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Analytical Method Development and Validation for Estimation of Metformin in Bulk and Pharmaceutical Dosage Form by UV Spectrometric Method

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ABSTRACT: A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of metformin hydrochloride in bulk forms and tablet dosage formulations. The spectrophotometric detection was as per carried out at an absorption maximum of 232 nm using phosphate buffer of pH 6.8 as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. The detector response for was linear over the selected concentration range 2 to 12µg/ml with a correlation coefficient of 0.994. The accuracy was carried out as per recovery study and found between 99.1 % to 100.45%. The results demonstrated that the excipients in the tablets did not interfere with the method and can be conveniently employed for routine quality control analysis of metformin in bulk and formulation.

Keywords: UV Spectroscopy; Method Development; Validation; Metformin and ICH Guideline.

INTRODUCTION: A spectroscopy method is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 µm. Ultraviolet-Visible spectrophotometry[1] UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer - Lambert law. Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration[2-3].

Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law[4].

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur[5].

Mathematically, Beer Lambert law is expressed as:

$$A=a\ b\ c$$

Where; A=absorbance or optical density a=absorptivity or extinction coefficient b=path length of radiation through sample (cm) c=concentration of solute in solution. Both b and a are constant so a is directly proportional to the concentration c.

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

$$A= A.\ 1/1cm.bc$$

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λmax), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal. The assay of single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal procedures. They are, use of standard absorptivity value, calibration graph and single or double point standardization. In standard absorptive value method, the use of standard A (1%, 1 cm) or E values are used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. In calibration graph method, the absorbances of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. The single point standardization procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration[6-7].

$$Ctest = (Atest \times Cstd)/Astd$$

Where Ctest and Cstd are the concentrations in the sample and standard solutions respectively and Atest and Astd are the absorbances of the sample and standard solutions respectively. For assay of substance/s in multi component samples by spectrophotometer; the following methods are being used routinely, which includes

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method validation
- Validation is concerned with assuring that a measurement process produces valid measurements.
- Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice.
- A measurement process producing valid measurements for an intended application is fit for purpose. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated,
- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- Whenever the method is changed and the change is outside the original scope of the method. Nowadays, there are several international renowned organisations offering guidelines on method validation and related topics.
- American Society for Testing and Material (ASTM)
- Codex Committee on Methods of Analysis and Sampling (CCMAS)
- European Committee for Normalization (CEN)
- Cooperation on International Traceability in Analytical Chemistry (CITAC)
- European Cooperation for Accreditation (EA)
- Food and Agricultural Organization (FAO)
- United States Food and Drug Administration (FDA)
- International Conference on Harmonization (ICH). ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc. Types of Analytical Procedures to be validated The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures: - Identification tests; - Quantitative tests for impurities' content; - Limit tests for the control of impurities; - Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. The objective of the analytical procedure should be clearly understood

since this will govern the validation characteristics which need to be evaluated[8-10].

Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Furthermore revalidation may be necessary in the following circumstances: -Changes in the synthesis of the drug substance; - Changes in the composition of the finished product; - Changes in the analytical procedure. Aim of Present Work This work deals with the validation of the developed method for the assay of metformin from its dosage form (tablets) using phosphate buffer of pH 6.8. Hence, the method can be used for routine quality control analysis and also stability. The aim and scope of the proposed work are as under:

- To develop suitable spectrophotometric method for assay of metformin tablet.
- Perform the validation for the method.[11]

Metformin is 1, 1-Dimethylbiguanide. It is official in IP, USP & BP. Metformin is a biguanide hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus not responding to dietary modification. Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5$.HCl and a molecular weight of 165.63. Metformin Hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. Metformin Hydrochloride used in the management of type 2 diabetes. Metformin Hydrochloride improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose.[12-14]

MATERIALS AND METHODS:

Instruments: The analysis was performed by using the analytical balance (Mettler), pH meter (Cyber scan), UV spectrophotometer (UV-Lambda 25, Perkin Elmer equipped with variable wavelength detector and data integration software).

Reagents and solutions: Metformin hydrochloride, potassium dihydrogen phosphate, sodium hydroxide analytical grade were used in entire research work.

Preparation of solvent system:

Potassium dihydrogen phosphate (KH_2PO_4): 6.8 gm of dipotassium hydrogen phosphate was weighed accurately and transferred into a 1000 ml volumetric flask containing 900 ml of water and mixed well till clear solution obtained. pH of solution was adjusted up to 6.8 by using Sodium hydroxide. Finally volume make up to 1000ml with water.

Standard stock solution of metformin: 100 mg of metformin weighted accurately and transferred into a 100 ml volumetric flask containing 60 ml water. Solution sonicated to dissolve metformin and cooled at room temperature then volume make up with water and mix well (Stock solution-1). Pipette 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml of standard stock solution, into a 10 ml volumetric flask, mix and diluted to volume with buffer solution. This solution contains 0.1 mg/ml of metformin.

Sample Stock Solution: Average weight of the tablets was determined and fine powder made with the help of mortar and pestle. Transferred accurately equivalent to one tablet weight into a 500 ml volumetric flask containing 250 ml water and sonicated till clear solution, finally cooled at room temperature. Final volume made with water and mixed well. Prepared solution then centrifuge at 3500 rpm for 5 minutes and used as standard test solution (Stock solution-2). Pipette 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml of standard stock solution, into a 10 ml volumetric flask, mix and diluted to volume with buffer solution. This solution contains 0.1 mg/ml of metformin.

Spectral study: The final stock solution scanned in UV spectrophotometer over the range 200-400nm (Figure 1).

RESULTS AND DISCUSSION: The methods discuss in the present work provide a convenient, precise and accurate way for estimation of metformin hydrocholride in bulk and pharmaceutical dosage form using phosphate buffer of pH 6.8. The absorption maximum of metformin was selected at 232nm for the analysis. Regression analysis shows linearity over the concentration range of 2-12µg/ml with correlation coefficient 0.994 (Figure 2).

The % RSD for repeatability (n=6) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as percentage recovery. Percentage recovery for was found within the range between 99.1 % to 100.45%. The % RSD value for was found to be less

than 2%. In this study estimation of metformin was carried out by UV spectroscopy method and all the validation parameters found satisfactorily. The result of developed method and validation was given in table 1.

Table 1: Result of method development and validation.

Sr. No.	Parameters	Observations
01.	SPECIFICITY (Interference of peaks)	No interference observed
02.	PRECISION 1. Precision of system (%RSD) 2. Precision of Method (%RSD)	 0.01% 1.21%
03.	INTERMEDIATE PRECISION 1. Precision of system (%RSD) 2. Precision of Method (%RSD)	 0.05%. 1.32%
04.	LINEARITY (Correlation coefficient)	0.994
05.	ACCURACY (% Recovery)	99.1%- 100.45%
06.	RUGGEDNESS (%RSD)	1.22%
07.	ROBUSTNESS	Complies all deliberated changes.

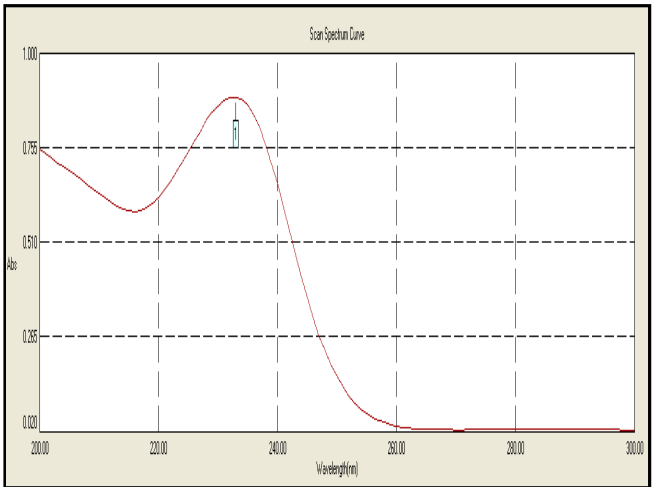

Figure 1: UV Spectra of Metformin.

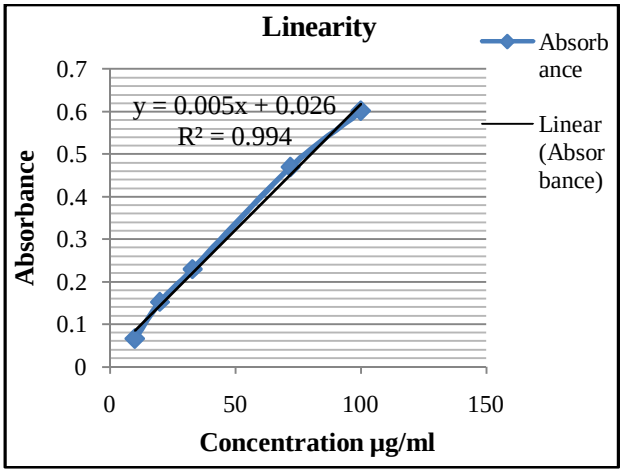

Figure 2: Calibration curve of metformin.

CONCLUSION: The analytical method for estimation of metformin has been developed and validated according to validation protocol of ICH guidelines. All parameters mentioned in the protocol were tested and they fulfilled the requirement of ICH analytical method validation for the drug. The results obtained are well within the set limit; indicates that the described analytical method is suitable for estimation of metformin in bulk as well as tablet formulation.

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