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Are analysts doing method validation in liquid chromatography?

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ABSTRACT

Method validation is being applied in the reported analytical methods for decades. Even before this protocol was defined, authors already somehow validated their methods without full awareness. They wished to assure the quality of their work. Validation is an applied approach to verify that a method is suitable and rugged enough to function as a quality control tool in different locations and times. The performance parameters and statistical protocols followed throughout a validation study vary with the source of guidelines. Before single laboratory validation, an analytical method should be fully developed and optimized. The purpose of the validation is to confirm performance parameters that are determined during method development, and it should provide information on how the method will perform under routine use. An unstable method may require re-validation. Further method development and optimization will be needed if validation results do not meet the accepted performance standards. When possible, the validation protocol should also be conducted as a collaborative study by multiple laboratories, on different instruments, reagents, and standards. At this point, it would be interesting to know how people are validating their methods. Are they evaluating all defined validation parameters? Are they indicating the followed guidelines? Is re-validation really currently used? Is validation performed by a single laboratory, or is it a collaborative work by several laboratories? Is it an evolving discipline? In this survey, we will try to answer these questions focused to the field of liquid chromatography.

1. Introduction

The aim of any analytical measurement is to obtain accurate, reliable, and consistent data to find the nature of a sample. These properties can be judged by the results obtained through method validation, which since long is an integral part of any good analytical practice. An analytical methodology should include besides the required data to solve the problem, at least the achievable sensitivity, accuracy, precision and range of application. Unless a method is used on a regular basis to provide confidence in its continued validity, it is essential to document that the method is still valid prior to analysis. Analytical methods should be validated, verified, or re-validated.

Analytical methods and techniques are constantly undergoing changes and improvements, since often they must stay at the cutting edge of the technology. It is also important to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria may need to be developed for each analyte. Moreover, the appropriateness of the technique may also be influenced by the ultimate aim of the study. When sample analysis for a given study is conducted at more than one site, it is necessary to validate the analytical method(s) at each site and provide appropriate validation information for different sites in order to establish inter-laboratory reliability. Also, while validation of each method is of interest by itself, there may be situations where comparison of methods will be necessary (e.g., when more than one method has been employed in a long-term study).

Method validation (also called method performance by some authors) implies not only the definition and evaluation of the classical validation or performance parameters (or characteristics): accuracy/recovery, precision (repeatability, intermediate precision and reproducibility), linearity and application range, limit of detection (LOD)/limit of quantitation (LOQ), selectivity/specificity, robustness, ruggedness, uncertainty, trueness, stability and system suitability studies, but also a detailed and extensive protocol on how to operate and transfer analytical methods and the involved procedures. All these validation parameters have been extensively

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commented and defined in the literature. Another performance parameter of an analytical method not usually included is the applicability (or scope).

Method validation is required to assure high quality and achieve acceptance of products by the international agencies. It is a mandatory requirement for accreditation as per ISO 17025 guidelines [1], and for registration of any pharmaceutical product or pesticide formulation. The main objective is to demonstrate that the procedure is suitable for its intended purpose [2–4]. In previous work, Rambla-Alegre et al. discussed the need of validating new developed procedures [5]. The conclusion of the authors was unequivocal: *No doubt, the answer is clearly yes. Even more, the analytical method validation should be a mandatory step to evaluate the ability of developed methods to provide accurate results for their routine application. Indeed, without results of adequate quality or reliability, the critical decisions that will be made during routine application of the method will be untrustworthy*. In that work, a complete and detailed definition and description of method validation parameters was provided.

This work shows a literature survey on how analysts are doing validation studies during method development in liquid chromatography (LC). This is not an easy task, due to the variety of existing fields (biological fluids, pharmaceuticals, impurities, microbials, food, and botanicals, among others), and applied analytical techniques. An additional problem is the number and variety of international renowned organizations offering guidelines on method validation.

2. The validation process

The validation process starts before an instrument is placed online, and continues long after method development and transfer. The validity of a specific procedure 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. Ideally, the validation protocol should be written following a thorough understanding of the method's capabilities and intended use. The validation protocol will list the acceptance criteria that the method can meet. Any failure to meet the criteria will require that a formal investigation be conducted.

Method validation is not a single event. It begins when an analyst has the initiative of implementing a new method in a laboratory, and obviously ends when the method is discontinued. The validation process is complex and time-consuming. Therefore, it must be broken down in several well-defined steps as described in Fig. 1.

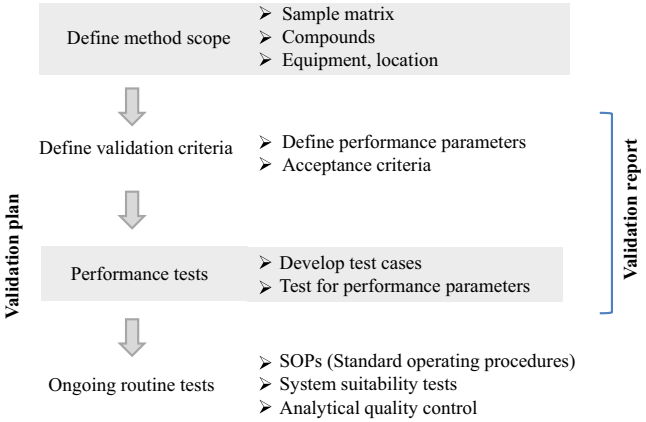

Fig. 1. Validation steps.

A validation plan is first developed. This should include owners, responsibilities and deliverables. The first step is to define the scope of the method. This includes the compounds and concentration ranges, sample matrix, specific equipment to be used, and location where the method should be performed. Once the target analysis is known, the performance parameters, performance tests and acceptance criteria should be defined. Test protocols are then developed with all experimental details, and the tests are executed according to the protocols. Tests results are compared with acceptance criteria. Finally, routine method procedures are developed to verify constant system performance at the time of analysis. Tests may include system suitability testing and the analysis of quality control samples. All experimental conditions and validation results must be documented in a validation report.

During the course of a product development program, a defined analytical method may undergo many modifications. These evolving changes require different levels of validation to demonstrate continuity of the validity of method performance. Three different levels/types of method validation are defined: full validation, partial validation, and cross-validation. Full validation is necessary when developing and implementing an analytical method for the first time for a new product. Partial validations are performed associated to modifications of validated analytical methods that do not necessarily require full revalidations. Cross-validation is a comparison of two analytical methods and is required when two or more analytical methods generate data within the same study.

Another topic in the field of validation is remediation of validated analytical methods. This is typically triggered by the need to improve existing methods used for controlling commercial products. The improvement may be required due to an unacceptable rate of method failures related to toxicity, environmental impact, lengthy run times, obsolete instruments or consumables, changing regulatory specifications, stability testing, or business interests, among others. Frequently, old methods have to be replaced by methods using newer technologies, creating a significant challenge for the industry in providing demonstration of method equivalency and a corresponding level of validation for the methods.

The biggest advantage of analytical method validation is that it builds a degree of confidence, not only to the developer but also to the user. Although the validation exercise may appear tedious, costly and time consuming, it eventually turns out to pay for itself, eliminating annoying repetitions and leading to better time management on the long term.

Finally, one of the key requirements for method validation (which is also one of the greatest challenges) is that only well-characterized reference standards (or materials) with well-documented purities should be used throughout the validation study. The degree of purity necessary depends on the intended use. The challenge stems from the fact that, in some cases, the tools used to characterize reference standards are being developed and validated at the same time as the reference standard itself. As part of method development, reference materials should be assessed for identity, purity, stability, and storage conditions. For all standards, the suitability for use should be ensured.

Reference standards can often be obtained from US Pharmacopeia (USP) and may also be available through the European Pharmacopoeia, Japanese Pharmacopoeia, World Health Organization (WHO), or National Institute of Standards and Technology (NIST). Reference standards for a number of biological products are also available from the Center for Biologics Evaluation and Research (CBER). Reference standards from other sources should be characterized by procedures including routine and beyond routine release testing as described in ICH Q6A.

If an appropriate certified reference material (CRM) is available, a single-laboratory test allows a laboratory to assess laboratory bias and method bias in combination, by analyzing the CRM a number of