Method validation in pharmaceutical analysis: from theory to practical optimization

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Abstract
The validation of analytical methods is required to obtain high-quality data. For the pharmaceutical industry, method validation is crucial to ensure the product quality as regards both therapeutic efficacy and patient safety. The most critical step in validating a method is to establish a protocol containing well-defined procedures and criteria. A well planned and organized protocol, such as the one proposed in this paper, results in a rapid and concise method validation procedure for quantitative high performance liquid chromatography (HPLC) analysis.

Introduction

Analytical methods can be considered as a complex and multi-step issue, ranging from sampling to generating the result. It is internationally recognized that the validation of methods is required to obtain high-quality data.[1]

According to Araujo (2009),[2] the word valid - from the Latin *validus* - means strong. To validate means to officially state that something that has been proven to be true is useful and of an acceptable standard.

The validation of analytical procedures intends to establish the performance characteristics of analytical applications through experimental tests, resulting in a suitable analytical method for its purpose.[3]

Considerations about method validation have been highlighted since the late 1940s when mathematics and statistics were considered to be essential prerequisites for the development of analytical methods. Its implementation in analytical laboratories was established in the late 1970s, reflecting the recognition of this process as an important way of obtaining standard methods. Currently, several international organizations are committed to continuous processing improvement.[2]

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High performance liquid chromatography (HPLC) is the most common analytical technique applied in pharmaceutical analysis. For the pharmaceutical industry, the demonstration that an analytical method is able to quantify the drug and its related compounds is crucial to ensure the product quality as regards both therapeutic efficacy and patient safety.

Although different areas of work have specific characteristics, the validation of analytical methods might be applied for different types of analyses, as it does not depend on the matrix of the sample or analytical technology employed.[4]

What to be considered in the validation study

Analytical procedures might be validated for investigating the identity of the analyte in a sample (identification tests); obtaining the analyte content (quantitative tests); and analyzing impurities (quantitative or limit tests).[5]

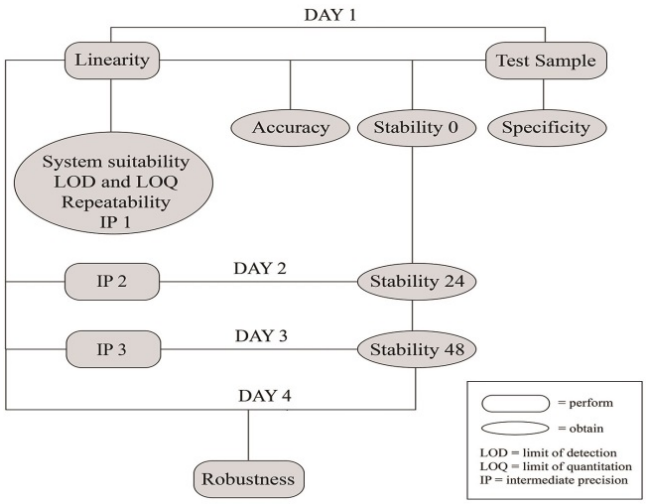
Aiming the validation of HPLC quantitative methods, the analytical performance characteristics to be considered are linearity, range, precision, accuracy, specificity and robustness, according to the International Conference on Harmonisation guideline Q2(R1).[5] Although not necessary for quantitative analytical procedures, detection and quantitation limits (LOD and LOQ) might be included as complementary tests, in addition to stability and system suitability.

Proposing an optimized validation scheme

The most critical step in validating a method is to establish a protocol containing well-defined procedures and criteria. Testing validation parameters consumes time and resources; however, a well-planned and organized protocol, such as the

one presented in Figure 1, results in a rapid and concise method validation procedure.

Figure 1. Scheme for a rapid quantitative HPLC method validation



The following parameters should be considered in order to obtain the proposed scheme:

1. Linearity

Linearity is the ability of obtaining results that are directly proportional to the analyte concentration in the sample, across the specified range, i.e., the interval between the upper and lower levels of analyte.[5] It might be evaluated through a calibration curve with 5 points in triplicate within a minimum range of 80 to 120% of the test concentration. It is estimated by the linearity's curve correlation coefficient, y-intercept, slope of the regression line and residual sum of squares.

This test should be run first, since its data might be used for assessing repeatability, intermediate precision (IP), accuracy, LOD, LOQ, stability and system suitability.

2. Precision

Precision is the closeness of agreement between individual results obtained from a repeatedly applied procedure in a homogeneous sample, comprising repeatability and IP.[5] It is expressed as the standard deviation or coefficient of variation.

2.1. Repeatability

Repeatability is defined as precision over a short interval of time under the same operating conditions, i.e., same day, same analyst and same equipment.[5] It is obtained by analyzing standard solutions of analyte at 3 different concentrations covering the specified range in triplicate. As shown in the scheme, it can be directly obtained from the linearity test.

2.2. Intermediate precision (IP)

IP expresses within-laboratory variation, considering different days, different analysts or different equipments.[5] In practice, it is repeatability performed for 3 times. Therefore, the first determination of IP can be obtained from the repeatability test; the second and third determinations are obtained by repeating the test for 2 more consecutive days.

3. Accuracy

Accuracy is the closeness of agreement between test results across the specified range and an accepted reference value.[5] It is obtained by the standard addition method, in which the sample is spiked with known quantities of the analyte. In practice, it can be evaluated by spiking a sample with the standard solutions of analyte at 3 different concentrations used in the linearity test in triplicate. It is expressed as the percentage of analyte recovery, standard deviation and relative standard deviation.

4. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of substances that are expected to be present, such as excipients, impurities and components of the mobile phase.[5] It might be assessed by verifying the absence of interfering peaks at the analyte retention time when spiking a sample with appropriate levels of impurities or excipients. Peak purity determination with a diode array detector can be performed to demonstrate that the target peak corresponds to a single component.

5. Robustness

Robustness is the capacity of the analytical method to remain unaffected by small variations in its parameters, indicating reliability.[5] Concerning liquid chromatography, variations such as mobile phase composition, mobile phase pH, column temperature, column lots, column suppliers and flow rate might be set.[5] It can be evaluated by the analysis of the standard solution of analyte at 100% of expected concentration in triplicate for each isolated condition and expressed as the standard deviation and relative standard deviation.

6. Limits of detection and quantitation

Both LOD and LOQ parameters can be directly obtained from the linearity test. The lowest amount of analyte which can be detected under the stated experimental conditions is the

LOD.[5] It can be estimated based on the signal-to-noise ratio of 3:1 or standard deviation of the y-intercepts divided by the slope of the calibration curve obtained in the linearity test, in a ratio of 3.3:1.[5]

LOQ is the lowest amount of analyte which can be quantitatively determined with precision and accuracy under the stated experimental conditions.[5] This parameter can be evaluated in the same way as for the LOD; however, in a ratio of 10:1 for both cases.[5]

7. Stability

Since chemical decomposition may occur during storage, drug stability should be evaluated by the analysis of sample and standard solutions during a preselected period of time, allowing the estimation of the maximum interval between sample preparation and analysis.

A short-term storage test might be performed by maintaining sample solution and standard solution of analyte at 100% at temperature of interest and testing at time, e.g., 0, 24 and 48 hours in triplicate. It is expressed as the standard deviation and relative standard deviation. The standard solution of analyte at 100% can be obtained from the linearity test.

8. System suitability

The system suitability is characterized by a set of tests applied to verify the reproducibility of the chromatographic system. It can be obtained by performing 5 injections of the standard solution of analyte at 100% and assessing the following parameters: peak tailing, resolution between peaks, theoretical plates, capacity factor and peak symmetry. It is expressed as the relative standard deviation. As in the stability test, system suitability can be evaluated using the standard solution of analyte at 100% from the linearity test.[3]

Final considerations

Method validation is an essential step of quantitative analysis, ensuring the level of measurement and increasing the confidence in the results. Reference guidelines present the definition of the validation parameters and provide recommendations on how to evaluate them; however, these guidelines do not present detailed information on how to test these parameters simultaneously.

In this paper, we aimed at introducing an idea of method validation optimization; however, different schemes considering the necessary parameters might be proposed in order to optimize the validation process. To our knowledge, this is the first study to address the optimization of method validation through a rapid and concise procedure, improving usual and customary processes. This working methodology can be applied easily to routine quality control, as well as scientific research, producing more rigorous results and enabling methodological problems solving.

The main advantage of the proposed scheme is the time optimization, since all the required data can be obtained in a maximum of four days of tests. Additionally, it leads to solvents and reference standards savings, consequently generating less chemical waste. Nevertheless, in practice minor adaptations might be required according to the laboratory routine, difficulty level of sample preparation and total analysis time.

Besides composing the first level of quality assurance (QA),[1] validation of methods provides valuable information about the specific characteristics of method performance and its critical steps.[6] Given the significance of obtaining reliable results in pharmaceutical analysis, further research is needed to improve the processes related to the validation of analytical methods.

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