Understanding and Implementing Efficient Analytical Methods Development and Validation

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Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

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To ensure compliance with quality and safety standards, the United States, Europe, Japan, and other countries have published compendia, or *pharmacopeias,* that describe official test methods for many marketed drug products. For example, compendial analytical methods found in *United States Pharmacopeia 25* (*USP 25*) are legally recognized analytical procedures under section 501 (b) of the Federal Food, Drug, and Cosmetic Act. For these compendial methods, *USP* provides regulatory guidance for method validation (1). In addition, validation of analytical methods is covered by the United States *Code of Federal Regulations* (*CFR*). Specific references are 21 *CFR* 211.165 (e) and 21 *CFR* 211.194 (a).

Method validation is defined as the process of proving (through scientific studies) that an analytical method is acceptable for its intended

use. Recent guidelines for methods development and validation for new noncompendial test methods are provided by the FDA draft document, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation" (2). This recent document applies to the method development and validation process for products included in investigational new drug (IND), new drug application (NDA) and abbreviated new drug application (ANDA) submissions. Therefore, expectations from regulatory agencies for method development and validation are clear.

In recent years, a great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe, and Japan. As part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation. The recent FDA methods validation draft guidance document as well as *USP* both refer to ICH guidelines (2). Analytical guidance documents recently published by the ICH are the following:

- stability testing (Q1)
- validation of analytical procedures (Q2)

- impurities in drug substances and products (Q3)
- specifications for new drug substances and products (Q6).

Additional regulatory guidance can be found on the FDA Web site www.fda.gov/cder/guidance and on the ICH Web site www.ich.org. These sites ensure access to current methods development and validation guidelines.

The methods validation documentation requirements for IND and NDA submissions are outlined in the chemistry, manufacturing and controls (CMC) guidance document (2). The current trend continues to be in the direction of phase-dependent methods development and validation. Nonvalidated screening methods are used to monitor the synthesis of active ingredients or to confirm their identity during discovery and preclinical research. Analytical methods are progressively optimized and a preliminary validation package is furnished as part of the IND application before Phase I safety trials are initiated. All analytical methods should be fully optimized and validation completed before the NDA is submitted at the end of Phase III studies.

Method validation is a continuous process. The goal is to ensure confidence in the analytical data throughout product development.

The method development and validation processes

The steps of methods development and method validation depend upon the type of method being developed. However, the following steps are common to most types of projects:

- method development plan definition
- background information gathering
- laboratory method development
- generation of test procedure
- methods validation protocol definition
- laboratory methods validation
- validated test method generation
- validation report.

A well-developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. As the methods development and validation processes advance, the information gathered is captured in the design and subsequent improvement of the method. Ideally, the validation protocol should be written only 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.

The required validation parameters, also termed *analytical performance characteristics*, depend upon the type of analytical method. Pharmaceutical analytical methods are categorized into five general types (3):

- identification tests
- potency assays
- impurity tests: quantitative
- impurity tests: limit

- specific tests.

The first four tests are universal tests, but the specific tests such as particle-size analysis and X ray diffraction are used to control specific properties of the active pharmaceutical ingredient (API) or the drug product.

Validation requirements depend upon the type of test method, including

- *specificity:* ability to measure desired analyte in a complex mixture
- *accuracy:* agreement between measured and real value
- *linearity:* proportionality of measured value to concentration
- *precision:* agreement between a series of measurements
- *range:* concentration interval where method is precise, accurate, and linear
- *detection limit:* lowest amount of analyte that can be detected
- *quantitation limit:* lowest amount of analyte that can be measured
- *robustness:* reproducibility under normal but variable laboratory conditions.

Only specificity is needed for an identification test. However, the full range of specificity, accuracy, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), precision, and robustness testing is needed for more-complex methods such as quantitative impurity methods.

The validated test method is included in the validation report that summarizes the results of the validation studies. Both the validation report and test method are submitted as parts of the NDA or ANDA.

Advances in technology and equipment

Recent progress in methods development has been largely a result of improvements in analytical instrumentation. This is especially true for chromatographs and detectors. Isocratic and gradient reverse-phase HPLC have evolved as the primary techniques for the analysis of nonvolatile APIs and impurities.

The HPLC detector of choice for many types of methods development is the photodiode array (PDA) detector because it can be used for both quantitative and qualitative analysis. The use of a PDA detector to determine peak purity of the active ingredient in stressed samples greatly facilitates the development of stability-indicating assays.

The emphasis on the identification of trace impurities and degradants has led to the increased use of hyphenated techniques such as liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–nuclear magnetic resonance spectroscopy (LC–NMR). This trend will continue with the need to better define degradation pathways.

The ultraviolet (UV) absorbance detector remains the most common HPLC detector for potency and impurity analysis. Once specificity has been demonstrated, the PDA detector is replaced with a variable wavelength detector and the HPLC effluent is monitored at fixed wavelengths. Stability-indicating and

impurity methods often are required to measure analytes within a wide concentration range. For example, process impurities and/or degradation products may be present at levels of 0.1%, and the main active ingredient typically is present at the nominal concentration (100%). This amount is well within the linear range of a fixed wavelength detector but not within the linear range for LC–MS detectors.

Recent FDA and ICH guidance about chiral drug products and impurities has posed new challenges for methods development scientists (3). However, recent advances in the use of chiral HPLC columns has greatly facilitated progress in this area. The advances are primarily a result of the introduction of chiral stationary phases (CSPs) prepared by reacting amylose or cellulose derivatives with silica. The new CSPs allow trace levels of enantiomeric impurities to be measured.

Gas chromatography remains the method of choice for the analysis of volatile compounds. Gas chromatography with mass spectrometry detection (GC–MS) is increasingly being used to identify impurities and to determine active ingredient peak purity in stressed samples.

Advances in laboratory robotics and automation also are beginning to be applied to methods development and validation. Development teams are using laboratory robotics to develop automated methods for high-volume tests.

An in-depth review of all the recent advances in analytical instrumentation is beyond the scope of this article. However, several methods should be noted. Advances in the use of nondestructive infrared (IR) and near-infrared spectroscopy (near IR) and NMR techniques are particularly promising for methods development scientists.

Issues and challenges

For a methods development and validation program to be successful, a holistic approach is recommended. A common challenge encountered during methods development and validation is that methods are typically developed by the R&D department, whereas validation is typically the responsibility of a validation group. It's important that the R&D and validation groups work as one team.

Various groups also may be responsible for ensuring the suitability of the methods to support early clinical phases and commercial manufacturing. The transfer of analytical methods from one group to another then becomes an important step for ensuring that the proper validation is in place to justify its intended use.

Because the method will be run by several groups during its progression from development to validation, the method must be robust. This means the method should provide reliable data, both on a wide range of equipment and in the hands of several chemists. A common weakness in development and validation of methods is that the methods are not robust enough. If robustness is not built into methods

early in development, then the result most likely will be loss of efficiency during routine QC testing and a lengthy and complicated validation process as well.

Another challenge encountered early in the development of methods intended to support stability studies is ensuring that the method is stability indicating. This process is typically achieved by conducting forced-degradation studies. The design and execution of these studies requires thorough knowledge of the product being tested as well as a good understanding of the analysis technique.

As mentioned previously, new regulatory guidelines are being published governing the expectations of regulatory agencies throughout the world for methods development and validation. Another challenge is that many pharmaceutical companies must upgrade methods to meet current regulatory standards. From a simple method improvement to a complete redevelopment and subsequent cross-over to an older method, the upgrade of analytical methods can be a daunting task. For this reason, one must be alert to current trends in regulatory guidelines and to adopt a proactive approach to changes that may affect development and validation programs.

Finally, one of the key requirements for methods validation (which is also one of the key challenges), is that only well-characterized reference materials with well-documented purities should be used during method validation activities. The challenge stems from the fact that, in some cases, the tools used to characterize reference standard materials are being developed and validated at the same time as the reference standard itself.

Conclusion

The efficient development and validation of analytical methods are a critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance. Experience is one of these factors—both the experience level of the individual scientists and the collective experience level of the development and validation department. A strong mentoring and training program is another important factor for ensuring successful methods development and validation. Companies must maintain an appropriate level of expertise in this important dimension of developing safe and effective drugs.

References

1. *USP 25–NF 20* (United States Pharmacopeial Convention, Rockville, MD, 2002), p. 2256.
2. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls," *Federal Register* (Notices) **65** (169), 52,776–52,777 (30 August 2000).
3. "International Conference on Harmonization; Draft Guidance on Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and Products: Chemical Substances," *Federal Register* (Notices) **65** (251), 83041–83063 (29 December 2000). **PT**