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Validation of Uv- Visible Spectrophotometric Analytical Method for Bcs Class Ii Drug

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ABSTRACT- Glibenclamide is a potent antihyperglycemic BCS class II drug which is clinically effective in controlling high sugar level of Noninsulin-Dependent Diabetes Mellitus (NIDDM type II) disease. A simple, precise, fast and selective uv- visible spectrophotometric method was developed and validated as per ICH guidelines Q2 (R1) (ICH, 2005)18-19 for the determination of glibenclamide in bulk as well as dosage formulations. An lambda max of 300nm was used for the spectrophotometric determination, using methanol as solvent. The method was validated for specificity, precision, robustness , linearity, and accuracy. The response for glibenclamide was linear in the selected concentration range 10 to 50 µg/ml with a correlation coefficient of 0.999. The accuracy was between 98.73 ± 1.10% to 101.13 ± 0.92%. The limit of detection (LOD) and limit of quantification (LOQ) are 0.35 and 1.09 µg/ml, respectively. The method was also precise, robust,reproducible and linear since all the samples showed less than 5% coefficient of variation value and statistically, theoretical and practical concentrations showed no significant difference. The method demonstrated that the excipients in the microemulsion was not causing any interference with drug and can be routinely employed for daily quality control analysis of glibenclamide in bulk drug as well as in microemulsion formulations.

Keywords- Glibenclamide, UV-Visible Spectroscopy, ICH guidelines, Validation, microemulsion

I. INTRODUCTION

Glibenclamide (also known as Glyburide) is a second generation sulfonylurea having chemical name 5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl) sulfamoyl] phenyl} ethyl)-2-methoxybenzamide (Figure 1). It is BCS CLASS II potent hypoglycemic agent (dose -2.5-20 mg/day) , used orally to treat hyperglycemia caused by non-insulin dependent diabetes mellitus type II (NIDDM - II)[1-2]. It is one of only three oral anti-diabetics in the World Health Organization 2019 Model List of Essential Medicines (the other being metformin and gliclazide) glibenclamide. Being bcs class II drug, the water solubility of glibenclamide is very poor but permeability is very high so the absorption of drug is irregular due to its variable dissolution profile which may also be affected by other factors like dosage form design, food habits or by patient related factors. [4- 7]. So there is requirement of dosage form which improves its solubility as well as regularize the dissolution profile for uniform and steady permeation of drug. Microemulsion may be one of the best choice for that because of its high dissolving efficacy as well as its liquid state for this drug. Microemulsions are widely used as pharmaceutical drug delivery systems of several hydrophobic drugs proving their suitability and clinical efficacy for drug delivery. Microemulsions are micron sized o/w or w/o transparent homogenous and thermodynamically stable systems [10-11]. They can dissolve wide variety of drugs either they are hydrophobic or hydrophilic [12-13].The analytical methods are used to determine quality of pure drug as well as dosage forms using official and validated methods. The hplc chromatographic technique has many advantages but it also have some limitations like high cost of instrumentation, its operation, comparatively long time for analysis and the needs the skill for processing of samples as well as handling the equipment [14]. Spectrophotometry is a relativly convenient and sophisticated analytical technique that is mostly used in labs for quality control purposes due to its simplicity, low operation cost and wide applications [15].The main purpose of this analytical process was to develop and validate a precise, robust, rapid, economical and accurate method for quantification of glibenclamide by ultaviolet visible - spectrophotometry. Subsequently, the developed method can be used to determine pure drug in bulk as well as in dosage forms, like microemulsion.

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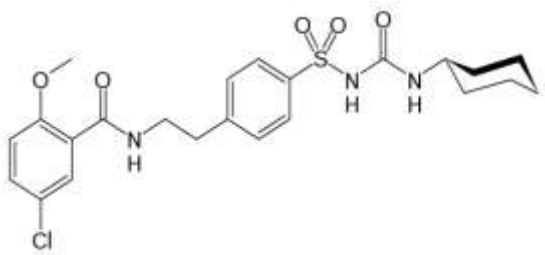

Figure-1 Structure of Glibenclamise

Literature study shows that Multiple number of analytical methods were developed for the quantitative determination of glibenclamide alone as well as with other drugs like TLC [16-18]; UV Spectrophotometry by derivatization technique or coupling with another reagent [19-20]; HPLC–UV & RP-HPLC [21-26]; Spectrofluorimetry method[27]; HPLC–fluorescence [28-29]; CE–UV [30]; LC/API (MS) [31] in bulk as well as marketed formulations. Most of these methods are time consuming and much expensive and some methods can not be used in microemulsion formulation due to use of its excipients like oil ,surfactant and cosurfactant . so it is not suitable for routine analysis for quality control in bulk drug as well as marketed formulations Therefore, present work is to develop and validate accurate ,simple, rapid ,robust and precise UV-Visible spectrophotometer analytical method which is not using any complexation agent, extraction or derivatization.

II. MATERIALS AND METHOD

2.1 Materials

Glibenclamide was obtained as a gift sample from Hetero Pharmaceutical Ltd, Hyderabad. All other chemicals used were AR grade and purchased from S.D. fine Chemicals.

2.2 Method Development

2.2.1 Instrument- Double beam UV Visible Spectrophotometer (SCHIMADZU CORPORATION UV PHARMSPEC-1700, JAPAN) attached with 10 mm quartz cells, that was used for the measurements of the absorption. A thermostatic magnetic stirrer (remi, India) were used.

2.2.2 Preparation of Standard Stock Solution Glibenclamide has a molecular weight of 445 and M.P.= 171 °C being characterized by white crystalline powder. Accurately weighed 100 mg of standard glibenclamide was added in 100ml volumetric flask having some volume of methanol and mixed well by magnetic stirrer to get transparent solution, then volume was make up to 100 ml by methanol(standard stock solution). Aliquots of different concentration was prepared from this standard stock solution, by suitable dilutions with methanolthe final concentrations were varying in between 10 and 50 µg/ml. Each standard solutions preparations were performed in triplicate for the validation of analytical method.

2.2.3 Method Optimization

2.2.3.1 Selection of Solvent -as published in literature, in the ultraviolet method the solvent have a very marked effect on the shape as well as quality of the peak[32-33]. Being a BCS class II drug glibenclamide is practically insoluble in water (pka-5.3) but highly soluble in methanol[34] , so methanol was found best to comply quality standards as well as non-interference of peak at selected wavelength.[35-37]

2.2.3.2 Selection of Wavelength-The wavelength of absorption maxima (ʎmax) of glibenclamide was determined using, aliquot of 100 µg/ml solution which was prepared by standard solution 1mg/ ml in methanol and diluted till 50 µg/ml and scanned in wavelength range of 200-400 nm using methanol as a blank. The absorption spectrum curve given at fig 2 showed maximum absorption at 300 nm for glibenclamide.

2.3Analytical Method Validation:- The developed method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005)[38]18,19 and all the parameters like linearity, Accuracy, Robustness, specificity, Precision, Limit of Detection (LOD) and Limit of Quantification (LOQ). were evaluated. All assays were analysed at 25 °C except the robustness, where solutions were stored at 4 °C and 37 °C before analysis.

2.3.1Linearity :-The verification of linearity of proposed method was performed by preparing different standard solutions of glibenclamide (10, 20, 30, 40 and 50 µg/ml $^{-1}$),they were analyzed in triplicate, to plot fifteen derived analytical curves. The curve of absorbance v/s concentration (figure 3) of glibenclamide was plotted and the curve was found linear as given in table 1. Linear regression analysis by applying least square method as well as analysis of variance (ANOVA) were used to analyse the linearity of the analytical curve.

2.3.2Specificity -The specificity of the proposed method was also evaluated by comparing the both UV spectra of blank microemulsion sample against glibenclamide loaded microemulsion sample. This was performed from 200 to 400 nm range and varified for any changes in absorbance at the selected wavelengths.

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2.3.3 Precision -Repeatability (intraday) and intermediate (interday) variations were studied to determine precision of the developed analytical method. Three different levels of glibenclamide concentrations at 15, 20 and 25 µg/m were analyzed to determine precision. Different concentration solutions were prepared at any two different times of single day in triplicate and analyzed for intra-day variations. The same method was also used for two different days to determine inter-day variations. The measure of precision was determined by appplying percentage relative standard deviation (%RSD) of the regression equation. Mean absorbance of the samples was also compared with the paired t-test at 95% level of significance.

2.3.4 Accuracy -is used to test the exact measurement of the analytical method. It was determined on the basis of recovery of known amount of glibenclamide added to the sample of microemulsion. Briefly, to determine the developed method accuracy different drug concentration levels were used : lower concentration (15µg/ml), intermediate concentration (20 µg/ml) and higher drug concentration (25 µg/ml). A known aliquot of glibenclamide stock solution was mixed with unloaded microemulsion in 10 ml volumetric flask containing equivalent to the quantity of glibenclamide in microemulsion and volume was made up with methanol. All the solutions were made in triplicate and analysed with the developed method . The RSD and drug percentage recovery were used to determine the accuracy of method. The following equation was used.. The statistical t test at 95% level of significance was applied to check accuracy of developed method.

$$\text{Recovery (\%)} = \frac{\text{Theoritical drug concentration}}{\text{Practical/found drug concentration}} \times 100 \quad \text{---------- Eq.- 1}$$

2.3.5 Robustness- Temperature and solvent suppliers were changed to check the robustness of the developed method for the glibenclamide samples (10 µg./mL). Prior to analysis, the samples were prepared and sealed to tubes and transferred to the refrigerator at 4 °C or stored at different temperatures (25 °C as well as 37 °C) for 24 hrs. The samples were analysed three times under the same conditions[39].

2.3.6 LOD and LOQ:- were determined to check detection and quantification limits. The DL is the minimum concentration which can be diagnosed but not quantified and QL is the minimum concentration which can be quantified. The LOD and LOQ was determined by using following equation using signal- to - noise ratio as per ICH, 2005 [38}

$$QL = 10\ SD / S$$
$$DL = 3.3\ SD / S \quad \text{---------- Eq.-2}$$

Where SD stands for the standard deviation of intercept of the calibration curve and S stands for slope .

III. PREPARATION AND APPLICATION OF THE MICROEMULSION FORMULATION:-

The glibencamide microemulsion was prepared with the help of oil ,surfactants, and cosurfactants by water titration method. Firstly required drug was dissolved in the optimized oil amount and then surfactant cosurfactant mixture was added to it to get homogenous mixture. Then double distilled water was added dropwise to it to get transparent desired microemulsion.[40] The formulation was stored for 72 hrs at 25 ^{0}c before analysis. This analytical method was developed to determine concentration of glibenclamide in microemulsion formulation. In the literature, the quantification of lipophilic molecules in microemulsion as well as other formulations like inclusion complexes , liposomes have been already performed with great success using the proposed spectrophotometry method[41-42]

IV. RESULTS AND DISCUSSION

Analytical method validation- The absorption spectrum of the glibenclamide in solvent (methanol) was prepared at concentrations ranging from 10 to 50 µg/ml[1] . The drug glibenclamide exhibited a maximum absorption peak (ʎmax) at 300 nm with a molar absorptivity (ε) of 3.60×10^4 $L.mol^{-1}.cm^{-1}$(Figure-2). In addition, methanol did not cause any interference near ʎmax. So Methanol can be a suitable solvent for development and validation of the proposed method as well as also support the reproducibility of the results.

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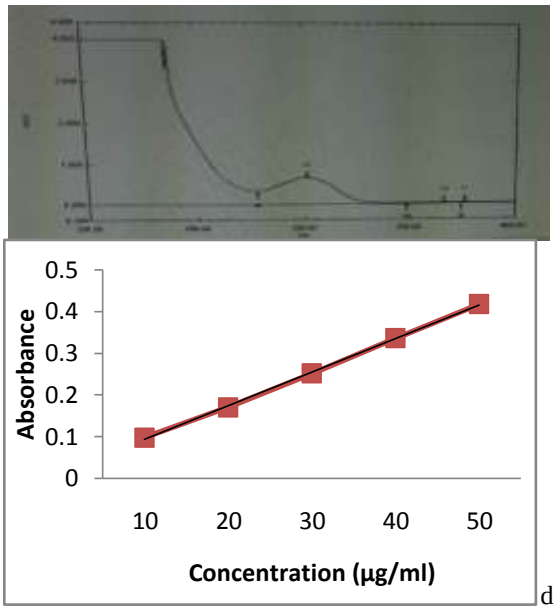

Figure 2. UV spectrum of Glibenclamide in methanol . **Figure-3** Standard curve or linearity curve of glibenclamide at 300 nm

The drug glibenclamide solutions ranging from 10 to 50 µg./mL presented a linear relationship (Table I), and the regression analysis data are tabled in Table II. The mean regression equation was developed by least squares treatment:

$y = 0.0081x - 0.0132$ ($r^2 = 0.999$, n=3) (Eq- 3).

Table-I Experimental results of validation parameters

Drug concentration(µg/ml)	Mean absorbance at 300nm ± S.D.(n=3)
10	0.098± 0.0010
20	0.171± 0.0024
30	0.252±0.0049
40	0.336±0.0020
50	0.418±0.0033

Limit of detection and limit of quantification

The 0.359 µg.mL-1 and 1.090 µg.mL-1, were the limit of detection (LOD) and the limit of quantification (LOQ) of the proposed method, respectively. These results confirmed the sensitivity of the method even at low concentrations of glibenclamide.

Table-II optical characterstics of glibenclamide statistical data of regression equation and validation parameters

Glibenclamide	Validation parameters
Optical characterstics	
-Molar absorptivity ε (L.mol−1.cm−1)	$3.6x10^4$
Regression analysis	
-Regression coeffecient (r^2)	0.999
-Intercept	0.0132 ($4.5x10^{-4}$
-slope	0.0081($2.5x10^{-4}$)
valiation parameters	
-ʎmax	300nm
-Range	(10-50 µg/ml)
-accuracy	98.73-101.13%
Precision(%RSD)	
-Intra day	1.14%
-Interday	1.78%
-Limit of detection(µg/ml)	0.3598
-Limit of quantification(µg/ml)	1.0903

Specificity describes the ability of method to check the presence of any one specific material irrespective of presence of another materials. In this method glibenclamide was easily analysed in microemulsion. A proper peak of glibenclamide was appeared at lambda max 300 nm. Addition to this, blank sample of microemulsion showed no peak at that wavelength (Figure 4) as well as there was no change in absorption peak of glibenclamide at 300 nm in the presence of the constituents of the microsomal formulation, thereby demonstrating the specificity of the method.

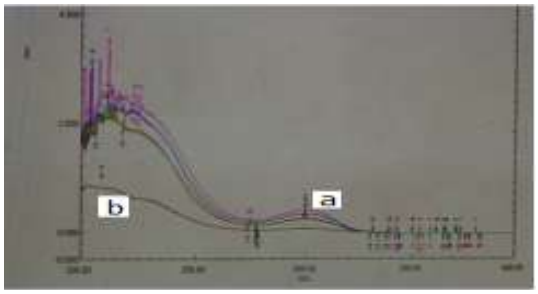

Figure-4 uv spectra of glibenclamide in microemulsion (a)as well as blank microemulsion (b)

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Accuracy - The accuracy was checked by recovery analysis of known quantities of glibenclamide (10, 20 and 30 µg/ml) spiked in loaded microemulsion. Glibenclamide recoveries ranged from 98.46 ± 0.01% to 101.13 ± 0.93%. The mean glibenclamide recovery values were nearly close to 100%, and its small standard deviation values confirms the high accuracy of the developed method. These results also specifies that small drug concentration changes can be accurately determined by the developed method.

Table III Recovery of glibenclamide in microemulsion to determine the accuracy of UV method

Amount of Glibenclamide added(µg/ml)	Amount of sample (µg/ml)	Total amount of sample (µg/ml	amount of sample after analysis ± SD (µg/ml)[a]	Recovery ± SD (%)	% RSD	$^{b}t_{cal}$	$^{b}t_{crit}$
10	20	15	14.76 ± 0.25	98.46±0.016	1.72	0.47	4.30
20	20	20	19.81 ± 0.173	99.16±0.005	0.58	0.49	4.30
30	20	25	24.54 ± 0.46	101.13±0.931	0.92	0.41	4.30

a -each value represent the mean of three measurements. $^{b}t_{cal}$ is the calculated value and $^{b}t_{crit}$ is the theoretical value based on the paired t-test at the level of significance of p = 0.05.

Precision The range of Repeatability (RSD%) was found from 0.79 to 1.78 (%) for three levels of glibenclamide concentrations (Table IV).The intra-day and inter- day precision result indicated the high precision because the RSD% values were less than 2.5% in all cases, either over a short period of time with in day or different days performed by same analyst or different. Whole process was performed in single laboratory [43].

Table IV. Repeatability and intermediate precision of developed analytical method

Precision					
	Glibenclamide added((µg/ml))	Glibenclamide found ± S.D. (µg/ml)	R.S.D.(%)	t_{cal}^{a}	t_{tab}^{b}
Intra day different time, same day	10	9.83± 0.078	0.79	0.66	2.57
	20	19.85± 0.140	0.71	0.69	2.57
	30	29.90± 0.341	1.14	0.52	2.57
Interday, same analyst	10	9.88± 0.1735	1.76	0.79	2.57
	20	20.07± 0.609	1.78	0.77	2.57
	30	29.94± 0.396	1.32	0.62	2.57
Interday, different analyst	10	9.94± 0.251	1.42	0.98	2.57
	20	19.89± 0.295	1.48	0.56	2.57
	30	30.04± 0.647	1.35	0.65	2.57

$^{a}t_{cal}$ is the calculated value; $^{b}t_{tab}$ is the theoretical value based on the t-test at the level of significance of p = 0.05 (n=6).

Robustness - The robustness of developed analytical method was proved (Table V), due to no statistically significant differences (Student´s t-test) were found during temperature variations as well as solvents from different manufacturers. The recovery of glibenclamide was satisfactory (>99%) from microemulsion samples kept at different temperatures so it was well proved that molar absorptivity of glibenclamide was temperature independent.

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Table-V Robustness of the developed method by using different solvent suppliers as well as temperature

Robustness				
	Glibenclamide added	**Glibenclamide found ± S.D. (µg/ml)**	**R.S.D.(%)**	**t_{cal} [b]**
solvent 1	20	19.88±0.246	1.23	0.56
solvent 2	20	19.91±0.474	2.38	0.42
4^0C	20	19.96±0.327	1.63	0.86
25^0C	20	19.81±0.294	1.48	0.05
37^0C	20	19.85±0.221	1.11	0.41

[a] t_{cal} is the calculated value. The theoretical value t_{crit} = 4.30 is based on the t-test at the level of significance of p = 0.05 (n=3).

V. CONCLUSION-

A fast, simple, accurate and precise UV spectrophotometric method was developed and validated for quantification of glb in microemulsion formulation. The proposed method performed satisfactory result for the intended drug analytical ability. Furthermore, it may be the alternative to the chromatographic assay, for the routine analysis of quality control as well as batch to batch drug content uniformity determination for microemulsion formulations.

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