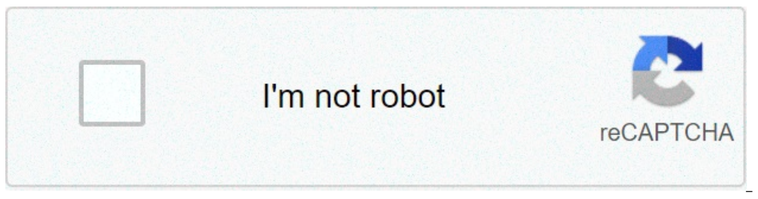

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Analytical method validation protocol template

As a summary in Part I, validation of analytical methods in any regulated environment is a critical part of the general validation process (1). The validation of the analytical method is part of the validation process that establishes, through laboratory studies, that the performance characteristics of the method meet the requirements for the analytical application provided and provides a guarantee of reliability during normal use; sometimes referred to as "the process of providing documented evidence that the method does what is intended to do." Regulated laboratories must validate the analytical method to be in accordance with the government or other regulators, as well as being good scientists. A well defined and well documented validation process not only provides proof that the system and method are suitable for its intended use, but can help transfer the method and meet regulatory compliance requirements. Michael Swartz Since the late 1980s, government agencies and other agencies (e.g., FDA, International Conference on Harmonization-ICH) have issued guidelines on validation methods. In 1987, the FDA designated the specifications in the current United States Pharmacopeia (USP) as those legally recognized in determining compliance with the Federal Food, Drug and Cosmetic Act (2). More recently, new information has been published, updated the previous guidelines and provided more details and harmonization with the international conference on(ich) guidelines (3-5). as provided in the guideline, validation of the analytical method is only part of the general validation process that includes at least four separate steps: Software validation, hardware validation (instrumentation) and qualification, validation of analytical method and system suitability. ira krull in part i of this article, we have dealt with software validation, analytical tool qualification (aiq,) and system suitability (1.) in part ii, we will discuss the specifications of validation of analytical methods, including terms and definitions, protocol/methodology, and how validation of analytical methods changes according to the type or intended purpose of the analytical method. terms and definitions of different parameters, generally referred to as analytical performance characteristics, can be examined during any method validation protocol and its intended use. These analytical performance characteristics, which we have over the years affectionately referred to as "the eight phases of validation of the analytical method," are illustrated in Figure 1. figure 1: characteristics of the validation performance of the analytical method. Although most of these terms are familiar and are used daily in any regulated high-performance liquid chromatography lab (hplc,) sometimes mean different things for different people. to avoid any confusion, it is necessary to have a complete understanding of the terms, definitions and methodology as discussed inlater sections. Examples of acceptance criteria are listed in Table I. Table I: Example of acceptance criteria of the validation protocol of the AccuracyAccuracy analytical method is the accuracy measurement of an analytical method or proximity of an agreement between an accepted reference value and the value found in a sample. Established through the range of method, accuracy is measured as per cent of analytic recovered from the essay. For narcotic substances, precision measurements are obtained by comparing the results of a standard reference material analysis, or by comparing a second well characterized method. For the analysis of the drug product, accuracy is evaluated by the analysis of synthetic mixtures beaten with known amounts of components. For the quantification of impurities, accuracy is determined by the analysis of samples (dope substance or drug product) with known amounts of impurities (if impurities are not available, see specificity). To document accuracy, the guidelines recommend that the data be collected from a minimum of nine determinations on a minimum of three levels of concentration covering the specified range (i.e. three concentrations, three replicas each). Data should be reported as the percentage recovery of the known quantity, added, or as the difference between the average and true value with trust intervals (e.g., ± 1 SD). Precision The accuracy of an analytical method is defined as the proximity of the agreement betweentest results from repeated analysis of a homogeneous sample. accuracy is commonly performed as three measurements: repeatability, intermediate accuracy and reproducibility. repeatability refers to the ability of the method to generate the same results in a short time interval under identical conditions (in-assay accuracy). to document repeatability, the guidelines suggest to analyze a minimum of nine determinations covering the specified range of the procedure (i.e. three levels or concentrations, three repetitions each) or a minimum of six 100% determinations of the test or destination concentration. repeatability results are typically reported in % rsd. Intermediate accuracy refers to the agreement between the results of changes within the laboratory due to random events that may occur when using the method, such as different days, analysts or equipment. to determine intermediate accuracy, you need to use an experimental project so that the effects (if present) of the individual variables can be monitored. intermediate precision results are typically generated by two analysts who prepare and analyze replicated sample preparations. each analyst would prepare their own standards and solutions, and could use a different hplc system for analysis. the percentage-difference in the average values between the results of the two analysts are subjected to statistical tests (for example, a student test) to examine whether there is a difference in the average values obtained.refers to the results of collaborative studies between different laboratories. Documentation in support of reproducibility studies should include standard deviation, relative standard deviation (or variation coefficient), and trust interval. To generate data to demonstrate reproducibility, a typical experimental design could include analysts of two laboratories (perhaps other than analysts involved in intermediate accuracy) re-examining and preparing replicated sample preparations, each analyst would prepare their standards and solutions and use a different HPLC system for analysis. The results are shown in RSD %, and the percentage-difference in the average values between the two analysts must be within the specifications. Statistical calculations can also be made to determine whether there is any difference in the average values obtained. Among the USP guidelines are the term robustness, defined as the degree of reproducibility of test results obtained from the analysis of the same samples in a variety of conditions, such as different laboratories, analysts, tools, reagent batches, test times spent, test temperature or days. Robustness is a measure of the reproducibility of test results in the variation of conditions normally provided by the laboratory to the laboratory and analyst to the analyst. The use of the term robustness, however, is falling in favor and is not used by the ICH, but is instead directed online guide Q2 (R1)The discussion of intermediate precision (5.) SpecificitySpecificity is the ability to accurately measure and specifically the analogue of interest in the presence of other components that may be expected to be present in the sample. The specificity takes into account the degree of interference from other active, excipient, impurities and degradation products. The specificity in a method ensures that the response of a peak is due to a single component (without peak coelutions). The specificity of an analytical data is commonly measured and documented by resolution, plate number (efficiency) and tail factor. For identification purposes, specificity is demonstrated by the ability to discriminate between other compounds in the sample or with reference materials known. For tests of analysis and impurities, specificity can be shown by the resolution of the two compounds more closely eluted. These compounds are usually the main component or active ingredient and a very eluted impurity. If impurities are available, it must be shown that the essay is not influenced by the presence of high-end materials (impurities or excipients.) If impurities are not available, the test results are compared to a second well-documented procedure. For example, the two results are compared. For impurities tests, the profiles of impurities are compared. The comparison of test results varies with the particular method, but may include visual comparison and retention times, peak areas (orand peak form. Starting with the publication of USP 24, and as a direct result of the ICH process, it is now recommended that a peak purity test based on the detection of photodiode-array (PDA) or mass spectrometry (MS) be used to demonstrate specificity in chromatographic analysis compared to a known reference material. Modern PDA technology is a powerful tool to assess specificity. PDA detectors can collect spectra in a range of wavelengths at each data point collected through a peak, and through software processes, each spectrum can be compared to determining peak purity. Used in this way, PDA detectors can distinguish spectral and minute chromatographic differences not easily observed by simple overlay comparisons. However, PDA detectors can be limited sometimes in peak purity assessment by a lack of UV response, as well as system noise and related concentrations of interfering substances. In addition, the more similar are the spectra, and the lower the relative absorptions, the more difficult it is to distinguish coeluted compounds. The MS detection exceeds many of the limitations of a PDA, and in many laboratories has become the method of detection of choice for validation of methods. MS can provide information of non-equivocal peak purity, exact mass, and structural and quantitative information. The combination of PDA and MS on a single HPLC tool can provide valuable orthogonal information to ensure interference is notduring validation of the method. Limit of detection and quantity The detection limit (LOD) is defined as the lowest concentration of an analytical in a sample that can be detected, but not necessarily quantified. It is a limit test that specifies if an analytic is higher or less than a given value. The quantation limit (LOQ) is defined as the lowest concentration of an analytical in a sample that can be accurately quantified and accurately acceptable in the operating conditions indicated in the method. In a chromatography laboratory, the most common way to determine both LOD and LOQ is using signal-noise ratios (S/N), commonly 3:1 for LOD, and 10:1 for LOQ. Another method that is gaining popularity is a means to calculate the limits based on the following formula: LOD/LOQ = K (SD/S) where K is a constant (3 for LOD, 10 for LOQ), SD is the standard deviation of the response, and S is the slope of the calibration curve. It should be noted that the determination of these limits is a two-phase process. Regardless of the method used to determine the limit, an appropriate number of samples must be analyzed at the limit, once calculated, to fully validate the performance of the limit method. Linearity and rangeLinearity is the ability of the method to provide test results directly proportional to the concentration of analytics within a given range. The range is the range between the higher and lower concentrations of an analytical (included) that have beenbe determined with acceptable accuracy, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results obtained by the method (e.g., ng/mL). Guidelines specify that you use at least five levels of concentration to determine the range and linearity, along with certain minimum intervals specified depending on the type of method. Table II summarizes the typical minimum ranges specified by the guidelines. The data to be reported generally include the equation of the calibration curve line, the determination coefficient (r2), the residues and the curve itself. Table II: Example of recommended minimum intervals Robustness The robustness of an analytical procedure is defined as a measure of its ability to obtain comparable and acceptable results when disturbed by small but deliberate variations of the procedural parameters listed in the documentation. Robustness provides an indication of the suitability and reliability of the method during normal use. During a robustness study the parameters are intentionally varied to see if the results of the method are affected. The keyword in the definition is deliberate. Examples of HPLC variations are illustrated in Tables III and IV for isocratic and gradient methods, respectively. Changes must be symmetrically chosen around a nominal value, or the specified value in the method, to form a range that slightly exceeds the variations that can be expected when the method isor transferred. For instrument settings, the specifications of manufacturers are sometimes used to determine variability. The range evaluated during the robustness study should not be selected to be so wide that the robustness test will end failing, but rather to represent the type of variability usually found in the laboratory. Inducing the method to the point of failure is not necessary. A practical advantage of robustness tests is that after robustness is demonstrated on a given range of parameters, the value of that parameter can be adjusted within that range to meet the suitability of the system without a requirement to reevaluate the method. Table III: Example of variations in the robustness of the isocratic separation Robustness should be tested late in the development of a method, and if not, it is generally one of the first parameters investigated during validation of methods. In the course of the method development process, however, attention should be paid to the identification of which chromatographic parameters are more sensitive to small changes so that when carrying out robustness tests the appropriate variables can be tested. Robustness studies are also used to establish system fitness parameters to ensure that the validity of the entire system (including both the tool and the method) is maintained during implementation and use of the method. In addition, if the results of a method or other measurements are likely to change the method parameters, these parameters shouldadequately controlled and a precautionary statement included in the method documentation. The common parameters of HPLC used to measure and document robustness include (for information on how to calculate, see reference 6) critical resolution peak couple Rscolumn plate number N (or peak width in slope elution) peak area (and height) and concentrationThe repetition injections will improve estimates (e.g., %RSD) of the effect of a parameter variation. In many cases, multiple peaks are monitored, especially when in the sample there are some combinations of acid, neutral or basic compounds. A common question that occurs during the development of analytical method validation protocols is defining robustness parameters compared to precision parameters or intermediate reproducibility. A simple thumb rule: If it is internal or written in the method (for example, temperature and flow rate) is a robustness parameter; if it is external to the method (for example, the analyst, the instrument number or the day) is an intermediate parameter (previously robust). In other words, you would write a method to reflect flow, temperature, wavelength, buffer composition and pH, but you would never write in a method: "Jim runs the method on Tuesday on the Lotto #42587 column on System Six" — these are all intermediate precision parameters. Stability of the solution Although not formally listed in the guidelines, it is also quite common to investigate sample and standard stability duringSample stability and standard stock reference solution is evaluated at different time intervals (for example, at the time 0, 3 and 7 days) after storage at room temperature and refrigeration. This information is used to determine how often standards are to be prepared, how they must be stored (and samples) and how quickly samples should be analyzed after preparation. The validation of the analytical method by means of the TypeSeveral method of methods are used to measure the active pharmaceutical ingredient (API) and impurities, related substances, and excipients and the USP recognizes that it is not always necessary to evaluate each analytical performance parameter for each method. The type of method and its intended use dictate what performance characteristics should be studied, as summarised in Table V. Both USP and ICH share analytical methods in four separate categories: Category 1: Analysis for quantification of the main components or active ingredients Category 2: Determination of impurities or degradation products Category 3: Determination of performance characteristics Category 4: Table IV identification test: Example of gradient of separation variations of robustness Category 1 tests are aimed at the analysis of the main components and include methods such as content uniformity and power analysis. These last methods, although quantitatively, are not usually interested in low concentrations of analytical, but only with the amountThe API in the drug product. Due to the simplicity of separation (the API must be resolved by all interferences, but any other peak in the chromoatogram should not be solved by each other,) the emphasis is on speed on resolution. For analysis in category 1, LOD and LOQ ratings are not necessary because the main component or active ingredient to measure is normally present at high concentrations. However, since you want quantitative information, all remaining analytical performance parameters are relevant. Category 2 Methods Category 2 tests aim to analyse impurities or degradation products (among other applications). These essays usually look at much lower analytic concentrations than category 1, and are divided into two subcategories: Quantitative and limit tests. If you want quantitative information, there is no need for LOD determination, but the remaining parameters are required. The methods used to support stability studies (referred to stability indication methods) are an example of a category 2 quantitative test. The situation is reversed for a limited test. Because quantation is not required, it is enough to measure the LOD and demonstrate specificity and robustness. For a category 2 limit test, it is only necessary to show that a compound of interest is present or not — i.e. above or under a certain concentration. Methods to support cleaning validation and EPA environmental methods oftenin this category. Although, as seen in Table V, it is never necessary to measure both LOD and LOQ for any given category 2 method, but it is common during validation to evaluate both characteristics. Category 3 Methods The parameters that must be documented for the methods of USP-assay Category 3 (test or methods specific to performance characteristics) depend on the nature of the test. Dissolution test is an example of a category 3 method. Because it is a quantitative test optimized for the determination of the API in a pharmacological product, the validation parameters evaluated are similar to a Category 1 test for a formulation designed for immediate release. However, for a prolonged release formulation, where it may be necessary to confirm that none of the active ingredient was released from the formulation until after a certain point of time, the parameters to be investigated would be more like a 2-quantity category test that includes LOQ. As analytical objectives may differ, the rating parameters of Category 3 depend on the actual method, as shown in Table V. Table V: Features of the analytical performance to be measured against the type of method (3) Category 4 MethodsCategory 4 identification tests are of qualitative nature, so only specificity is required. For example, identification can be performed by comparing retention time or a spectrum to that of a known reference standard. Freedom from interference is all that is necessary in terms ofseparation. Conclusions In today's global market, validation can be a long and expensive process, involving regulatory, government and sanctioning entities from around the world. A well-defined and well-documented validation process gives regulatory agencies proof that the system (instrument, software, method and controls) is suitable for its intended use. The validation of the method evolves constantly and is only a part of this general process. The bottom line is that all parties involved should be confident that an HPLC method will give results sufficiently precise, precise and reproducible for the analysis task at hand, and the validation of the method is only one of the tools to be used to achieve this task. Michael Swartz "Validation Viewpoint" Co-Editor Michael E. Swartz is with Ariad Pharmaceuticals, Cambridge, Massachusetts and a member of the editorial board of LCGC. Ira S. Krull "Validation Viewpoint" Co-Editor Ira S. Krull is an associate professor of chemistry at the Northeastern University of Boston, Massachusetts and a member of the editorial advisory committee of LCGC. Direct correspondence on this column at lcgcedit@lcgcmag.com. References(1) M.E. Swartz and I.S. Krull, LCGC North America 27(11), 989–995 (2009). 2) United States Food and Drug Administration, Guidelines for the presentation of samples and analytical data for the validation of methods, February 1997. 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