



Sudan University of Science and Technology

Collage of Graduate Studies

**DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC
METHOD FOR ESTIMATION OF GLIBENCLAMIDE**

تطوير واعتماد طريقة مطيافية لتقدير الجلايبينكلاميد

A Thesis Submitted For The Partial Fulfillment Of The
Requirements Of MSc In Chemistry

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DEDICATION

To:

My parents

My Family

My friends

Acknowledgements:

All praise is raised to Allah the merciful and the helper who gave me the strength and determination to complete this work.

I would like to seize this opportunity to express my deep gratitude to Dr .AlFateh Ahmed Hassan who been the ideal thesis supervisor for helpful advice.

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I also feel very much obliged to the assistance, support or advice of many persons without which this work could not have been completed.

ABSTRACT

A simple, selective, linear, precise and accurate UV-VIS method was developed and validated for rapid assay of glibenclamide in pharmaceutical dosage forms.

The validation parameter such as (linearity, precision, accuracy detection limit, quantitation limit) was validated according to the international conference of Harmonization.

In the linearity the regression equation found to be $Y = 0.0575X$ with a correlation coefficient $R^2 = 0.9996$

Statistical data reveals the method is accurate, selective and precise for the quantitation of Glibenclamide.

The method was successfully applied for the routine analysis of Glibenclamide in tablet dosage.

المستخلص :

طريقه مطيافيه بسيطه ، انتقائيه ، خطيه ودقيقه طورت واعتمدت لتقدير الجلايبيينكلاميد في الاشكال الصيدلانيه

المتغيرات المستخدمه للتأكد من صحة الطريقه المستخدمه في تقدير الجلايبيينكلاميد مثل (الخطية ، الدقه ، تم اعتمادها والتحقق من صحتها وفقا لتوصيات المؤتمر الدولي للتنسيق

في الخطية وجدت معادلة الانحدار $Y = 0.0575X$

ومعامل الانحدار $R^2 = 0.9996$

البيانات الاحصائيه اثبتت دقة وانتقائية وحساسية الطريقه لتقدير الجلايبيينكلاميد .

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List of abbreviation

Abbreviation	Full form
ICH	International Conference on Harmonization
API	Active pharmaceutical ingredient
CFR	Code of Federal Regulations
cGMP	Current good manufacturing practices
AQC	analytical quality control
IND	Investigational New Drug
NDA	New Drug Application
MAA	Marketing Authorization Application
FDA	Food and drug administration
USP	United states pharmacopeia
IUPAC	International Union of Pure and Applied Chemistry
DL	Detection limit
QL	Quantitation limit
ppm	parts per million
ppb	parts per billion
VMP	Validation Master Plan
QC	Quality control

Chapter one

Introduction

1. INTRODUCTION

1.1 Definitions of Analytic Method Development and Validation :

1.1.1 Analytic method development, validation, and transfer are key elements of any pharmaceutical development program. This technical brief will focus on development and validation activities as applied to drug products. Often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency.

These method-related activities are interrelated. They are iterative, particularly during early drug development phases. Parts of each process may occur concurrently or be refined at various phases of drug development. Changes encountered during drug development may require modifications to existing analytic methods. These modifications to the methods, in turn, may require additional validation or transfer activities, as shown below (Figure 1).

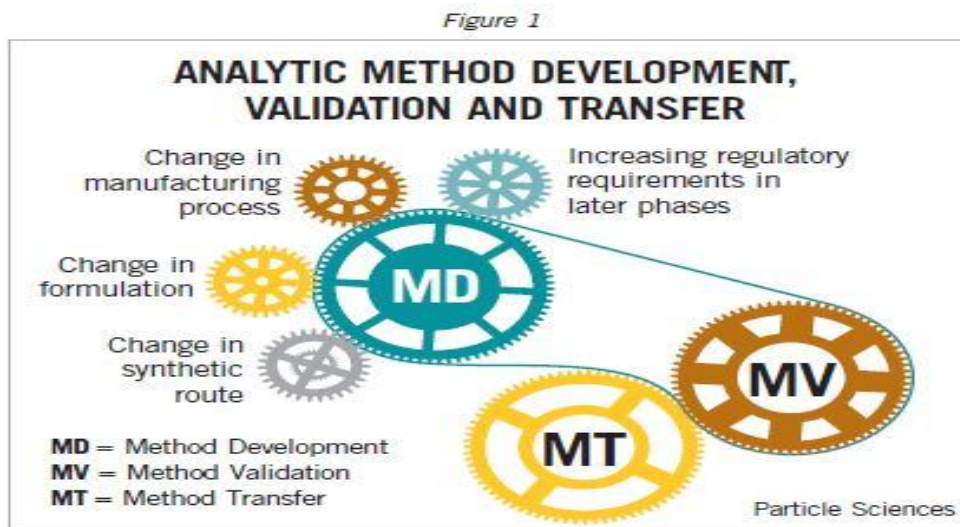


Figure 1: Analytical method development validation and transfer

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the “process of

demonstrating that analytical procedures are suitable for their intended use”¹. Method transfer is the formal process of assessing the suitability of methods in another laboratory. Each of these processes contributes to continual improvement of the methods and results in more efficient drug development.

Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. According to the International Conference on Harmonization (ICH), the most common types of analytic procedures are: (i) identification tests, (ii) quantitative tests of the active moiety in samples of API or drug product or other selected component(s) in the drug product, (iii) quantitative tests for impurities’ content, (iv) limits tests for the control of impurities.

1.1.2 Method development (Figure 2) is a continuous process that progresses in parallel with the evolution of the drug product. The notion of phase-appropriate method development is a critical one if time, cost, and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behavior. They should be suitable to support pre-clinical safety evaluations, [pre-formulation studies](#), and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased active pharmaceutical ingredient (API) and drug product knowledge. The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines.

Figure 2

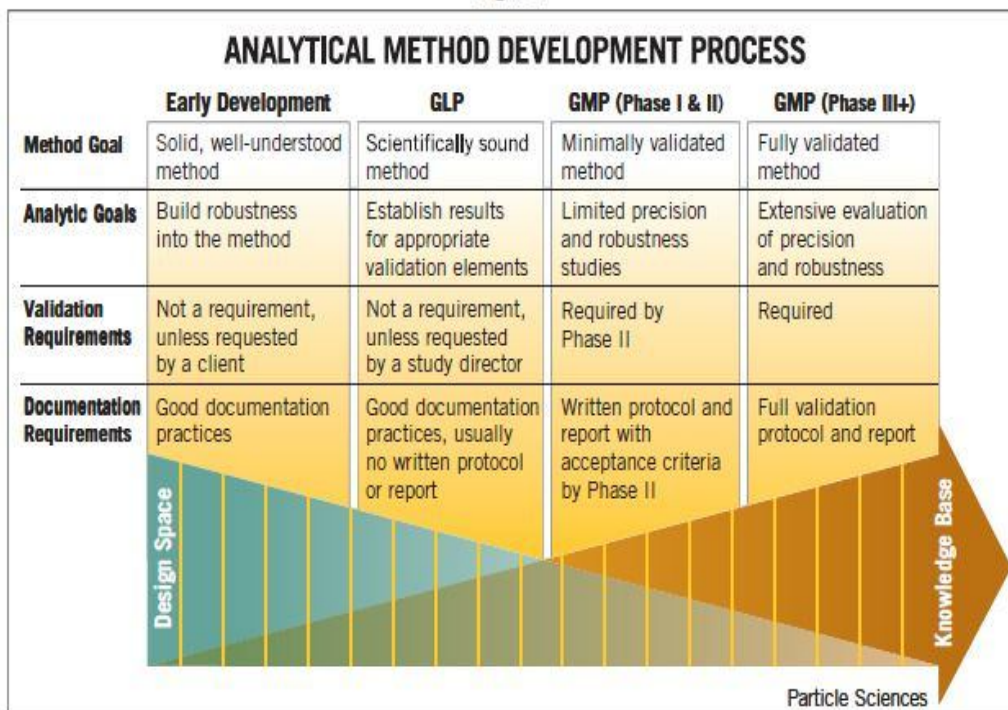


Figure 2: Analytical method development process

Scouting experiments are frequently performed during method development to establish the performance limits of the method, prior to formal validation experiments. These may include forced degradation studies, which are an integral part of development of a stability-indicating method. API is typically subjected to degradation by acid, base, peroxide, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products, while providing insight into the main mechanisms of degradation. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients.

Additional experiments help to define the system suitability criteria that will be applied to future analytic sample sets. System suitability tests are a set of routine checks to assess the functionalities of the instrument, software, reagents, and analysts as a system³. Final method system

suitability parameters may be determined from evaluations of method robustness performed under statistical design of experiments. The goal is to identify the critical parameters and to establish acceptance criteria for method system suitability.

1.1.3 Validation is defined as ‘finding or testing truth of something ‘. When analytical methods are used to generate results about the characteristics of drug related samples it is vital that the results are trustworthy: they may be used as the basis for decisions relating to administering the drug to patients, a validation study is performed on an analytical method to ensure that the results are always obtained .

1.1.4 Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the Pharmaceutical Manufacturing Handbook: Regulations and Quality, edited by Shayne Cox Gad

1.2 Why validate analytical procedures :

There are many reasons for the need to validate analytical procedures. Among them Are regulatory requirements, good science, and quality control requirements. The Code of Federal Regulations (CFR) 311.165c explicitly states that “the accuracy, Sensitivity, specificity, and reproducibility of test methods employed by the firm shall Be established and documented” of course, as scientists, we would want to apply Good science to demonstrate that the analytical method used had demonstrated Accuracy, sensitivity, specificity, and reproducibility. Finally management of the quality control unit would definitely want to ensure that the analytical methods that the department uses to release its products are properly validated for its intended Use so the product will be safe for human use.

1.3 Analytical method validation requirements for its intended use:

All analytical methods that are intended to be used for analyzing any clinical samples will need to be validated. Validation of analytical methods is an essential but time - consuming activity for most analytical

development laboratories. It is therefore important to understand the requirements of method validation in more detail and the options that are available to allow for optimal utilization of analytical resources in a development laboratory.

1.4 Current good manufacturing practices in twenty - first century

The overarching philosophy in current good manufacturing practices (cGMPs) of the twenty - first century and in robust modern quality systems is quality should be built into the product, and testing alone cannot be relied on to ensure product quality. From the analytical perspective, this will mean that analytical methods used to test these products should have quality attributes built into them. To have quality attributes built into the analytical method will require that fundamental quality attributes be applied by the bench - level scientist. This is a paradigm shift that requires the bench - level scientist to have the scientific and technical understanding, product knowledge, process knowledge, and/or risk assessment abilities to appropriately execute the quality functions of analytical method validation. It will require (1) the appropriate training of the bench - level scientist to understand the principals involved with method validation and able to validate an analytical method and understand the principals involved with the method validation, (2) proper documentation and understanding and interpreting data, and (3) cross - functional understanding of the effect of their activities on the product and the customer (the patient). It is the responsibility of management to verify that skills gained from the training are implemented in day - to - day performance.

1.5 Strategy for the validation of methods :

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. Possible steps for a complete method validation are listed in Table 1. This proposed procedure assumes that the instrument has been selected

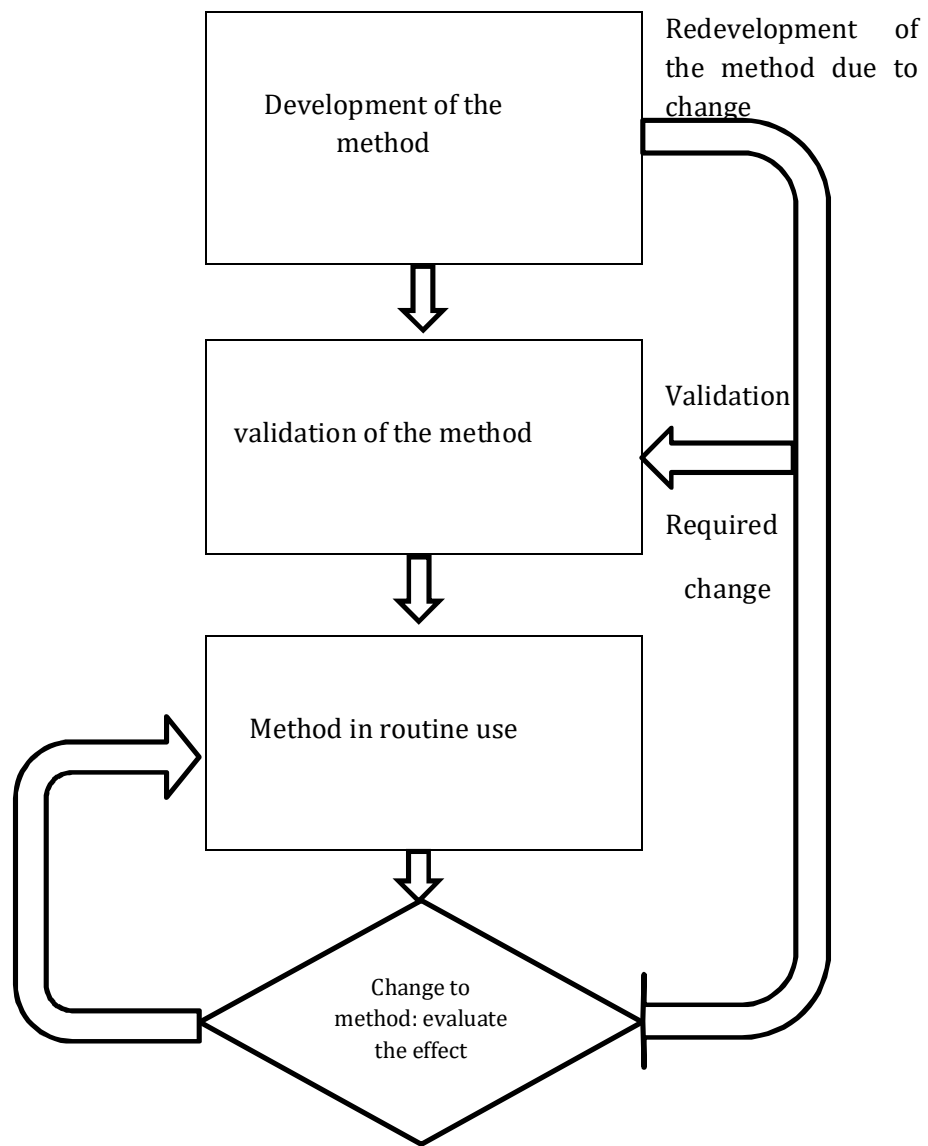
and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughput; turnaround time; and environmental, health and safety requirements.

Develop a validation protocol, an operating procedure or a validation master plan for the validation

1. For a specific validation project define owners and responsibilities.
2. Develop a validation project plan
3. Define the application, purpose and scope of the method
4. Define the performance parameters and acceptance criteria
5. Define validation experiments
6. Verify relevant performance characteristics of equipment
7. Qualify materials, e.g. standards and reagents for purity, accurate amounts and sufficient stability
8. Perform pre-validation experiments
9. Adjust method parameters or/and acceptance criteria if necessary
10. Perform full internal (and external) validation experiments
11. Develop SOPs for executing the method in the routine
12. Define criteria for revalidation
13. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine
14. Document validation experiments and results in the validation report

1.6 Cycle of analytical methods

The analytical method validation activity is not a one - time study. This is illustrated and summarized in the life cycle of an analytical procedure in Figure 3 .



Change is covered by existing validation

Change is not covered by existing validation

Figure 3: Cycle of analytical methods

1.7 Analytical method validation characteristics :

Method will be developed and validated for use to analyze samples during the early development of an active pharmaceutical ingredient (API) or drug product. As drug development progresses from phase 1 to commercialization, the analytical method will follow a similar progression. The final method will be validated for its intended use for the market image drug product and transferred to the quality control laboratory for the launch of the drug product. However, if there are any changes in the manufacturing process that have the potential to change the analytical profile of the drug substance and drug product; this validated method may need to be revalidated to ensure that it is still suitable to analyze the API or drug product for its intended purpose.

Once an analytical method is developed for its intended use, it must be validated. The extent of validation evolves with the drug development phase. Usually, a limited validation is carried out to support an Investigational New Drug (IND) application and a more extensive validation for New Drug Application (NDA) and Marketing Authorization Application (MAA). Typical parameters recommended by FDA, USP, and ICH are as follow:

1.7.1 Linearity:

ICH defines linearity of an analytical procedure as the ability (within a given range) to obtain test results of variable data (e.g., absorbance and area under the curve) which are directly proportional to the concentration (amount of analyte) in the sample. The data variables that can be used for quantitation of the analyte are the peak areas, peak heights, or the ratio of peak areas (heights) of analyte to the internal standard peak. Quantitation of the analyte depends on it obeying Beer's law for the spectroscopic method over a concentration range. Therefore, the working sample concentration and samples tested for accuracy should be in the linear range. There are two general approaches for determining the linearity of the method. The first approach is to weigh different amounts of standard

directly to prepare linearity solutions at different concentrations. However, it is not suitable to prepare solution at very low concentration, as the weighing error will be relatively high. Another approach is to prepare a stock solution of high concentration. Linearity is then demonstrated directly by dilution of the standard stock solution. This is more popular and the recommended approach. Linearity is best evaluated by visual inspection of a plot of the signals as a function of analyte concentration. Subsequently, the variable data are generally used to calculate a regression line by the least - squares method. At least five concentration levels should be used. Under normal circumstances, linearity is acceptable with a coefficient of determination (r^2) of ≥ 0.997 . The slope, residual sum of squares, and y intercept should also be reported as required by ICH. The slope of the regression line will provide an idea of the sensitivity of the regression, and hence the method that is being validated. The intercept will provide an estimate of the variability of the method. For example, the ratios percent of they intercept with the variable data at nominal concentration are sometimes used to estimate the method variability. For the determination of potency assay of a drug substance or a drug product, the usual range of linearity should be $\pm 20\%$ of the target or nominal concentration. For the determination of content uniformity, it should be $\pm 30\%$ of the target or nominal concentration.

1.7.2 Precision :

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions. Precision is usually investigated at three levels: repeatability, intermediate precision, and reproducibility. For simple formulation it is important that precision be determined using authentic homogeneous samples. A justification will be required if a homogeneous sample is not possible and artificially prepared samples or sample solutions are used.

Intermediate precision is defined as the variation within the same laboratory. The extent to which intermediate precision needs to be

established depends on the circumstances under which the procedure is intended to be used. Typical parameters that are investigated include day - to - day variation, analyst variation, and equipment variation. Depending on the extent of the study, the use of experimental design is encouraged. Experimental design will minimize the number of experiments that need to be performed. It is important to note that ICH allows exemption from doing intermediate precision when reproducibility is proven. It is expected that the intermediate precision should show variability that is in the same range or less than repeatability variation. ICH recommends the reporting of standard deviation, relative standard deviation (coefficient of variation), and confidence interval of the data

1.7.3 Accuracy

The International Convention on Harmonization (ICH) defines the accuracy of an analytical procedure as the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value and the value found. For drug substance, accuracy may be defined by the application of the analytical procedure to an analyte of known purity (e.g., a reference standard). For the drug product, accuracy will be determined by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure. The ICH document also recommends assessing a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations/three replicates).

Accuracy is usually reported as percent recovery by the assay (using the proposed analytical procedure) of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. The range for the accuracy limit should be within the linear range.

Typical accuracy of the recovery of the drug substance is expected to be about 99 – 101%. Typical accuracy of the recovery of the drug product is

expected to be about 98 – 102%. Values of accuracy of recovery data beyond this range need to be investigated as appropriate.

1.7.4 Repeatability

Repeatability is a measure of the precision under the same operating conditions over a short interval of time, that is, under normal operating conditions of the analytical method with the same equipment. It is sometimes referred to as intra - assay precision. The ICH recommends that repeatability be assessed using a minimum of nine determinations covering the specified range for the procedure (e.g., three concentrations/three replicates as in the accuracy experiment) or using a minimum of six determinations at 100% of the test concentration. Reporting of the standard deviation, relative standard deviation (coefficient of variation), and confidence interval is required. The assay values are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result. Table 1 provides an example set of repeatability data.

1.7.5 Reproducibility

Reproducibility measures the precision between laboratories. This parameter is considered in the standardization of an analytical procedure (e.g., inclusion of procedures in pharmacopeias and method transfer between different laboratories). To validate this characteristic, similar studies need to be performed at different laboratories using the same homogeneous sample lot and the same experimental design. In the case of method transfer between two laboratories, different approaches may be taken to achieve the successful transfer of the procedure. The most common approach is the direct - method transfer from the originating laboratory to the receiving laboratory. The originating laboratory is defined as the laboratory that has developed and validated the analytical method or a laboratory that has previously been certified to perform the procedure and will participate in the method transfer studies. The receiving laboratory is defined as the laboratory to which the analytical

procedure will be transferred and that will participate in the method transfer studies. In the direct - method transfer, it is recommended that a protocol be initiated with details of the experiments to be performed and acceptance criteria (in terms of the difference between the means of the two laboratories) for passing the method transfer. Table 2 provides examples of a set of method transfer data between two laboratories.

1.7.6 Selectivity and specificity

The ICH defines specificity as the ability to assess unequivocally an analyte in the presence of components that may be expected to be present. In many publications,

are often used interchangeably. However, there are debates over the use of specificity over selectivity and some authorities, for example, the International Union of Pure and Applied Chemistry (IUPAC), have preferred the term selectivity, reserving specificity for those procedures that are completely selective. For pharmaceutical application, the above definition of ICH will be used. For identity test, compounds of closely related structures which are likely to be present should be discriminated from each other. This could be confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. Furthermore, the identification test may be applied to material structurally similar or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sound scientific judgment with a consideration of the interferences that could occur. The specificity for an assay and impurity tests should be approached from two angles:

- 1. When Impurities Are Available** The specificity of an assay method is determined by comparing test results from an analysis of sample containing the impurities, degradation products, or placebo ingredients with those obtained from an analysis of samples without the impurities, degradation products, or placebo ingredients. For a stability - indicating assay method, degradation peaks need to be resolved from the drug substance. However, these impurities do not need to be resolved from each other.

For the impurity test, the determination should be established by spiking drug substance or drug product with the appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Representative chromatograms should be used.

2. If Impurities Are Not Available. Specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well - characterized procedure or other validated analytical procedure (orthogonal method). This should include samples stored under relevant stress conditions (light, heat, humidity, acid/base hydrolysis and oxidation). For the assay method, the two results should be compared; for impurity tests, the impurity profiles should be compared. Peak homogeneity tests should be performed using PDA or mass spectrometry to show that the analyte chromatographic peak is not attributable to more than one component. Figure 2 illustrates the selectivity of a method to resolve known degradation peaks from the parent peak.

1.7.7 Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical procedure. For the assay of drug substance or finished drug product, it is normally recommended to have a range of 80 – 120% of the nominal concentration.

For content uniformity, a normal range would cover 70 – 130% of the nominal concentration, unless a wider and more appropriate range (e.g., metered - dose inhalers) is justified.

For dissolution testing, a normal range is $\pm 20\%$ over the specified range. If the acceptance criterion for a controlled - release product covers a region from 20% after Peak area 1 h, and up to 90% after 24 h, the validated range would be 0 – 110% of the label claim. In this case, the lowest appropriate quantifiable concentration of analyte will be used as the lowest limit as 0% is not appropriate.

1.7.8 Detection limit

The detection limit (DL) is a characteristic for the limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection is usually expressed as the concentration of the analyte in the sample, for example, percentage, parts per million (ppm), or parts per billion (ppb). There are several approaches to establish the DL. Visual evaluation may be used for no instrumental (e.g., solution color) and instrumental methods. In this case, the DL is determined by the analysis of a series of samples with known concentrations and establishing the minimum level at which the analyte can be reliably detected. Presentation of relevant chromatograms or other relevant data is sufficient for justification of the DL. For instrumental procedures that exhibit background noise, it is common to compare measured signals from samples with known low concentrations of analyte with those of the blank samples. The minimum concentration at which the analyte can reliably be detected is established using an acceptable signal - to - noise ratio of 2: 1 or 3: 1. Presentation of relevant chromatograms is sufficient for justification of the DL.

Another approach estimates the DL from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the y intercepts of the regression lines done in the range of the DL. This estimate will need to be

subsequently validated by the independent analysis of a suitable number of samples near or at the DL:

$$DL = 3\sigma$$

Where σ is the standard deviation of the response.

1.7.9 Quantitation limit

The quantitation Limit (QL) is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. QL is defined as the concentration of related substance in the sample that will give a signal - to - noise ratio of 10 : 1. The QL of a method is affected by both the detector sensitivity and the accuracy of sample preparation at the low concentration of the impurities. In practice, QL should be lower than the corresponding ICH report limit. International conference of harmonization recommends three approaches to the estimation of QL. The first approach is to evaluate it by visual evaluation and may be used for no instrumental methods and instrumental methods. QL is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantitated with acceptable accuracy and precision. The second approach determines the signal - to - noise ratio by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. QL is the minimum concentration at which the analyte can be reliably quantified at the signal - to - noise ratio of 10 : 1. The third approach estimates QL by the equation

$$QL = 10\sigma / S$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The value of σ may be estimated by (1) calculating the standard deviation of the responses obtained from the measurement of the analytical background response of an appropriate number of blank samples or (2) calculating the residual standard deviation of the regression line from the calibration curve using samples containing the analyte in the range of the QL. Whatever approach is applied, the QL should be subsequently validated by the analysis of a suitable number of samples prepared at the QL and determining the precision and accuracy at this level.

1.7.10 Robustness/ruggedness

The definition for applied is "The robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained result(s), and a robustness test as an experimental set-up to evaluate the robustness of a method. The term ruggedness is frequently used as a synonym several definitions for robustness or ruggedness exist which is, however, all closely related. The one nowadays most widely applied in the pharmaceutical world is the one given by the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) and which was given above. Only in Ref. a distinction between the terms ruggedness and robustness is made and ruggedness is defined there as the degree of reproducibility of the test results obtained under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times,

different assay temperatures, different days, etc. The latter definition will not be applied since detailed guidelines exist for the estimation of the reproducibility and the intermediate precision. The international conference of harmonization (ICH) guidelines also recommends that "one consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution tests) is established to ensure that the validity of the analytical procedure is maintained whenever used". The assessment of the robustness of a method is not required yet by the ICH guidelines, but it can be expected that in the near future it will become obligatory. Robustness testing is nowadays best known and most widely applied in the pharmaceutical world because of the strict regulations in that domain set by regulatory authorities which require extensively validated methods. Therefore most definitions and existing methodologies, e.g. those from the ICH, can be found in that field, as one can observe from the above. However, this has no implications for robustness testing of analytical methods in other domains and this guideline is therefore not restricted to pharmaceutical methods.

Robustness tests were originally introduced to avoid problems in inter laboratory studies and to identify the potentially responsible factors. This means that a robustness test was performed at a late stage in the method validation since inter laboratory studies are performed in the final stage. Thus the robustness test was considered a part of method validation related to the precision (reproducibility) determination of the method. However, performing a robustness test late in the validation procedure involves the risk that when a method is found not to be robust, it should be redeveloped and optimized. At this stage much effort and money have already been spent in the optimization and validation, and therefore one wants to avoid this. Therefore the performance of a robustness test has been shifting to earlier points of time in the life of the method. The Dutch Pharmacists Guidelines, the ICH Guidelines as well as some authors working in bio-analysis consider robustness a method validation topic performed during the development and optimization phase of a method, while others consider it as belonging to the development of the analytical procedure.

The robustness test can be viewed as a part of method validation that is performed at the end of method development or at the beginning of the validation procedure.

The exact position has relatively little influence on how it is performed.

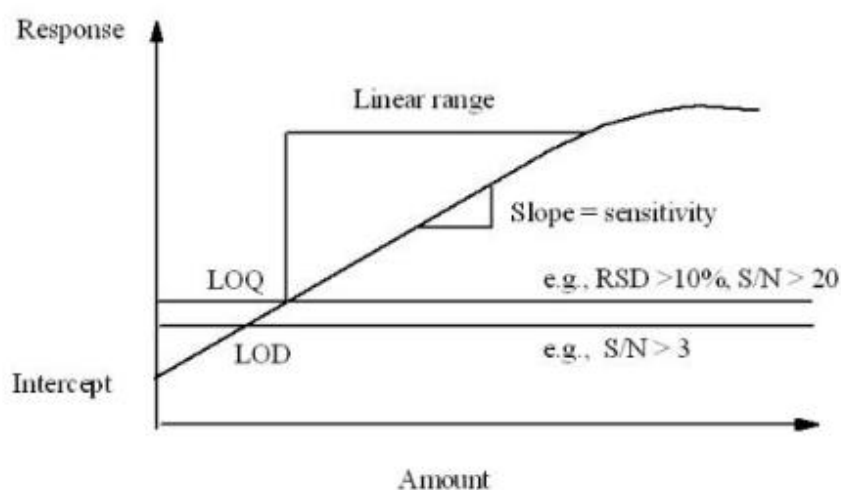


Figure4: Definitions for linearity, range, LOQ, LOD

1.8 Process of analytical method validation :

The typical process that is followed in an analytical method validation is chronologically listed below:

1. Planning and deciding on the method validation experiments
2. Writing and approval of method validation protocol
3. Execution of the method validation protocol
4. Analysis of the method validation data
5. Reporting the analytical method validation

6. Finalizing the analytical method procedure

The method validation experiments should be well planned and laid out to ensure efficient use of time and resources during execution of the method validation. The best way to ensure a well - planned validation study is to write a method validation protocol that will be reviewed and signed by the appropriate person (e.g., laboratory management and quality assurance). The validation parameters that will be evaluated will depend on the type of method to be validated. Analytical methods that are commonly validated can be classified into three main categories: identification, testing for impurities, and assay.

Execution of the method validation protocol should be carefully planned to optimize the resources and time required to complete the full validation study. For example, in the validation of an assay method, linearity and accuracy may be validated at the same time as both experiments can use the same standard solutions. A normal validation protocol should contain the following contents at a minimum:

- (a) Objective of the protocol
- (b) Validation parameters that will be evaluated
- (c) Acceptance criteria for all the validation parameters evaluated
- (d) Details of the experiments to be performed
- (e) Draft analytical procedure

The data from the method validation data should be analyzed as the data are obtained and processed to ensure a smooth flow of information. If an experimental error is detected, it should be resolved as soon as possible to reduce any impact it may have on later experiments. Analysis of the data includes visual examination of the numerical values of the data and chromatograms followed by statistical treatment of the data if required.

Upon completion of all the experiments, all the data will be compiled into a detailed validation report that will conclude the success or failure of the validation exercise. Depending on the company's strategy a summary of

the validation data may also be generated. Successful execution of the validation will lead to a final analytical procedure that can be used by the laboratory to support future analytical work for the drug substance or drug product.

1.9 Information required in analytical procedure :

The minimal information that should be included in a final analytical procedure is as follows:

- (a) Rationale of the analytical procedure and description of the capability of the method. Revision of analytical procedure should include the advantages offered by the new revision.
- (b) Proposed analytical procedure. This section should contain a complete description of the analytical procedure in sufficient detail to enable another analytical scientist to replicate it. The write - up should include all important operational parameters and specific instructions, for example, preparation of reagents, system suitability tests, precautions, and explicit formulas for calculation of the test results.
- (c) List of permitted impurities and its levels in an impurity assay.
- (d) Validation data. Either a detailed set or summary set of validation data is included

1.10 Type of analytical procedure :

Note: - characteristic not normally evaluated +characteristic normally evaluated.

- (a) In cases where reproducibility has been performed, intermediate precision is not needed.
- (b) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).

- (c) May be needed In some cases.
- (e) Revision history.
- (f) Signature of author, reviewer, management, and quality assurance.

1.11 Method verification :

The U.S. Food and Drug Administration (FDA) regulation 21 CFR 211.194(a)(2) specifically states that users of analytical methods in the U.S. Pharmacopeia/National Formulary (USP/NF) are not required to validate the accuracy and reliability of these methods but merely verify their suitability under actual conditions of use.

USP has issued guidance for verification in general chapter < 1226 >. This proposal provides general information to laboratories on the verification of compendial procedures that are being performed for the first time to yield acceptable results utilizing the laboratories' personnel, equipment, and reagents.

Verification consists of assessing selected analytical validation characteristics described earlier to generate appropriate, relevant data rather than repeating the validation process for commercial products. The guidance in this general chapter is applicable to applications such as titrations, chromatographic procedures (related compounds, assay, and limit tests), and spectroscopic tests. However, general tests (e.g., water, heavy metals, residue on ignition) do not typically require Verification.

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1.12 Method revalidation :

There are various circumstances under which a method needs to be revalidated. Some of the common situations are described below:

1. During the optimization of the drug substance synthetic process, significant changes were introduced into the process. To ensure that the analytical method will still be able to analyze the potentially different profile of the API, revalidation may be necessary.

2. If a new impurity is found that makes the method deficient in its specificity, this method will need to be modified or redeveloped and revalidated to ensure that it will be able to perform its intended function.
3. A change in the excipient composition may change the product impurity profile. This change may make the method deficient in its specificity for the assay or impurity tests and may require redevelopment and revalidation.
4. Changes in equipment or suppliers of critical supplies of the API or final drug product will have the potential to change their degradation profile and may require the method to be redeveloped and revalidated.

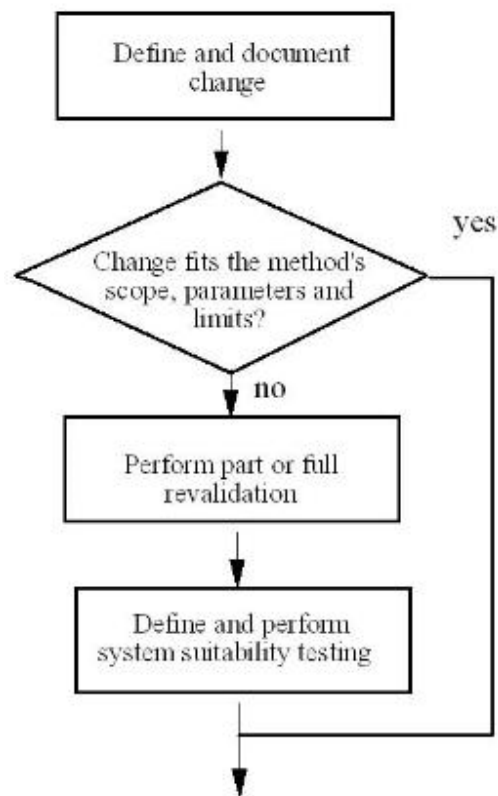


Figure 5: Flow diagrams for revalidation

1.13 Steps in method validation

Successful acceptance of the validation parameters and performance criteria, by all parties involved, requires the cooperative efforts of several departments, including analytical development, QC, regulatory affairs and the individuals requiring the analytical data. The operating procedure or the Validation Master Plan (VMP) should clearly define the roles and responsibilities of each department involved in the validation of analytical methods.

The scope of the method and its validation criteria should be defined early in the process. These include the following questions:

What analytes should be detected?

What are the expected concentration levels?

What are the sample matrices?

Are there interfering substances expected, and, if so, should they be detected and quantified?

Are there any specific legislative or regulatory requirements?

Should information be qualitative or quantitative?

What are the required detection and quantitation limits?

What is the expected concentration range?

What precision and accuracy is expected?

How robust should the method be?

Which type of equipment should be used? Is the method for one specific instrument, or should it be used by all instruments of the same type?

Will the method be used in one specific laboratory or should it be applicable in all laboratories at one side or around the globe?

What skills do the anticipated users of the method have?

The method's performance characteristics should be based on the intended use of the method. It is not always necessary to validate all analytical parameters that are available for a specific technique. For example, if the method is to be used for qualitative trace level analysis, there is no need to test and validate the method's limit of quantitation, or the linearity, over the full dynamic range of the equipment. Initial parameters should be chosen according to the analyst's experience and best judgment. Final parameters should be agreed between the lab or analytical chemist performing the validation and the lab or individual applying the method and users of the data to be generated by the method. Table 2 gives examples of which parameters might be tested for a particular analysis task.

The scope of the method should also include the different types of equipment and the locations where the method will be run. For example, if the method is to be run on a specific instrument in a specific laboratory, there is no need to use instruments from other vendors or to include other laboratories in the validation experiments. In this way, the experiments can be limited to what is really necessary.

	Major compounds	Major compounds and traces	Traces	Traces
	quantitative	quantitative	qualitative	qualitative
limit of detection	no	no	Yes	no
limit of quantitation	no	yes	No	yes
linearity	yes	yes	No	yes
range	yes	yes	No	no
precision	yes	yes	No	yes
accuracy	yes	yes	No	yes
specificity	yes	yes	Yes	yes

Table 1: Validation parameters for specific task

1.14 Introduction to spectroscopic methods :

It 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. The regions of electromagnetic spectrum are shown in table 2. Ultraviolet-Visible spectrophotometry 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 quantitative spectrophotometric analysis is Beer -Lambert law.

1.14.1 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.

1.14.2 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.

Region	Wavelength
Far (or vacuum)ultraviolet	10-200 nm
Near ultraviolet	200-400 nm
Visible	400-750 nm
Near infrared	0.75- 2.2 μm
Mid infrared	2.5-50 μm
Far infrared	50-1000 μm

Table 2: Regions of electromagnetic spectrum.

1.14.3 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. 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)

$$A = A_{1\%}^{1\text{cm}} bc$$

Quantification of medicinal substance using spectrophotometer may be carried out by preparing solution in transparent solvent and measuring its absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λ_{max}), 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 absorptivity 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 absorbance's 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.

$$C_{\text{test}} = (A_{\text{test}} \times C_{\text{std}}) / A_{\text{std}}$$

Where C_{test} and C_{std} are the concentrations in the sample and Standard solutions respectively and A_{test} and A_{std} are the absorbance's 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 [4],

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method

Dilution solvent should be as similar to mobile phase as possible.

1.15 Introduction to glibenclamide:

1.15.1 **Glibenclamide** also known as **glyburide** is an **antidiuretic drug** in a class of medications known as **sulfonylureas**, closely related to **sulfonamide antibiotics**. It was developed in 1966 in a cooperative study between **Boehringer Mannheim** (now part of **Roche**) and **Hoechst** (now part of **Sanofi-Aventis**).

Glyburide is a sulfonamide **urea** derivative with ant hyperglycemic activity that can potentially be used to decrease cerebral edema. Upon administration, glyburide binds to and blocks the **sulfonylurea** receptor type 1 (SUR1) subunit of the **ATP**-sensitive inwardly-rectifying **potassium** (K(**ATP**)) channels on the membranes of pancreatic beta cells. This prevents the inward current flow of positively charged **potassium** (K⁺) ions into the cell, and induces a **calcium ion** (Ca²⁺) influx through voltage-sensitive **calcium** channels, which triggers exocytosis of insulin-containing granules. In addition, glyburide also inhibits the SUR1-regulated nonselective cation (NC) Ca-**ATP** channel, melastatin 4 (transient receptor potential cation channel subfamily M member 4; (TRPM4)), thereby preventing capillary failure and brain swelling. SUR1-TRPM4 channels are formed by co-assembly of SUR1 with TRPM4 in neurons, astrocytes, and capillary endothelium during cerebral ischemia. Upon ischemia-induced **ATP** depletion, channels open which results in **sodium** influx, cytotoxic edema formation, capillary fragmentation and necrotic cell death. SUR1-TRPM4 is not expressed in normal, uninjured tissues.

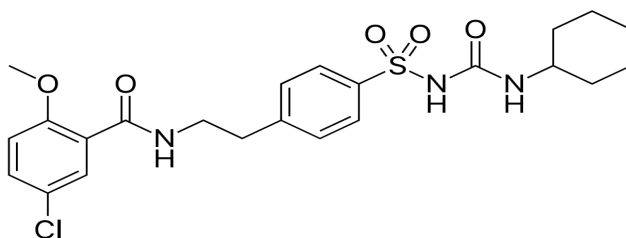


Figure 6: Structure of Glibenclamide

1.15.2 Chemical data :

<u>Formula</u>	C ₂₃ H ₂₈ ClN ₃ O ₅ S
<u>Molar mass</u>	494.04 Mol

Table 3: Chemical data of glibenclamide

1.15.3 Side effects and contraindications :

This drug is a major cause of drug-induced **hypoglycemia**. The risk is increased against other **sulfonylureas**. Cholestasis **jaundice** is noted.

Glibenclamide may be not recommended in those with G6PD deficiency, as it may cause acute hemolysis recently published data suggest glibenclamide is associated with significantly higher annual mortality when combined with metformin than other insulin-secreting medications, after correcting for other potentially confounding patient characteristics. The safety of this combination has been questioned.

1.15.4 Mechanism of action :

Glyburide appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. The mechanism by which glyburide lowers blood glucose during long-term administration has not been clearly established. With chronic administration in Type II diabetic patients, the blood glucose lowering effect persists despite a gradual decline in the insulin secretory response to the drug. Extra pancreatic effects may be involved in the mechanism of action of oral sulfonylurea hypoglycemic drugs. The combination of glyburide and metformin may have a synergistic effect, since both agents act to improve glucose tolerance by different but complementary mechanisms.

Some patients, who are initially responsive to oral hypoglycemic drugs, including micronase, may become unresponsive or poorly responsive over time. Alternatively, micronase Tablets may be effective in some patients who have become unresponsive to one or more other sulfonylurea drugs.

In addition to its blood glucose lowering actions, glyburide produces a mild diuresis by enhancement of renal free water clearance. disulfiram-like reactions have very rarely been reported in patients treated with micronase tablets.

1.15.5 Research :

Glibenclamide improves outcome in animal stroke models by preventing brain swelling and enhancing neuroprotection. A retrospective study showed, in type 2 diabetic patients already taking glyburide, NIH stroke scale scores were improved on discharge compared to diabetic patients not taking glyburide.

1.15.6 Trade names :

Glibenclamide is available as a **generic**, is manufactured by many pharmaceutical companies and is sold in doses of 1.25, 2.5 and 5 mg under many brand names including Daonil, Diabeta, Euglucon, Gilemal, Glidanil, Glybovin, Glynase, Maninil, Micronase and Semi-Daonil. It is also available in a fixed-dose **combination drug** with **metformin** that is sold under various trade names, e.g. Bagomet Plus, Benimet, Glibomet, Gluconorm, Glucored, Glucovance, Metglib and many others

1.16 Summery :

This chapter summarizes the validation parameters that are required according to the requirements of ICH. The paradigm shift under cGMP in the twenty -first century that requires the bench - level scientist to have the scientific and technical understanding, product knowledge, process knowledge, and/or risk assessment abilities to appropriately execute the quality functions of analytical method validation is presented in detail. The method validation process and the minimum requirements to be included in a regulatory method are also discussed. An overview of phase - appropriate method validation, method verification, and method revalidation are presented to stimulate ideas and the thought process to follow when such situations are encountered

1.17 Aims and objectives :

Based on the importance and advantages of UV-Vis method in pharmaceutical industry this work aimed to:

- To validate UV-VIS procedure for Glibenclamide Analysis
- To simplify the idea of validation and show how it carried practically.
- To estimate all validation parameters (Precision , Accuracy ,Linearity , Range , Selectivity ,sensitivity , limit of detection ,limit of quantitation) of UV method for Glibenclamide .

Chapter two

Experimental

2 Experimental

2.1 Instrumentation and conditions:

- 2.1.1 Absorption spectral measurements were carried out with a UV – Visible spectrophotometer (Shimadzu Model 1800) using UV Probe software version 2 was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells.
- 2.1.2 Digital heated ultrasonic path.
- 2.1.3 Sensitive balance Adam Equipment's.

2.2 Materials

- 2.2.1 Glibenclamide (GLB) were supplied by India and used as such.
- 2.2.2 Methanol used was from **sharlab** fine chemicals **Ltd,India**.
- 2.2.3 Water used was generated by reverse osmosis.
- 2.2.4 Glibenclamide Tablet used was supplied by Unimed Pharmaceutical
- 2.2.5 Ethanol
- 2.2.6 Acetonitrile
- 2.2.7 Isopropyl alcohol
- 2.2.8 Buffer 4
- 2.2.9 Buffer 7
- 2.2.10 Buffer 9.2

2.3 Apparatus

- 2.3.1 Volumetric flask 200 ml (Class A) (Calibrated).
- 2.3.2 Volumetric flask 100 ml (Class A) (Calibrated).
- 2.3.3 Volumetric flask 50 ml (Class A) (Calibrated).
- 2.3.4 Funnel
- 2.3.5 Cylinder class A 100 ml
- 2.3.6 Graduated pipette 10 ml
- 2.3.7 Graduated pipette 10 ml
- 2.3.8 Mortars
- 2.3.9 Beakers
- 2.3.10 Pipette filler 5 ml
- 2.3.11 Pipette filler 10 ml
- 2.3.12 Conical flasks 100 ml
- 2.3.13 Filter Paper (What man Filter Paper)Size(12.5 cm- 90 mm)
- 2.3.14 Spatula double ended .

2.4 Methods

2.5 Preparation of standard solutions

The Glibenclamide reference standard solution (1000 mg L⁻¹) was prepared by accurately weighing 100.0 mg of glibenclamide reference in a 100.0 mL volumetric flask. The volume was completed with methanol. This flask was sonicated for 25 min. this solution was diluted in volumetric flask with methanol to obtain a final solution containing 100 mg L⁻¹ of GLB.

2.5.1 Preliminary solubility studies of drugs

Solubility of the drug was determined at 28±1 C. A small quantity of standard drugs were dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, and pH 4, 7, 9.2 buffer solutions. By the solubility studies we determined that the drug was dissolved in methanol.

2.5.2 Determination of maximum absorption λ_{max} .

From the stock solutions, a working standard was prepared. The absorption spectrum for GLB the absorption spectrum was recorded using 10 mg L⁻¹ solution and the maximum absorption was found to be 230nm. The UV spectra of GLB drugs were shown in [figure 8](#)

Chapter three

Results and discussion

3 RESULTS AND DISCUSSION

3.1 Determination of maximum absorbance :

Sr.No.	Wavelength nm	Absorbance
1	300.4	0.073
2	274.6	0.033
3	230	0.629
4	202.2	1.212
5	194.2	0.559
6	278.2	0.029
7	272	0.03
8	219.8	0.521

Table 4 Determination of maximum absorbance

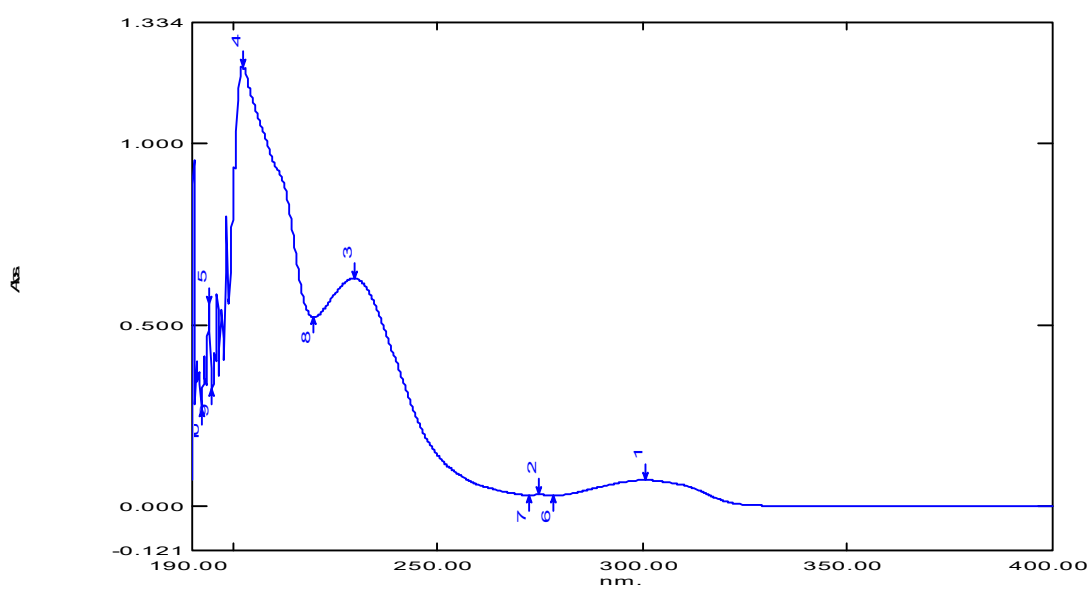


Figure 7 UV spectrum of glibenclamide

3.2 Method validation parameters :

3.2.1 Linearity

The linearity was determined by plotting concentration against corresponding absorbance. The calibration curve was defined in the concentration interval in which the intensity of the spectrophotometer response was linearly proportional to the concentration of the analyzed substance:

General equation of regression line:

$$A = a.C + b \quad (1)$$

Where A is the absorbance; C , concentration of sample; a , slope of the curve; and, b , y intercept of the curve.

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method and the correlation coefficient (r) indicated the linearity of the method.

Sample No.	Concentration (ppm)	Absorbance
1	2	0.119
2	4	0.227
3	6	0.347
4	8	0.463
5	10	0.572
SD = 0.161527211		
R ² = 0.9996		

Table 5: Determination of linearity

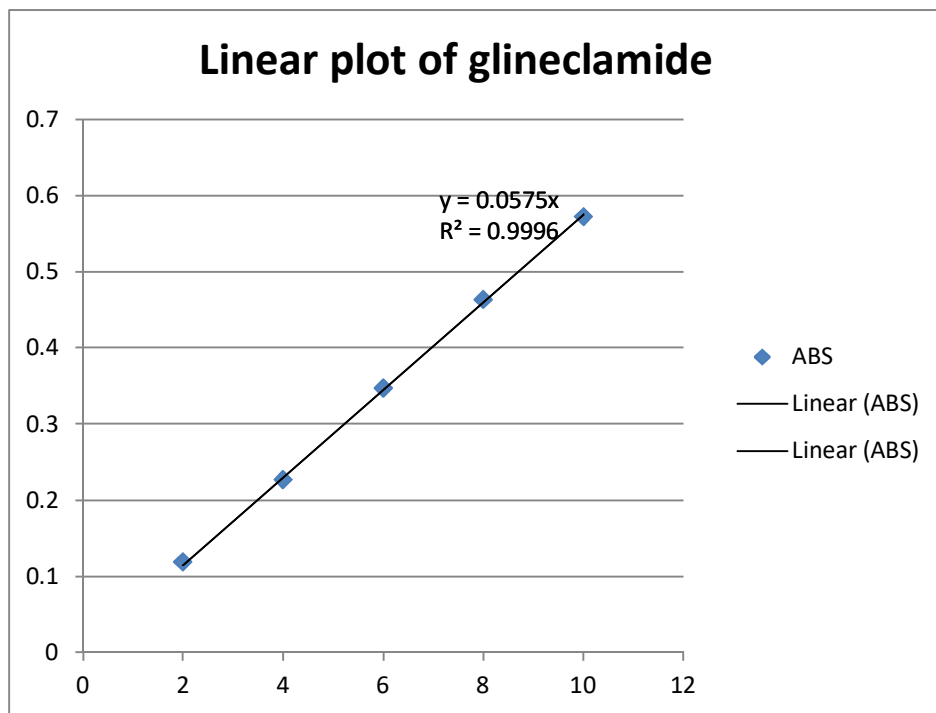


Figure 8: Linear plot of glibenclamide

Correlation Coefficient = 0.9996

Slope = 0.0575

Intercept = 0

Acceptance criteria

Typically, a square of correlation coefficient ($r^2 > 0.99$) demonstrate linearity. In addition y-intercept must not be significantly different from zero

3.2.2

Range:

Sample No.	Concentration (ppm)	Absorbance
1	1	0.051
2	2	0.118
3	4	0.239
4	6	0.346
5	8	0.452
6	10	0.571
7	20	1.131

Table 6: Determination of linearity range

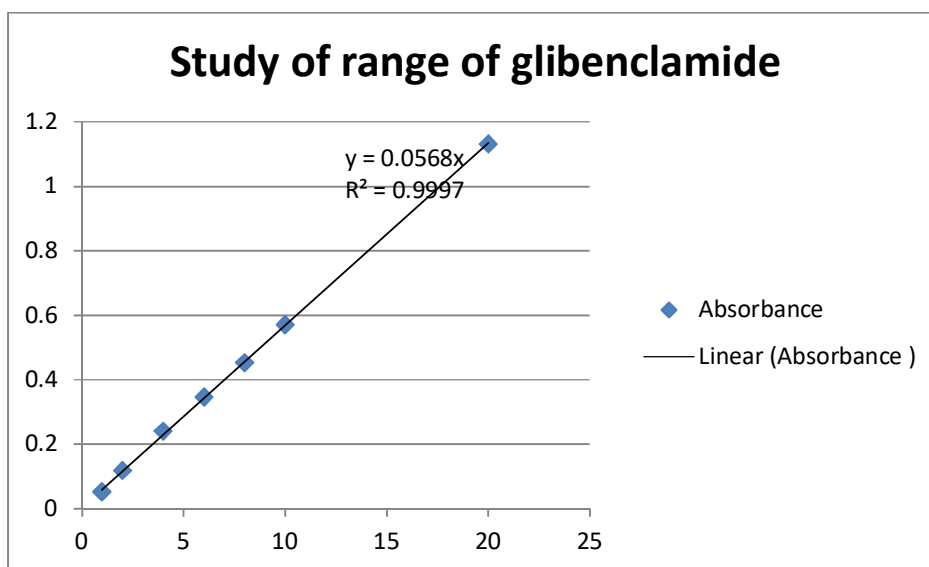


Figure 9: Range of linearity

Correlation Coefficient	0.9997
Slope	0.0568
Intercept	0

3.2.3 Precision

The intra-day precision was determined by analyzing the samples of glibenclamide at concentrations of 5.0 $\mu\text{g mL}^{-1}$. Determinations were performed with 6 replicates on the same day. The precision is expressed as relative standard deviation (RSD) amongst responses. In order to be considered precise, the RSD of the method should be less than 2.0%.

3.2.3.1 Repeatability :

Concentration	6 PPM	8 PPM	10 PPM
	0.345	0.451	0.575
	0.346	0.452	0.571
	0.346	0.451	0.572
	0.344	0.451	0.572
	0.344	0.452	0.576
	0.345	0.451	0.582
Mean	0.345	0.451333	0.5732
SD	0.000816	0.000471	0.002167948
%RSD	0.236666	0.104447	0.3782185

Table 7: Determination of repeatability

3.2.3.2 Intermediate precision :

concentration	Time	
	12.30 p.m.	3.30 p.m.
standard 10 ppm	0.575	0.565
standard 10 ppm 2	0.571	0.564
standard 10 ppm 3	0.572	0.569
standard 10 ppm 4	0.572	0.571
standard 10 ppm 5	0.576	0.571
standard 10 ppm 6	0.582	0.566
Mean	0.574666667	0.5676667
SD	0.004082483	0.0030768
%RSD	0.710408858	0.5420073
Average %RSD	0.710408858	

Table 8: Determination of intermediate precision

3.2.4 Accuracy

The accuracy of the method was evaluated through the recovery test. Recovery tests were performed by adding known amounts of standard solutions to samples followed by analyses using the proposed method. Aliquots of standard and samples solutions were transferred to 10 mL volumetric flasks and final volumes were completed with methanol. The percentage of recovery (R) was calculated as indicated by Association of Official Analytical Chemists International.

$$R = [(C_F - C_U) / C_A] \times 100 \quad (2)$$

Where C_F represents the concentration of analyte measure in fortified test sample; C_U , the concentration of analyte measure in unfortified test sample; and, C_A , the concentration of analyte added to fortified test sample.

concentration	absorbance	conc ppm	Recovery %	Mean	%RSD
80%	0.231	4.0173913	99.56709957		
80%	0.23	4	100		
80%	0.231	4.0173913	99.56709957		
		Avg	99.71139971	99.71%±0.204%	0.2046619
		SD	0.204071221		
		RSD	0.204661876		
100%	0.279	4.8521739	97.04347826		
100%	0.289	5.026087	100.5217391		
100%	0.288	5.0086957	100.173913		
		Avg	99.24637681	99.24± 1.57%	1.5760207
		SD	1.564143486		
		RSD	1.576020743		
120%	0.344	5.9826087	99.71014493		
120%	0.35	6.0869565	101.4492754		
120%	0.341	5.9304348	98.84057971		
		Avg	100	100.0%±1.085%	1.085
		SD	1.084538373		
		RSD	1.084538373		

Table 9: Determination of accuracy

The measured recovery should be 98% to 102% of the amount added.

3.2.5 Specificity & Selectivity

Specificity is the ability of the method to accurately measure a compound in the presence of other components such as impurities, degradation products and matrix components. The specificity of the proposed method was evaluated through the analysis of a placebo solution, which it was prepared with the excipients of the pharmaceutical formulation. Thus, the mixture of component inert was prepared in their usual concentration employed in tablets than the method was applied in order to check if any component of the formulation could generate a response or a read with absorption band similar to the drug. And the placebo didn't show any absorbance at the specified wave length.

3.2.6 Limit of detection :

The results of Linearity were used to find out the detection limit using the method of standard deviation of absorbance's and the slope ,in which the detection limit is expressed by the following relation according to International Conference on Harmonization guidelines.

$$\text{LOD} = 3.3 \text{ SD}_b / a$$

Where SD_b represents the standard deviation

And a is the slope of calibration curve.

3.2.7 Limit of quantitation :

The results of linearity were used to find out the quantitation limit using the method of standard deviation of the absorbance's and the slope in which it expressed by the following relation

$$\text{LOQ} = 10 \text{ SD}_b / a$$

LOD	LOQ
0.046 $\mu\text{g/mL}$	0.143662 $\mu\text{g/mL}$

Table 10: Determination of LOD&LOQ

3.2.8 Robustness:

Method robustness was performed by applying small changes in the wave length.

Effect of different wave length of light on 10 ppm solution of GLP:

	Wave length	absorbance	Concentration ppm	Assay	Mean	RSD %
Sample 1	230	0.573	9.965217391	99.652174		
	229	0.572	9.947826087	99.478261		
	231	0.571	9.930434783	99.304348	99.47±0.142%	0.142%
	Avg			99.478261		
	SD			0.1419994		
	%RSD			0.1427442		
Sample 2	230	0.571	9.930434783	99.304348		
	229	0.571	9.930434783	99.304348		
	231	0.566	9.843478261	98.434783	99.01±.41%	0.41%
	Avg			99.014493		
	SD			0.409917		
	%RSD			0.4139969		
Sample 3	230	0.565	9.826086957	98.26087		
	229	0.564	9.808695652	98.086957		
	231	0.569	9.895652174	98.956522	98.43±0.46%	0.46%
	Avg			98.434783		
	SD			0.4601307		
	%RSD			0.4674472		

Table 11: Determination of robustness

3.3 CONCLUSION

In this study, the developed and validated UV-spectrophotometric alternative method for the determination of glibenclamide in pharmaceutical formulations has the advantage of being fast, simple, cost-effective with high precision, and accuracy. These advantages encourage the application of this method in routine analysis of glibenclamide.

The proposed method for the determination glibenclamide in solid dosage form was found to be precise, selective, rapid and economical. Glibenclamide exhibited maximum absorption at 230 nm and obeyed Beer's law in the concentration range of 2-20 µg/ml. the proposed method for the determination of glibenclamide showed linear regression

$Y = 0.0575x$ with a correlation coefficient (R^2) of 0.9997(Figure 9). A relative standard deviation of

0.236666, 0.104447 and 0.3782185 % was observed on analysis of six replicate samples of concentration 6, 8 & 10 ppm respectively.

Our studies revealed a recovery percentage of 99.24 – 100.0 %, which indicates that the developed method was simple, rapid and precise. The proposed method can be used for the drug analysis in routine quality control & method proves to be more economical than the published standard methods.

3.4 Recommendations

3.4.1 Develop a validation master plan or an operating procedure for method validation.

3.4.2 For individual method validation projects, develop a validation project plan

3.4.3 Define intended use of the method and performance criteria.

3.4.4 Check all equipment and material for performance and quality.

3.4.5 Perform validation experiments.

3.4.6 For standard methods: check scope of the standard with your own requirements.

3.4.7 For non-routine methods: develop and use generic methods and customize them for specific non-routine tasks.

3.4.8 Develop an operating procedure for method transfer between laboratories.

3.4.9 Analytic method development and validation are continuous and interconnected activities conducted throughout the drug development process. The practice of validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Although apparently contradictory, validated methods produce results within known uncertainties. These results are crucial to continuing drug development, as they define the emerging knowledge base supporting the product.

3.4.10 The time and effort that are put into developing scientifically-sound, robust, and transferrable analytic methods should be aligned with the drug development stage. The resources that are expended on method validation must be constantly balanced with regulatory requirements and the probability for product commercialization.

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