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Analytical method development and validation of Glimepiride in pharmaceutical dosage form by UV visible spectrophotometric method

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ABSTRACT

The main objective of the present work is to develop new, simple, sensitive, accurate and economical analytical methods for the estimation of Glimepiride and validate the proposed methods in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of Glimepiride in pharmaceutical dosage forms by UV Spectrophotometry. The Analytical method was developed by Studying Different Parameters. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Glimepiride was found in concentration range of 5µg-25µg and correlation coefficient (r) was found to be 0.999 and Regression coefficient (r^2) 0.999 %Recovery studies were carried out and the percentage recovery was found to be in the range of 100.8% - 101%. %RSD of Absorbance for Intraday, Inter day precision was 1 and 0.95% RSD for Ruggedness was found to be less than 2. LOD and LOQ of calibration curve of drug prepared in 0.1N NaOH were found to be 0.5and 1.5µg/ml, respectively. Hence the suggested UV Spectrophotometric method can be used for routine analysis of Glimepiride in API and Pharmaceutical dosage form.

Keywords: Glimepiride, UV Spectrophotometric, Tablets, validation, method development.

INTRODUCTION[1-6]

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present). Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge. Pharmaceutical Analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. The term “Pharmaceutical analysis” is otherwise called quantitative pharmaceutical chemistry. Pharmaceutical analysis includes both qualitative and quantitative analysis of drugs and pharmaceutical substances starts from bulk drugs to the finished dosage forms. The technique employed in quantitative analysis is based upon the quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained. In the modern practice of medicine, the analytical methods are used in the analysis of chemical constituents found in human body whose altered concentrations during disease states serve as diagnostic aids and also used to analyze the medical agents and their metabolites found in biological system.

Method validation

Method validation is the process to confirm that analytical procedure employed for a specific test is suitable for its intended use. Method needs to be validated or revalidated

- Before their introduction into routine use
- Whenever the conditions changes for which the method has been validated, e.g., instrument with different characteristics

- Whenever the method is changed, and the change is outside the original scope of the method.

All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters.

6. METHOD DEVELOPMENT
Materials and Methods

UV Spectrophotometry Method Development
Solvent selection

In the start of the method development for this drug, different solvents were tested such as water, methanol, 0.1N NaOH, Phosphate buffer and Acetonitrile. In order to select suitable solvent for determination of Glimepiride, various solvents were selected for the solubility studies and it was found that Glimepiride was freely soluble in 0.1N NaOH, sparingly soluble in Methanol, water…etc. In the present investigation, 0.1N NaOH was used for all the dilutions. Due to greater solubility and reproducible readings of maximum absorbance, 0.1N NaOH was taken under consideration for further work.

Selection of wavelength

The absorbance of the solution containing Glimepiride at 10 µg/ml was determined in the UV range 200-400 nm using an appropriate blank. The maximum absorbance was found to be 233nm.

Assay
Preparation of Glimepiride standard solution

Weigh accurately1 mg of standard substances of Glimepiride [1mg=1000µg]. Dissolve 1 mg of Glimepiride in to a 100 ml of 0.1N NaOH solution [stock solution of Glimepiride [Stock solution of Glimepiride100µg/ml].

1mg/100ml

1000µg/100ml=10 µg/ml

The10 µg/ml solution was observed and found absorbance in UV.

Preparation of stock solution of Tablet formulation

Weigh accurately 20 tablets of marketed formulation [Amaryl 1mg]. Calculate average weight. Weigh accurately 1 mg equivalent weight of powder in to a100 ml of 0.1N NaOH solution [Stock solution of mixture 100µg/ml]

1mg/100ml

1000µg/100ml=10 µg/ml

The10 µg/ml solution was observed and found absorbance in UV.

METHOD VALIDATION PARAMETERS
LINEARITY OF TEST METHOD

Table 1: Glimepiride solution preparation

Name of the Drug	Standard Stock solution of Glimepiride 100µg/ml]	Dilute up to	Total Concentration we get
Glimepiride	0.5 ml from 100µg/ml	10ml	5µg/ml
	1 ml from 100µg/ml	10ml	10µg/ml
	1.5 ml from 100µg/ml	10ml	15µg/ml
	2ml from 100µg/ml	10ml	20µg/ml
	2.5 ml from 100µg/ml	10ml	25µg/ml

Procedure

Each Concentration was Observed into the UV spectrophotometric system and Absorbance were measured at 233nm against solvent blank. Plot a graph of Absorbance versus concentration (on X-axis concentration and on Y-axis Absorbance) and the correlation coefficient was calculated. And the absorbance values were shown in Table 4. The obtained absorbance values were plotted against the concentration of Glimepiride to get the calibration graph and were represented as Fig no: 10. The regression equation and correlation coefficient were determined and are given in Table 1. Correlation coefficient should be not less than 0.999.

Accuracy

The accuracy study was performed for 80%,100% and 120 % for Glimepiride. Each level was observed in triplicate into UV spectrophotometric System. The Concentration of each level was used for calculation of % recovery. Results are tabulated in Table no 2. The Percentage recovery was found to be within the limits (98-102%).

Table 2: Preparation of 80,100,120% solution of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Glimepiride	Dilute Upto	Total conc we get
80%	1 ml from 100µg/ml	0.8. ml from 100µg/ml	10ml	18 µg/ml
100%	1 ml from 100µg/ml	1 ml from 100µg/ml	10ml	20 µg/ml
120%	1 ml from 100µg/ml	1.2 ml from 100µg/ml	10ml	22 µg/ml
Make three same dilution at each % level				

PRECISION

To evaluate Precision of the method, Precision was performed on Different days by maintaining same conditions.

Table 3: Preparation of 100% solution[10µg/ml]of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Glimipiride	Dilute Upto	Total conc we get
100%	0.5 ml from 100µg/ml	0.5 ml from 100µg/ml	10ml	10 µg/ml

Intra-day precision: The 10 µg/ml solution was observed for six times and found absorbance for all 6 times In UV. The % RSD for absorbance, Concentration Found, % Assay for 6 Sample was found to be within the specified limits.

Inter-day precision: The10 µg/ml solution was observed for six times and found absorbance for all 6 times In UV. The % RSD for absorbance, Concentration Found, % Assay For 6 Sample was found to be within the specified limits. Results are tabulated in Table 3. The % Relative Standard Deviation of % Assay of Glimepiride from the six sample preparations should be not more than 2.0%. The individual % Assay of Glimepiride should not be less than 98.0% and not more than 102.0%.

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RUGGEDNESS

Analyst to analyst variability

To evaluate Ruggedness of the method, Ruggedness was performed by using two different analysts by maintaining same conditions.

Table 4: Preparation of 100% solution [10µg/ml]of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Glimepiride	Dilute Up to	Total concentration we get
100%	0.5 ml from 100µg/ml	0.5 ml from 100µg/ml	10ml	10 µg/ml

Analyst 1: The10 µg/ml solution was observed for six times and found absorbance for all 6 times In UV .The % RSD for absorbance, Concentration Found, % Assay for 6 Sample was found to be within the specified limits.

Analyst 2: The10 µg/ml solution was observed for six times and found absorbance for all 6 times In UV .The % RSD for absorbance, Concentration Found, % Assay For 6 Sample was found to be within the specified limits. Results are tabulated in Table 4. The % Relative Standard Deviation of % Assay of Glimepiride from the six sample preparations should be not more than 2.0%. The individual % Assay of Glimepiride should not be less than 98.0% and not more than 102.0%.

Limit of detection and Limit of quantification

Each Concentration was observed into the UV spectrophotometric system and Absorbance was measured at 233nm against solvent blank.

Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

RESULT AND DISCUSSION

Table 5: Optical Characteristic results

S. No	Parameter		Result
1	Absorption Maximum		233nm
2	Linearity Range[µg/ml]		5-25[µg/ml]
3	Standard Regression Equation		Y=0.0393x+0.0108
4	Slope		0.0393
5	Intercept		0.0108
6	Correlation coefficient(r)		0.99
7	Regression coefficient (r^2)		0.999
8	Accuracy(% Recovery)	For 80%	100.8%
		For 100%	101%
		For 120%	101%
9	Precision(% RSD of Absorbance)	Intra Day	1%
		Inter Day	0.95%
10	Ruggedness(%RSD of Absorbance)	Analyst 1	1.25%
		Analyst 2	1.25
11	LOD	0.5µg/ml	
12	LOQ	1.5µg/ml	
13	STNDARD DEVIATION	0.031	

14	STANDARD ERROR	0.013
15	STANDARD ERROR INTERCEPT	0.000994987

Linearity plot

The plot of Concentration [x] versus the absorbance [y] data of Glimipiride is a straight line

Y= mx + c

Slope (m) = 0.0393

Intercept (c) = 0.0108

Regression coefficient (r^2)=0.999

Correlation coefficient(r)= 0.9

Validation Criteria

The Correlation Coefficient should be not less than 0.999. Correlation coefficient (r) is 0.99. Regression Coefficient (r^2) is 0.99. These values meet the Validation Criteria. Hence these method is applicable.

Spectrophotometric results data for Accuracy study

Table 6: Observation table for accuracy

Concentration Taken[18ppm] 80%		Concentration Taken[20ppm] 100%		Concentration Taken [22ppm] 120%	
Absorbance	**Concentration Found [Absorbance-Intercept/Slope]**	**Absorbance**	**Concentration Found [Absorbance-Intercept/Slope]**	**Absorbance**	**Concentration Found [Absorbance-Intercept/Slope]**
0.7293	18.28	0.805	20.20	0.893	22.44
0.7284	18.25	0.815	20.46	0.891	22.39
0.7175	17.98	0.801	20.10	0.874	21.96

Table 7: Results of Accuracy for concentration 80%, 100%, 120%

Sample no	Recovery at about (in %)	% Recovery [concentration Found/concentration taken*100]	Mean% recovery	Standard Deviation	% Relative standard deviation
1	80	101.5	100.8	0.8	0.79
2	80	101			
3	80	99.9			
1	100	101	101.2	0.92	0.91
2	100	102.3			
3	100	100.5			
1	120	102	101.01	1.19	1.17
2	120	101.7			
3	120	99.8			

The Percentage recovery was found to be within the limits (98-102%). The results obtained for recovery at 80%, 100%.120% are within the limits. Hence method is accurate

Spectrophotometric results data for Precision study

Intra-day precision: [Day-1]

Table 8: Results of Method Precision (Day-1)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.3984	9.86	98.6
10 µg/ml	0.4103	10.16	101.6
10 µg/ml	0.3994	9.88	98.8
10 µg/ml	0.4005	9.91	99.1
10 µg/ml	0.3997	9.89	98.9
10 µg/ml	0.4012	9.95	99.5
Average	0.4	9.941	99.4
SD	0.004	0.11	1.11
%RSD	1	1	1

The % Relative Standard Deviation of % Assay of Glimepiride from the six sample preparations should be not more than 2.0%. The individual % Assay of Glimepiride should not be less than 98.0% and not more than 102.0%. The %RSD obtained is within the limits. Hence the method is accurate.

Inter-day precision: [Day-2]

Table 9: Results of Method Precision (Day-2)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.3991	9.88	98.8
10 µg/ml	0.3987	9.87	98.7
10 µg/ml	0.4052	10.03	100.3
10 µg/ml	0.4082	10.36	103.6
10 µg/ml	0.3998	9.89	98.9
10 µg/ml	0.4012	9.93	99.3
Average	0.40	9.9	99
SD	0.003833	0.18	1.8
%RSD	0.95	1.8	1.8

Spectrophotometric results data for Ruggedness study

Analyst 1:

Table 10: Results of Method Ruggedness (Analyst-1)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.3991	9.8	98
10 µg/ml	0.3957	9.7	97
10 µg/ml	0.4051	10.03	100.3
10 µg/ml	0.3982	9.8	98
10 µg/ml	0.3992	9.8	98
10 µg/ml	0.41	10.1	101
Average	0.40	9.87	98.7
SD	0.005	0.15	1.5
%RSD	1.25	1.5	1.5

The % Relative Standard Deviation of % Assay of Glimepiride from the six sample preparations should be not more than 2.0%. The individual % Assay of Glimepiride should not be less than 98.0% and not more than 102.0%. The %RSD obtained is within the limits. Hence the method is accurate

Analyst 2:

Table 11: Result of Method Ruggedness (Analyst-2)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.3997	9.89	98.9
10 µg/ml	0.3962	9.81	98.1
10 µg/ml	0.4041	10.00	100
10 µg/ml	0.4002	9.90	99
10 µg/ml	0.3989	9.87	98.7
10 µg/ml	0.3892	9.85	98.5
Average	0.398	9.88	98.8
SD	0.005	0.06	0.6
%RSD	1.25	0.6	0.6

The % Relative Standard Deviation of % Assay of Glimepiride from the six sample preparations should be not more than 2.0%. The individual % Assay of Glimepiride should not be less than 98.0% and not more than 102.0%. The %RSD obtained is within the limits .Hence the method is accurate

Spectrophotometric results data for LOD, LOQ study

Table 12: Absorbance values for calibration curve of Glimepiride at 233nm for LOD, LOQ Study

Name of the Drug	Actual concentration [µg/ml]	Absorbance
Glimepiride	0.1	0.012
	0.2	0.032
	0.3	0.053
	0.4	0.073
	0.5	0.091

SUMMERY

The objective of the proposed work was to develop some new and sensitive analytical methods for the determination of Glimepiride and to validate the methods according to ICH guidelines and applying the same for its estimation in pharmaceutical formulations.

In the start of the method development for this drug, different solvents were tested such as water, methanol, 0.1N NaOH, acetonitrile and Phosphate buffer (pH 7.4). Due to greater solubility and reproducible readings of maximum absorbance, 0.1N NaOH was taken under consideration for further work. The UV spectrum of Glimepiride was obtained by using 0.1N NaOH as a solvent and then validated. The λ_{max} was determined by scanning 10µg/mL solution of drug in 0.1N NaOH in the range of 200-400 nm. The wavelength 233 nm was selected because it showed maximum absorbance by the solvent 0.1N NaOH.

Calibration curve of drug were prepared in 0.1N NaOH at λ_{max} 233 nm. The absorbance values with their standard deviations at different concentration in the range of 5-25 µg/mL are given in table no.11. And calibration curve was prepared by plotting graph between absorbance and concentration (µg/ml)

The accuracy of the method was determined by recovery studies by adding known amount of the pure drug to the formulation. Thus, for accuracy, % recovery studies were carried out and the percentage recovery was found to be in the range of 100.8% - 101%, which was within the recommended limits, indicating that the method has required accuracy.

The precision of an analytical method or a test procedure is referred to as the degree of closeness of the result obtained by the analytical method or the test procedure to the true value. For evaluation of the precision, the %RSD was determined and the % assay was calculated. For intra-day precision, the %RSD for Absorbance was found to be 1 and % assay was found to be 99.4. For inter-day precision, the %RSD for Absorbance was found to be 0.95 and % assay was found to be 99. The results are given in Table no 14 & 15. It is suggested that the analytical method may be considered validated in terms of precision if the precision around the mean value does not exceed 2% RSD. The results obtained in intra-day precision and inter-day precision expresses the precision of the method.

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slots by using two different systems by different analysts and analyzed under similar operational and environmental conditions. The % RSD reported was found to be less than 2. The results were shown in the table 16 & 17, indicating that the method is rugged.

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under a stated experimental condition and the limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. These two parameters are required for assay validation as per ICH Q2A guidelines. Limit of detection and limit of quantitation of calibration curve were calculated which was based on the standard deviation of y-intercept of regression line (SD) and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3 (SD/S) and LOQ =10 (SD/S). LOD and LOQ of calibration curve of drug prepared in 0.1N NaOH were found to be 0.5and 1.5µg/ml, respectively.

Linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. Linearity data was prepared in 0.1 N NaOH. The extract was found to obey Beer's law in the concentration range of 5-25 µg/mL with correlation coefficient (r) values 0.99. The regression equations were calculated as y = 0.0393x + 0.0108. The results were shown in Figure 10 and table 4. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity, slope (b), intercept (C), correlation coefficient (r) obtained from different concentrations.

An efficient UV Spectrophotometric method and was developed and validated for estimation of Glimepiride in tablet dosage forms.

CONCLUSION

The method was developed using 0.1 N NaOH as solvent and the λ_{max} was found to be 233 nm. The method was validated with respect to linearity, precision, accuracy, sensitivity and ruggedness. The calibration plot for the method was constructed. The method was established according to ICH guideline and definition. Accuracy was investigated by analyzing marketed formulations and percentage recovery was found to be within the limits. Therefore it can be said that the method were highly accurate. The interday and intraday relative standard deviation (RSD) values with low percentage RSD values were obtained. This indicated that the precision of the method was found to be good. The proposed method based on UV spectrophotometer is precise, accurate, simple to perform and economy in practice. It do not require expensive or sophisticated and chemicals in contrast with Spectrophotometric method.

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