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A COMPLETE REVIEW ON METHOD VALIDATION

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ABSTRACT

Method validation is the technique used to affirm that the analytical method hired for a particular take a look at is appropriate for its meant use. Results from approach validation may be used to decide the quality, reliability, and consistency of analytical results; it's far an fundamental a part of any top analytical practice. Analytical techniques want to be established or revalidated. Method validation has obtained giant interest withinside the literature and from commercial committees and regulatory agencies. The proposed assessment protected a method for the validation approach, steps in approach validation, and distinctive parameters for approach validation.

KEYWORDS: Method validation, parameters, regulatory agencies, strategy, steps.

INTRODUCTION

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.

1. Analytical methods need to be validated or revalidated.
2. Before their introduction into routine use
3. 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
4. Whenever the method is changed, and the change is outside the original scope of the method.
5. Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies.

The U.S. FDA CGMP[1] request in Section 211.165 (e) methods to be validated: The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with Section 211.194 (a). These requirements include a statement of each method used in testing the sample to meet proper standards of accuracy and reliability as applied to the tested product. The U.S. FDA has also proposed industry guidance for analytical procedures and methods validation.[2]

ISO/IEC 17025 includes a chapter on the validation of methods[3] with a list of nine validation parameters. The International Conference on Harmonization (ICH)[4] has developed a consensus text on the validation of analytical procedures. The document includes definitions for 8 validation characteristics. ICH also developed guidance with the detailed methodology.[4]

The U.S. EPA prepared guidance for method's development and validation for the Resource Conservation and Recovery Act.[5] The AOAC, the EPA, and other scientific organizations provide methods that are validated through multi-laboratory studies.

The USP has published specific guidelines for method validation for Compound evaluation.[6] USP defines eight steps for validation.

Accuracy

Precision

Specificity

Limit of detection

Limit of quantitation

Linearity and range

Ruggedness

Robustness

The FDA has also published guidance for the validation of bio analytical methods.[7] the most comprehensive document is the conference report of the 1990 Washington conference. Analytical methods validation: Bioavailability, bioequivalence, and pharmacokinetic studies, which was sponsored by, among others, the American Association of Pharmaceutical Scientists, the AOAC, and the U.S. FDA. The report presents guiding principles for

validating studies of both human and animal subjects. The report has also been used as a basis for the FDA industry guidance document.[7]

Representatives of the pharmaceutical and chemical industry have published papers on the validation of analytical methods. Hokanson[8,9] applied the life cycle approach, developed for computerized systems, to the validation and revalidation of methods. Green[10] gave a practical guide for analytical method validation, with a description of a set of minimum requirements for a method. Renger et al.[11] described the validation of a specific analytical procedure for the analysis of theophylline in a tablet using high-performance thin-layer chromatography. The validation procedure in this particular article is based on the requirements for EU multistate registration.

Wegscheider[12] has published procedures for method validation with a special focus on calibration, recovery experiments, method comparison, and investigation of ruggedness. Seno et al.[13] have described how analytical methods are validated in a Japanese QC laboratory. The AOAC[14] has developed a peer-verified methods validation program with detailed guidelines on exactly which parameters should be validated. Winslow and Meyer[15] recommend the definition and application of a master plan for validating analytical methods. Breaux et al. have published a study on analytical methods development and validation.[16] The key point is to develop methods for easy validation and revalidation. Krause published a guide for analytical method transfer, comparability, maintenance, and acceptance criteria for the testing of biopharmaceuticals.[17]

This primer gives a review and a strategy for the validation of analytical methods for both methods developed in-house as well as standard methods, and a recommendation on the documentation that should be produced during, and on completion of, method validation. It also describes what is important when transferring a method.

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. 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.

STEPS IN METHOD VALIDATION[18]

Develop a validation protocol, an operating procedure or a validation master plan (VMP) for the validation.

For a specific validation, project defines owners and responsibilities.

Develop a validation project plan.

Define the application, purpose, and scope of the method.

Define the performance parameters and acceptance criteria.

Define validation experiments.

Verify relevant performance characteristics of equipment.

Qualify materials, for example, standards and reagents for purity, accurate amounts, and sufficient stability.

Perform pre-validation experiments.

Adjust method parameters or/and acceptance criteria if necessary.

Perform full internal (and external) validation experiments.

Develop SOPs for executing the method in the routine.

Define criteria for revalidation.

Define type and frequency of system suitability tests and/or analytical quality control checks for the routine.

Document validation experiments and results in the validation report.

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 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 laboratory or analytical chemist performing the validation and the laboratory or individual applying the method and users of the data to be generated by the method.

The validation experiments should be carried out by an experienced analyst to avoid errors due to inexperience. The analyst should be very well versed in the technique and operation of the instrument. Before an instrument is used to validate a method, its performance specifications should be verified using generic chemical standards. Satisfactory results for a method can be obtained only with equipment that is performing well. Special attention should be paid to those equipment characteristics that are critical for the method. For example, if detection limit is critical for a specific method, the instrument's specification for baseline noise and, for certain detectors, the response to specified compounds should be verified.

Any chemicals used to determine critical validation parameters, such as reagents and reference standards, should be.
Available in sufficient quantities
Accurately identified
Sufficiently stable

Checked for exact composition and purity.

Any other materials and consumables, for example, chromatographic columns, should be new and be qualified to meet the column's performance criteria. This ensures that one set of consumables can be used for most experiments and avoids unpleasant surprises during method validation.

Operators should be sufficiently familiar with the technique and equipment. This will allow them to identify and diagnose unforeseen problems more easily and to run the entire process more efficiently.

If there is little or no information on the method's performance characteristics, it is recommended to prove the suitability of the method for its intended use in initial experiments. These studies should include the approximate precision, working range, and detection limits. If the preliminary validation data appear to be inappropriate, the method itself, the equipment, the analysis technique, or the acceptance limits should be changed. Method development and validation are, therefore, an iterative process. For example, in liquid chromatography, selectivity is achieved through the selection of mobile phase composition. For quantitative measurements, the resolution factor between two peaks should be 2.5 or higher. If this value is not achieved, the mobile phase composition needs further optimization. The influence of operating parameters on the performance of the method should be assessed at this stage if this was not done during development and optimization of the method.[19]

There are no official guidelines on the correct sequence of validation experiments, and the optimal sequence may depend on the method itself. Based on the author's experience, for a liquid chromatographic method, the following sequence has proven to be useful.

Selectivity of standards (optimizing separation and detection of standard mixtures if selectivity is insufficient)

Linearity, limit of quantitation, and limit of detection, range

Repeatability (short-term precision) of retention times and peak areas

Intermediate precision

Selectivity with real samples

Trueness/accuracy at different concentrations

Ruggedness (inter laboratory studies).

The more time-consuming experiments, such as accuracy and ruggedness, are included toward the end. Some of the parameters, as listed under 2 to 6, can be measured in combined experiments. For example, when the precision of peak areas is measured over the full concentration range, the data can be used to validate the linearity.

During method validation, the parameters, acceptance limits, and frequency of ongoing system suitability tests or QC checks should be defined. Criteria should be defined to indicate when the method and system are beyond statistical control. The aim is to optimize these experiments so that, with a minimum number of control analyses, the method and the complete analytical system will provide long-term results to meet the objectives defined in the scope of the method.

Once the method has been developed and validated, a validation report should be prepared that includes the following.

Objective and scope of the method (applicability, type)

Summary of methodology

Type of compounds and matrix

All chemicals, reagents, reference standards, QC samples with purity, grade, their source or detailed instructions on their preparation

Procedures for quality checks of standards and chemicals used

Safety precautions

A plan and procedure for method implementation from the

Method development laboratory to routine analysis

Method parameters

Critical parameters taken from robustness testing

Listing of equipment and its functional and performance requirements, for example, cell dimensions, baseline noise, and column temperature range. For complex equipment, a picture or schematic diagram may be useful.

Detailed conditions on how the experiments were conducted, including sample preparation. The report must be detailed enough to ensure that it can be reproduced by a competent technician with comparable equipment.

Statistical procedures and representative calculations

Procedures for QC in routine analyses, for example, system suitability tests

Representative plots, for example, chromatograms, spectra, and calibration curves

Method acceptance limit performance data

The expected uncertainty of measurement results

Criteria for revalidation

The person(s) who developed and validated the method

References (if any)

Summary and conclusions

Approval with names, titles, date and signature of those responsible for the review, and approval of the analytical test procedure.

VERIFICATION OF STANDARD METHODS

A laboratory applying a specific method should have documented evidence that the method has been appropriately validated. This holds for methods developed in-house, as well as for standard methods, for example, those developed by organizations such as the EPA, the American Society for Testing and Materials, ISO or the USP.

A number of questions usually arise about the validation of standard methods: First, should these methods be revalidated in the user's laboratory and, if so, should method revalidation cover all experiments, as performed during initial validation? Second, which documentation should be available or developed in-house for standard methods? Official guidelines and regulations are not explicit about validating standard methods. Only CITAC/EURACHEM guide[18] includes a short paragraph that reads as follows.

The validation of standard or collaboratively tested methods should not be taken for granted, no matter how impeccable the method's pedigree - the laboratory should satisfy itself that the degree of validation of a particular method is adequate for the required purpose and that the laboratory is itself able to match any stated performance data.

There are two important requirements in this excerpt

1. The standard's method validation data are adequate and sufficient to meet the laboratory's method requirements
2. The laboratory must be able to match the performance data as described in the standard. Further advice comes from FDA's 21 CFR 194 Section (a) 2: "If the method employed is in the current revision of the United States Pharmacopeia, National Formulary, Association of Official Analytical Chemists, or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not

modified, a statement indicating the method and reference will suffice. The suitability of all testing methods used shall be verified under actual conditions of use." The spirit of this text is in line with the two requirements listed above.

This section elaborates on what these statements mean in practice, and it gives a strategy for validating standard methods. Like the validation of methods developed in-house, the evaluation and verification of standard methods should also follow a documented process that is usually the validation plan. Results should be documented in the validation protocol. Both documents will be the major source for the validation report.

An example of a step-by-step plan for the evaluation and validation of standard methods is shown as a flow diagram in Figure 1. As a first step, the scope of the method, as applied in the user's laboratory, should be defined. This should be done independently of what is written in the standard method and should include information such as.

The type of compounds to be analyzed

Matrices.

The type of information required (qualitative or quantitative)

Detection and quantitation limits Range

Precision and accuracy as specified by the client of the analytical data and

The type of equipment - its location and environmental conditions.

As a second step, the method's performance requirements should be defined in considerable detail, again irrespective of what has been validated in the standard method. General guidelines on validation criteria for different measurement objectives and procedures for their evaluation are discussed later in this chapter.

The results of these steps lead to the experiments that are required for adequate method validation and to the minimal acceptance criteria necessary to prove that the method is suitable for its intended use. Third, required experiments and expected results should be compared with what is written in the standard method. In particular, the standard method should be checked for the following items.

Have the reported validation results been obtained from the complete procedure or from just a part of it? Sometimes, the validation data from the published method have been obtained from the chromatographic analysis but have not included sample preparation steps. The diagram in Figure 2 can be used for this check. A complete validation of the analytical

procedure should include the entire process from sampling, sample preparation, analysis, calibration, and data evaluation to reporting

Has the same matrix been used?

Did the validation experiments cover the complete concentration range as intended for the method in the user's laboratory? If so, has the method's performance been checked at the different concentration ranges?

Has the same equipment (brand, model) been used as available in the user's laboratory, and, if not, was the scope of standard method regarding this item broad enough to include the user's equipment? This question is very important for a gradient HPLC analysis, where the HPLC's delay volume can significantly influence the method's selectivity

Have performance characteristics, for example, the limit of quantitation, been checked in compliance with the most recent guidelines, as required for the user's laboratory (e.g., the ICH guideline[4] for pharmaceutical laboratories)? If not, does the test procedure have equivalency to the guideline?

If the scope, the validation parameters or the validation results do not meet the user's requirements, adequate validation experiments should be defined, developed and carried out. The extent of these experiments depends on the overlap of the user requirements with the scope and results as described in the standard method. If there is no overlap, a complete validation should be carried out. In the case of a complete overlap, validation experiments may not be necessary.

If method validation experiments are unnecessary, the user should prove the suitability of the method in his or her laboratory. This evidence should confirm that the user's equipment, the people, the reagents, and the environment are qualified to perform the analysis. The experiments may be an extract of the full method validation and should focus on the critical items of the method. Guidelines for these tests should have been developed during method development. If not, they should be developed and carried out at this stage.

REVALIDATION

Most likely, some method parameters have to be changed or adjusted during the life of the method if the method performance criteria fall outside their acceptance criteria. The question is whether such change should be defined for each method, either based on experience with similar methods or else investigated during method development. These ranges should be verified during method validation in robustness studies and should be part of the method characteristics. Availability of such operating ranges makes it easier to decide when a method

should be revalidated. A revalidation is necessary whenever a method is changed, and the new parameter lies outside the operating range. If, for example, the operating range of the column temperature has been specified to be between 30°C and 40°C, the method should be revalidated if, for whatever reason, the new operating parameter is 41°C.

Revalidation is also required if the scope of the method has been changed or extended, for example, if the sample matrix changes or if operating conditions change. Furthermore, revalidation is necessary if the intention is to use instruments with different characteristics, and these new characteristics have not been covered by the initial validation. For example, an HPLC method may have been developed and validated on a pump with a delay volume of 5 mL, but the new pump has a delay volume of only 0.5 mL.

Part or full revalidation may also be considered if system suitability tests, or the results of QC sample analysis, lie outside preset acceptance criteria and where the source of the error cannot be traced back to the instruments or any other cause.

Whenever there is a change that may require part or full revalidation, the change should follow a documented change control system. A flow diagram of such a process is documented in Figure 3. The change should be defined, authorized for implementation, and documented. Possible changes may include.

New samples with new compounds or new matrices

New analysts with different skills

New instruments with different characteristics

New location with different environmental conditions

New chemicals and/or reference standards and

Modification of analytical parameters.

An evaluation should determine whether the change is within the scope of the method. If so, no revalidation is required. If the change lies outside the scope, the parameters for revalidation should be defined. After the validation experiments, the system suitability test parameters should be investigated and redefined, if necessary.

PARAMETERS FOR METHOD VALIDATION

The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature. Unfortunately, some

of the definitions vary between the different organizations. An attempt at harmonization was made for pharmaceutical applications through the ICH,[4] where representatives from the industry and regulatory agencies from the United States, Europe, and Japan defined parameters, requirements and, to some extent, methodology for analytical methods validation. The parameters as defined by the ICH and by other organizations and authors are described in brief in the following paragraphs.[21]

Accuracy

Accuracy is the agreement between the test results obtained by the proposed method and the true value. It expresses the correctness of the method.

It is expressed as percentage by the assay of known amount of substance. Accuracy also evaluated by recovery studies, in which known amount of drug is added to previously analyzed pharmaceutical preparation of the drug and tested for the recovery of the added drug.

The absolute error is a measure of the accuracy of the measurement; it is then calculated as, Absolute error=Mean error (True value−Measured value)/True value×100

Precision

Precision refers to the agreement among the individual test results when a method is applied repeatedly to the same sample. It is a measure of degree of repeatability or reproducibly of a method. The precision of an analytical procedure is usually expressed as relative standard deviation (RSD), which is calculated as, RSD=S.D./Mean×100

Specificity

Specificity of a method refers to the ability of the method to measure accurately and specifically the substance of interest in the sample as impurities, degradation products. For this, the test results of analysis of samples containing other ingredients are compared with the samples without containing ingredients.

Linearity

The linearity of an analytical method is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

Range

The range of an analytical method is the interval between the upper and lower concentration of analyte in the sample. Beer's law response - concentration curve should be linear at least 5-6 points in the range.

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated.

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variations in procedure.

Ruggedness

The degree of reproducibility of test results obtained by analyzing the same sample under variety of normal test conditions is known as ruggedness.

Sensitivity

Sensitivity refers to the smallest quantity that can be accurately measured. It also indicates the capacity of the method to measure small variations in concentration. In the case of UV and visible spectrophotometric methods, an estimate known as Sandell's sensitivity' is used to evaluate the sensitivity of the method.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time.

Reproducibility

Reproducibly expresses the precision in the laboratories.

CONCLUSION

This review gives complete knowledge of method validation and parameter including in it. 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. Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies.

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Conflict of interest

None.

REFERENCES

1. Fields, Tim. "Defining the" c" in cGMPs." Journal of GXP Compliance, 2007; 11.3: 36-42.
2. Drug, Dry Powder Inhaler DPI. "Guidance for industry." Center for Drug Evaluation and Research (CDER), 1998; 1000.
3. ISO, EN. "IEC 17025: General requirements for the competence of testing and calibration laboratories." International Organization for Standardization and International Electrotechnical Comission, 2005.
4. ICH Q8. "International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use." 2005.
5. Patil, Sunila T., Rajesh A. Ahirrao, and Sunil P. Pawar. "A short review on method validation." Journal of Pharmaceutical and BioSciences, 2017; 5.
6. Okeke, Claudia C. "Update on the Proposed Revision Process for United States Pharmacopeia Chapter< 797> Pharmaceutical Compounding-Sterile Preparations." International journal of pharmaceutical compounding, 2007; 11.4: 302.
7. FDA, US. "Guidance for industry, bioanalytical method validation, 2018;" (2001).
8. Hokanson, Gerard C. "A LIFE CYCLE APPROACH TO THE VALIDATION OF ANALYTICAL METHODS DURING PHARMACEUTICAL PRODUCT DEVELOPMENT. II: CHANGES AND THE NEED FOR ADDITIONAL VALID ATION." Pharmaceutical technology, 1994; 18.10: 92-92.

9. Hokanson, Gerard C. "A life cycle approach to the validation of analytical methods during pharmaceutical product development, Part I: The initial validation process.", 1994; 118-130.
10. Green, J. Mark. "Peer reviewed: a practical guide to analytical method validation." Analytical chemistry, 1996; 68.9: 305A-309A.
11. Renger, B., et al. "Validation of analytical procedures in pharmaceutical analytical chemistry: HPTLC assay of theophylline in an effervescent tablet." JPC. Journal of planar chromatography, modern TLC, 1995; 8.4: 269-278.
12. Rajput, Diksha, Vaishnav Rajat, and Anju Goyal. "Validation of Analytical Methods for Pharmaceutical Analysis." International Journal of Pharmaceutical Erudition**,** 2013; 3.1: 31-40.
13. Seno, S., S. Ohtake, and H. Kohno. "Analytical validation in practice at a quality control laboratory in the Japanese pharmaceutical industry." Validation in Chemical Measurement. Springer, Berlin, Heidelberg, 1997; 56-61.
14. Association of Official Analytical Chemists. AOAC® peer-verified methods program: manual on policies and procedures. AOAC International, 1998.
15. Winslow, Paul A., and Richard F. Meyer. "Defining a master plan for the validation of analytical methods." Journal of Validation Technology, 1997; 3: 361-368.
16. Breaux, Jay, Kevin Jones, and Pierre Boulas. "Analytical methods development and validation." Pharm. Technol, 2003; 1: 6-13.
17. Krause, S. O. "A guide to testing biopharmaceuticals Part II: acceptance criteria and analytical maintenance." PHARMACEUTICAL TECHNOLOGY EUROPE, 2006; 18.6: 29.
18. Co-operation on International Traceability in Analytical Chemistry, and EURACHEM. Guide to quality in analytical chemistry: an aid to accreditation. na, 2002.
19. Vessman, Jörgen. "Selectivity or specificity? Validation of analytical methods from the perspective of an analytical chemist in the pharmaceutical industry." Journal of pharmaceutical and biomedical analysis, 1996; 14.8-10: 867-869.
20. Huber, Ludwig, and Stephan A. George. Diode array detection in HPLC. M. Dekker, 1993.
21. Patil, Sunila T., Rajesh A. Ahirrao, and Sunil P. Pawar. "A short review on method validation." Journal of Pharmaceutical and Bio Sciences, 2017; 5.