120	0.8454
8	140	0.9666

Validation Of Proposed Method According To ICH Guidelines:

Table 4: Linearity, E1%1 CM, Absorptivity (L gm-1 cm-1), Molar Absorptivity (L mol-1 cm-1)

S.NO.	Concentration (µg/ml)	Absorbance at 300 nm	$E^{1\%}_{1\,CM}$	Absorptivity ($L\ gm^{-1}\ cm^{-1}$)	Molar Absorptivity ($L\ mol^{-1}\ cm^{-1}$)
1.	10	0.0733	73.3	7.33	3621.04
2.	20	0.1228	61.4	6.14	3033.18
3.	40	0.3009	75.22	7.522	3715.89
4.	60	0.4338	72.3	7.23	3571.65
5.	80	0.5996	74.95	7.495	3702.55
6.	100	0.7289	72.89	7.289	3600.79
7.	120	0.8954	74.62	7.462	3686.25
8.	140	0.9666	69.04	6.904	3410.61
		Mean	**71.715**	**7.1715**	**3542.745**

Precision
Repeatability:
 Pipette out 2.0, 3.0, 4.0ml standard solution and was transferred into a series of nine 10 ml volumetric flasks. Dilute it to 10 ml with ethanol to get 40, 60, 80µg/ml solutions. Absorbance of the resultant solutions was measured at 300 nm using ethanol as blank. The result obtained and summarized in the table 5.

Table 5: Study of Repeatability

Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml)	Mean Conc.(µg/ml)	SD	RSD
20	0.1451	19.7	19.86	0.00037	0.25088
	0.1463	19.9			
	0.1439	19.6			
	0.1458	19.8			
	0.1430	19.4			
	0.1529	20.8			

Intra-Day Precision:

Pipette out 2.0, 3.0 and 4.0ml working solution and was transferred into separate 10 ml volumetric flasks and made up the volume to 10ml with ethanol to get the concentrations 40, 60 and 80µg/ml respectively. Absorbance of the resultant solutions was measured at 300 nm using ethanol as blank. Such three revisions were performed within a day at 3 and 6 hrs interval. The result was summarized in the table 6.

Table 6: Study of Intra-Day Precision

Conc. (µg/ml)	Absorbance			Observed Conc.(µg/ml)			Mean Conc.(µg/ml)	SD	RSD
	0 hrs.	3 hrs.	6 hrs.	0 hrs.	3 hrs.	6 hrs.			
40	0.2988	0.2968	0.2928	41.1	40.8	40.3	40.73	0.00014	0.049
60	0.4537	0.4341	0.4405	61.5	60.0	60.9	60.8	0.00086	0.196
80	0.5957	0.5750	0.5814	82.4	79.6	80.5	80.83	0.00082	0.139
								Mean	**0.128**

Inter-Day Precision:

Pipette out 2.0, 3.0 and 4.0ml working solution and was transferred into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with ethanol to get solution of concentrations 40, 60 and 80µg/ml respectively. Absorbance of the resultant solutions was measured at 300 nm using ethanol as blank. Such three studies were performed for day one day two day three intervals. The result was summarized in the table 7.

Table 7: Study of Inter-Day Precision

Conc. (µg/ml)	Absorbance			Observed Conc.(µg/ml)			Mean Conc.(µg/ml)	SD	%RSD
	0 hrs.	24hrs.	28hrs.	0 hrs.	24hrs.	48hrs.			
40	0.3006	0.2968	0.2928	41.4	40.8	40.3	40.83	0.00014	0.1679
60	0.4404	0.4341	0.4405	60.8	60.0	60.9	60.56	0.00139	0.3201
80	0.5818	0.5750	0.5814	80.8	79.6	80.5	80.3	0.00082	0.1398
								Mean	**0.2092**

Accuracy

Pipette out 0.5 ml standard solution and was transferred into 10 ml volumetric flasks. Nine such transfers were made. Spike three of volumetric flask with the solutions with 0.8ml of working solution (Prepared from Formulation) and dilute each to 10 ml with ethanol to get 18µg/ml solutions. Spike another

three of the solutions with 1ml of working solution and dilute each to 10 ml with ethanol to get 20 µg/ml solutions. Spike last three of the solutions with 1ml of working solution and dilute each to 10 ml with ethanol to get 22

µg/ml solutions. Absorbance of the resultant solutions was measured at 300 nm using ethanol as blank. The obtained results were summarized in the table 8.

Table 8: Study of Accuracy

Recovery at	Nominal Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml)	% Recovery
80%	110=50+60	0.7988	109.8	98.82
80%	110= 50+60	0.8022	110.0	100
80%	110 = 50+60	0.7922	109.2	99.27
100%	120 = 60+ 60	0.8660	120.0	100
100%	120 = 60+60	0.8651	119.4	99.5
100%	120 = 60+60	0.8644	118.2	98.5
120%	130 = 60+70	0.9066	130.0	100
120%	130 = 60+70	0.8954	129.5	99.61
120%	130=60+70	0.8951	129.1	99.30
			Mean	**99.45**

Specificity

Specificity study was carried out by observing any interference in absorbance of drug in the presence of common excipients like starch, talc, lactose, magnesium stearate etc. Absorbance of

10 µg/ml drug solution with and without excipients was measured at 300 nm using ethanol as blank. The results obtained were summarized in the table 9.

Table 9: Study of Specificity

Nominal conc. (µg/ml)	Without Excipients		With Excipients		% Interference
	Absorbance	Observed Conc.(µg/ml)	Absorbance	Observed Conc. (µg/ml)	
10	0.0724	9.76	0.0730	9.94	0.99
10	0.0697	9.66	0.0720	9.74	0.97
10	0.0730	9.88	0.0738	10.06	1.00
10	0.0732	9.90	0.0735	10.02	1.00
10	0.0728	9.80	0.0728	9.90	0.99
10	0.0720	9.72	0.0724	9.89	0.98
				Mean	**0.99**

Estimation of glibenclamide in pharmaceutical dosage form (daonil, 5mg):
20 tablets were weighed and the average weight of the tablets were calculated. The tablets were powdered and weighed accurately. A quantity of powdered containing about 20 mg of Glibenclamide and was transferred it into 50 ml volumetric flask and 15 ml ethanol was added, sonicate for 15 minutes and was made

up the volume to 50 ml with solvent then mix and filter that solution. Taken 2.5 ml of the filtrate and made up the volume to 25 ml with ethanol. Further dilute 1.6 ml of the resulting solution to 10 ml with ethanol. Measure the absorbance of this resulting solution at 300 nm. The above procedure was repeated for three times. The result obtained was summarized in the table 10.

Table 10: % Assay of Glibenclamide in pharmaceutical dosage form (DAONIL, 5mg)

Sr. No.	Absorbance	Conc. (µg/ml)	Dil. Factor	Weight Taken (mg)	Avg. Weight (mg)	Label Claim (mg)	% Assay
1	0.0740	9.9	5000	50	50	5	99
2	0.0762	10.2	5000	50	50	5	102
3	0.0714	9.8	5000	50	50	5	98
4	0.0728	9.8	5000	50	50	5	98
5	0.0754	10.1	5000	50	50	5	101
						Mean	99.6

4. CONCLUSION

A simple and sensitive spectrophotometric method for quantitative determination of Glibenclamide in either pure form or in pharmaceutical dosage for was developed. Glibenclamide showed maximum absorbance at 300 nm in solvent. It has linear response in the entire range of 10 to 140µg/ml with correlation coefficient of 0.999 .The linear regression equation obtained is y = 0.0069x + 0.0123.The method has good precision < 2% and accuracy is 99.45 No significant interference was observed in the absorbance of the drug in the presence of common excipients. The method was statistically validated according to ICH. The method was employed for the quantitative determination of tablet dosage form.

5. REFERENCE

1. Mend H.J., "Vogel's, Textbook Quantitative Chemical Analysis", 5th edition Pearson education (Singapore) Pvt. Ltd. Indian branch, Delhi; 2003:3-8,630.
2. Skoog D.A., Holler F.J., et al., "Introduction to UV Spectroscopy in principle of Instrumental Analysis", 5th Edition, Thomson Brooks Cole Publication; 2004:1-17,301.
3. Sharma Y.R., "Ultraviolet and visible Spectroscopy in elementary organic spectroscopy", 1st edition, S. Chand & Company Ltd, New Delhi; 2004:9-60.
4. "Text on Validation of Analytical Procedure Q2A in; ICH Harmonized Tripartite Guidelines"; 1996:1-6.
5. Willard H.H., et al, "Instrumental method of analysis", 7th Edition, New Delhi, CBS Publishers and Distributors; 1988:118-139.
6. "Text on Validation of analytical procedure Q2B in; ICH Harmonized Tripartite Guidelines"; 1996:7-13.
7. 7. Adams J. Wade, Krueger Scott D, "Specific and sensitive high-performance liquid chromatographic determination of glyburide"; 1979;68(9):1138-1140.
8. Abdel-Hamid ME and Suleiman MS, "A rapid high performance liquid chromatography assay of Glibenclamide in serum"; 1989:14181-188.
9. Santurio Valdes Joser, Porto Esther, "Determination of glibenclamide in human plasma by solid-phase extraction and high-performance liquid chromatography"; 1996:682(2):364-370.
10. H.Emilsson, "High-performance liquid chromatographic determination of glibenclamide in human plasma and urine"; 2001:93-102.
11. Niopas I and Daftslos AC. "High performance liquid chromatographic method for the determination of Glibenclamide in human plasma and its application to pharmacokinetic studies. J Pharm Biomed Anal."; 2002;28:653-7.
12. F.S. Bandarkar, "Simultaneous estimation of Glibenclamide, Gliclazide, and Metformine hydrochloride from bulk and commercial products using a validated ultrafast liquid chromatography technique"; 2002;33(20).
13. Aburuz. S, Millership. J, McElnay. J, "SPE and HPLC methods for the simultaneous

determination of metformin and glipizide, gliclazide, glib enclamide or glimperide in plasma"; 2004:5512.

14. Deeb EL S , "Fast HPLC method for the determination of glimepiride, glibenclamide, and related substances using monolithic column and flow program", J Sep Sci.,2004;29(11):1571-7.

15. Chaturvedi P, Sharma R., "Development and validation of an RP-HPLC method for simultaneous analysis of a three-component tablet formulation containing metformin hydrochloride, pioglitazone hydrochloride, and Glibenclamide", 2008;20(3).

16. Gurav Suhas, Singh Sachin, "Method evelopment and validation of Glibenclamide in bulk and pharmaceutical dosage form by using UV-VIS spectrophotometric method",2011.

17. S. Prashant, Kumar Pradeep, "Development and validation of HPLC method for the determation of Glibenclamide in rat serum", 2011.

18. Eapen Cicy, G Prashant and Rai Amita, "Development of UV Spectrometric Method of Glibenclamide (Glyburide) in Bulk and Pharmaceutical Formulations",2012;4:356-360.

19. Jayanthi M, S.V.Thirunavukkarasu S. V, "Development and Validation of RP-HPLC Method for Determination of Glibenclamide in Pharmaceutical Dosage Forms"; 2012;4(2):593-601.

20. Bilal Abida, Rehman Kanwal, Hamid Akash Muhammad, "Development and Validation of Analytical Method for Qualitative and Quantitative Determination of Glibenclamide in Different Brands of Tablet Dosage form Using UV-Visible Spectroscopy"; 2013.

21. *B K Koteshwar, Naha Anup, Saikiran Sunil, "Method Development and Validation of Glibenclamide in Tablet Dosage Form by using RP-HPLC"; 2013:2(4):226-230.*

22. Patil M. Sidharth, Chilkawar N. Ramchandra, Panchal Vinod, "Validated simple and sensitive UV spectrophotometric method for estimation of Glibenclamide in bulk and pharmaceutical formulation"; 2013;2(5):2627-2634.

23. Edla Subhashini and Syama Sundhar B, "Development a rapid of RP-HPLC for the simultaneous estimation of Metformin and Glibenclamide in their combined tablet form"; 2014.

24. Chavhan Bhavika*, Patil P. B, Bavaskar S, "Development and validation of analytical method for simultaneous estimation of Glibenclamide and Metformin hcl in bulk and tablets using UV-VIS spectroscopy", 2015;4(11):1257-1266.

25. Elkady F Ehab, A. Mahmoud, "Validated Liquid Chromatographic Method for Simultaneous Determination of Metformin, Pioglitazone, Sitagliptin, Repaglinide, Glibenclamide and Gliclazide", 2015.

26. Ibrahim M. Magda, "Development and validation of a RP-HPLc method for the simultaneous determation of Carvedilol, Glimepride or Glibenclamide in binary combinations"; 2015;5(08):2791-2802.

27. Issarani Neelkant, Nagori Roshan, "Development a simple and sensitive ultraviolet spectrophotometric method for quantitative estimation of glibenclamide in presence of lipid turbidity has been developed, to avoid false estimation due to diffraction by turbidity", 2016.

28. Prasad Neelkant, "Ultraviolet-visible spectrophotometric method for estimation of the glibenclamide in presence of liposomal/proliposomal turbidity", 2016; 23(4):318-322.

29. Umadevi. N, Babu Sudheer, "Spectrophotometric method development and validation for the estimation of Glibenclamide in pure form and in dosage form", 2017:59.

30. S. D. Rajendran, "RPHPLC method for the estimation of glibenclamide in human serum"; 2017;69(6):796-799.

31. Islam Hasibul Sheikh , Alauddin Md, "Development and validation of stability-indicating reverse phase-HPLC (RP-HPLC) method for simultaneous estimation of Rosuvastatin (RSV) and Glibenclamide (GBC)", 2018;7(5):22-37.