Analytical Methods Validation

In-Process Control Methods for the Manufacture of Active Pharmaceutical Ingredients

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The authors propose a strategy for classifying and validating in-process testing methods.

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In-process methods are key components of quality control in a chemical manufacturing plant. These methods ensure that a production reaction step conducted by trained operators within the entire validated process will produce a quality chemical entity in the expected yields. The presence of impurities and related compounds (derived from the reaction or secondary reactions) is a critical parameter that determines a synthetic material's quality.

Chemical processing differs from product manufacturing. For example, the manufacture of a finished product typically involves a molecular entity that is stable under normal conditions and can be stored for prolonged periods without losing its physical and chemical characteristics. Most chemical reactions, however, require very tight controls and close monitoring of their progress because any of several potential result paths may be followed if conditions are not monitored closely. Other factors such as temperature and pressure are critical parameters for the successful completion of the chemical conversion process.

Each chemical reaction is unique. Consider the combination of reactants and the resulting end products, for example. One must examine conditions such as temperature, light, heat, environment, and the reaction vessel's surface. In addition, whether the reaction is chemical or biological is an important factor. Therefore, each process must be analyzed separately and classified according to the International Conference on Harmonization (ICH) Q7A guidance. This task is important because a reaction step may generate an impurity that may be carried over to the active pharmaceutical ingredient (API), regardless of how far apart that process may be from the API.

ICH's Q7A guidance briefly mentions analytical methods validation and does not discuss the in-process control methods for each reaction. The guidance does indicate, however, that as the process gets closer to the manufacture of the key intermediate and the API, the current good manufacturing practices (CGMP) requirements become more demanding.

This article outlines a plan for classifying and validating in-process testing methods and is intended as a foundation for assessing the parameters and acceptance criteria needed for validation.

Matrix and interferences

To characterize a reaction by means of an analytical method, it may be necessary to prepare a matrix, which functions similarly

Definitions

Although the International Conference on Harmonization established several definitions in its Q7A and Q2B guidances, the following terms are not defined in those documents:

- *Intermediate-release method*: a method developed and used for the release of an isolated intermediate material produced during the manufacture of an active pharmaceutical ingredient (API) before the material is used, stored, or shipped. It may contain more than one specification for product release.
- *In-process control method:* a method developed and used for monitoring the progress of a reaction (*e.g.,* disappearance or formation of intermediates) or a critical attribute of a reaction such as moisture or pH;
- *Marker:* a stable material, characterized by nuclear magnetic resonance, mass spectrometry, or another scientifically recognized characterization method, that is related to the process being monitored. Its identity must be reconfirmed periodically;
- *Step* (or *stage*): the synthesis progression for converting one molecular entity to another in an API manufacturing process. This step may have several conditions. A step is complete when the converted intermediate either is isolated or is stable enough that it can be converted *in situ* to another molecule.

to a placebo for a finished product. A matrix is the combination of the reactants without the main component or precursor being converted. Because of the nature of some reactions, the combination of reagents may not be possible. On the other hand, adducts or complexes can be formed, which would not otherwise be formed in the presence of a component being converted into a product. The preparation of the matrix must be judged by the scientists working with the reaction process.

Classification of methods

For monitoring purposes, analytical methods can be classified according to the manufacturing step in which they are applied. The document indicates that the GMP requirements become more stringent as the synthesis steps approach the API. Beginning with the introduction of the starting material into the process, manufacturing processes can be divided into three classes, which reflect the practices established in the ICH Q7A guidance.

- intermediates production (*e.g.,* alkylation, hydrogenation)
- isolation and purification (*e.g.,* washing, crystallization)
- physical processing and packaging (*e.g.,* micronization).

For the purposes of this article, intermediates production is subdivided into intermediates and key intermediates production. The classification of the methods (*e.g.,* in-process controls and intermediate-release methods) is determined by how far the stage or step is removed from the API. Figure 1 represents the application of the ICH guidance to these classes.

The ICH Q2B guidance enables chromatographic resolution to be used as an indicator of specificity for critical separations, which means that peak purity is not necessary. Furthermore, peak purity should not be a consideration because samples are submitted only to confirm the disappearance of the starting material and the formation of the desired adduct. No peak will be as pure as required when the analysis is a crude reaction mixture.

Solution and standard stability should be included as part of some of the studies. The length of the stability study is defined by the process requirements.

Class 1. The Class 1 classification is exclusive to methods used for in-process control (and monitoring) of intermediate steps during an API manufacturing process. The classification pertains to reactions that are at least two steps from the processing of the key intermediate. Because the formation or source of impurities should be known and each impurity identified, it is possible that in some instances, the classification becomes Class 2 several steps before the key intermediate production.

Class 2. Class 2 is exclusive to methods used for in-process control (and monitoring) of intermediate steps during an API manufacturing process. The classification pertains to those reactions that precede the formation of the key intermediate.

Class 3. This classification includes methods used as intermediate-release methods when the product formed is an intermediate that will be used further after isolation or supplied as a starting material for another synthesis. This classification pertains to the key intermediate or isolated entity that eventually will be converted to an API.

An example of how processes would be classified is shown in Figure 2. Substance D is the key intermediate, one step before the API formation. Substance E represents the final API molecule before purification. Substance B, for example, could be subject to intermediate-release method testing if the material is isolated and the starting material is used in a parallel synthesis. In this case, the purification step does not involve any chemical conversion and the API is structurally identical to Substance E. The purification step can be recrystallization, micronization, or any other physical manipulation of the active that does not involve a chemical conversion or change in chemical structure. In addition, a reaction sequence may involve the isolation of an intermediate that is several steps away from the formation of the key intermediate. The intermediate would be classified as an intermediate-release method or Class 3.

Validation of methods. The suitability of all methods used as in-process control methods and as intermediate-release methods should be verified and documented under actual conditions of use. Each category has a recommended suitability procedure defined. The degree of analytical validation performed must reflect the purpose and stage of the API production process. All analytical equipment must be qualified before it is used for method validation. Complete records must be maintained for any and all equipment modifications made to validate analytical methods.

The validation process may require that intermediates be characterized, isolated, and used as reference markers for establishing the relative retention times. The preparation of a matrix or reaction mixture without the active may help establish unknown peaks and potential interferences. The matrix must be treated according to the procedures established for in-process control monitoring methods.

If an intermediate is not isolated, but reacted *in situ* to a later step, isolation may not be necessary for its characterization if it is an unstable entity. Should it be a stable molecule, however, its isolation and characterization may be necessary.

Chromatographic methods

Chromatographic methods are validated according to their classification, as discussed previously. The method validation pro-

Table I: Summary of the requirements, per classification, for chromatographic methods.

Performance parameter	Class 1	Class 2	Class 3
System suitability			X
Accuracy			X
Precision			X
Quantitation limit		X	X
Detection limit	X		X
Specificity	X	X	X
Linearity		X	X
Robustness			X
Standard and solution stability		X	X

tocol should include a discussion of the method's classification and the justification for the classification. The validation described for each classification is for quantitative chromatographic methods. Chromatographic identification and semiquantitative techniques such as thin-layer methods must be validated (described later in this article). These methods require the determination of accuracy in their semiquantitative level. Table I summarizes the requirements of each classification.

Class 1. Description. Class 1 methods are limit tests and must be validated accordingly. This validation should include a demonstration of the method's detection limit specificity and determination because these steps are far removed from the formation of the key intermediate and API. Therefore, one must be able to identify the peak of interest, properly resolved from the starting materials (reactants). The method should confirm the disappearance of the reactants or the formation of the adduct.

Requirements. The testing needed to monitor the intermediate steps during API manufacture falls into Class 1. According to ICH Q7A, this classification suits reaction steps far removed from the formation of the API and for which CGMP requirements are not as demanding.

As is typical for every method, system suitability is a requirement and usually includes injecting a marker for methods using response normalization for quantitation. Precision is not necessarily a requirement at this stage. Knowledge of the relative retention times of the reactants being monitored is essential, however.

The method's sensitivity should be established once the target entity monitored is quantified and the limit is established. The limits can be established by following ICH Q2B recommendations, which suggest a linearity experiment. At this stage, running a three-point linearity experiment around the target value can be used to estimate the detection limit. For example, consider a reaction in which the transformed or consumed reactant will be monitored until the area of the peak of its signal is 0.5% of the product being formed. The linearity experiment can be executed to include 0.25, 0.50, and 0.75% of the reactant. Linear regression results would provide the intercept's slope and standard error, and thus, the estimation of the detection limit for this classification. Alternatively, estimation solutions can be prepared. When a signal-to-noise (3.3:1) ratio is obtained for the solution, the figure becomes the detection limit for the method.

Class 2. Description. Class 2 method validation includes the limit-test requirements described previously (specificity, detection limit), the determination of the method quantitation limit, and the demonstration of the method linearity covering the entire range of the method (*i.e.,* reactants and adduct being formed to determine the specified limit). Thus, the detection limit and quantitation limit values should be calculated from the linearity data generated during the execution of the validation protocol. The testing required for monitoring the formation of the key intermediate falls under Class 2.

As with Class 1, a marker solution can be injected to establish the system-suitability criteria such as resolution and tailing factors. Again, precision may not be an issue because the criticality of this monitoring is the appearance or disappearance of a reactant or an adduct relative to each other.

The determination of the quantitation limit would be part of the limit-test requirement. In this case, because we are further into the ICH 7A-suggested criteria for CGMPs, it is recommended that the linearity experiment be conducted over its full range. This range would be from the quantitation limit to at least 125% of the concentration range monitored. If the target value lies far from the quantitation limit (*e.g.,* the quantitation limit is 0.01% and the target concentration is 5%), then a three-point calibration should be run close to the quantitation limit. This step would be followed by a normal five-point linear regression from 50 to 125% of the target. It is useful to compare the precision between the curves and to compare the response factors obtained from both curves.

Solution and standard stability are essential components at this stage of the synthesis. The CGMP requirements mandate that these solutions be stable for the time of use. Any degradation could generate false data and miscalculations/misidentifications of unknowns. The length of stability will be defined by the process requirements.

Class 3. Description. In essence, Class 3 methods are finished-product (release) methods that control the final product of the manufacturing process. As such, the extent of validation for this type of method approximates the extent of validation for the finished product methods described in the *US Pharmacopeia* (*USP*). Class 3 methods validation includes linearity, accuracy, precision, specificity, and intermediate precision. The ranges applied to each parameter are subject to internal procedure requirements. The rationale for the ranges used must be clearly explained in the methods validation protocol. The impurity method parameters will be applied when the reactant is the quantified chromatographic component.

Requirements. The testing required for the monitoring reactions for the formation of the final API molecule falls into Class 3. The following are the specific requirements for validating such methods:

- The standard must be fully characterized by scientifically recognized methods.
- *USP* standards should be used if they are available.

Semiquantitative thin-layer chromatography methods are in this category. This technique is a two-dimensional chromato-

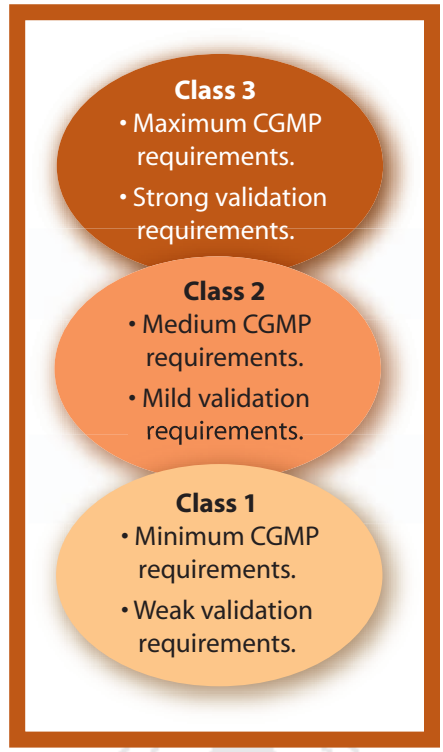

Figure 1: The classes to which an API manufacturing process is divided, according to the International Conference on Harmonization Q7A guidance document.

graphic procedure. As such, the system must be tested and the procedure must be clear.

The accuracy and linearity of the technique being qualified as a semiquantitative method must be verified. The reference standards must be prepared at concentrations ranging from a quantitation limit to at least 125% of the concentration targeted in the analysis. This information is a visual calibration for the method.

On the other hand, specifications must be set for the response factor (R_f) of the spots of reference material and any other reference substances that are critical for the test. This process will establish the conditions for system suitability. Furthermore, the sensitivity of the method must be determined to enable visual calibration to be established and a reliable limit to be set for the test.

Along these lines, the composition of the mobile phase and the length of the plate run are critical to this test. The concentration established for the test must be such that the separation yields good resolution (R) between the closest eluting components of the mixture. The process also involves changes in chromatographic plates and analyst-to-analyst comparison. Therefore, robustness also is required.

Table II: Summary of the requirements for nonchromatographic methods.

Performance parameter	Titration	Atomic absorption	UV-*vis*
Calibration		X	X
Accuracy	X	X	X
Precision	X*	X	X*
Quantitation limit		X	X
Detection limit		X	X
Specificity	X		X
Linearity	NA	X	NA
Standard and solution stability			X

*Intermediate precision.
NA indicates not applicable

A comparison of a unique R_f for the main substances monitored or quantified with the R_f of other major components of the reaction would yield the test's specificity. Once again, standard and solution stability are critical as controls for the methodology and for ensuring reliable results.

Nonchromatographic instrument methods

Among nonchromatographic methods, titration can be used for monitoring reaction sequences. Titration methods will require validation beyond equipment qualification.

Linearity and accuracy for the technique are important. Linearity is established by preparing three-point calibration curves. Regarding accuracy, the titration would require the determination of matrix contributions, if any, to the end point of the procedure. The preparation of spiked solutions, as well as the titration of blanks containing matrix elements, is required for validating the reliability of the technique. Thus, precision can be assessed from the experiments conducted under linearity and accuracy.

The reaction and its stoichiometric relationship can be presented to assess the specificity of the method. If a method is not specific, an explanation or the rationale for using a nonspecific method must be provided in the method validation protocol.

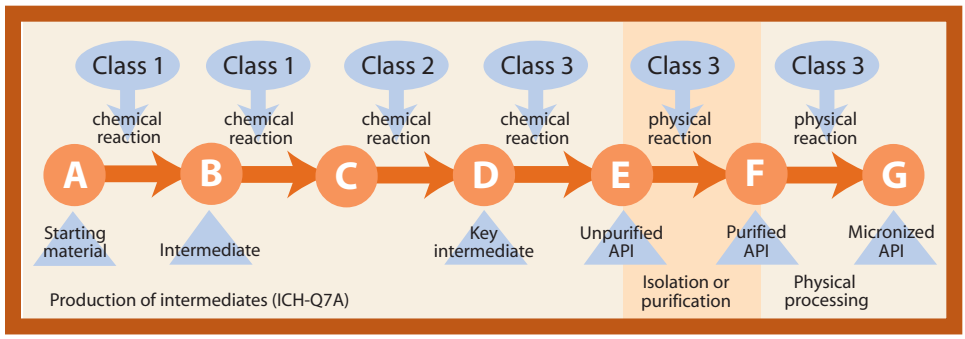

Figure 2: An example of how processes can be classified.

In-process spectrophotometric methods

With the exception of chromatographic methods, in-process spectrophotometric methods are those for which the end detection technique is spectrophotometric. When these methods are used for identification purposes, only sensitivity, standard, and solution stability data are required. The calibration of each technique is required before analytical work can begin.

The validation of atomic absorption techniques should include linearity, accuracy, precision, and specificity. Although specificity is inherent to the technique, the protocol and its report should indicate the analysis specificity of the particular metal.

Furthermore, the sensitivity (detection limit) and standard and solution stability also should be included. For system suitability, the %RSD of a standard's replicate sampling measurements usually must be reported. At minimum, most systems include a calibration curve and a standard confirmation reading. Systems that do not report %RSD values and/or include a calibration curve must be addressed in the methods validation protocol and report. Table II summarizes the validation requirements for nonchromatographic methods.

UV-*vis* spectrophotometry should include linearity, accuracy, precision, sensitivity (detection limit), and standard and solution stability. This technique is nonspecific. A calibration curve is an essential component for analyses such as the American Pharmaceutical Association's standard for absorption of light (color) and requires the preparation of primary standards. If a calibration curve must cover a range of time (*e.g.,* 7 days), the statement's validity must be established and documented.

Conclusion

Monitoring chemical processes for the formation of an API is the first step to ensuring quality in pharmaceutical manufacturing. Having reliable and reproducible methods will enable the production plant to guarantee the consistency of drugs batch after batch. Furthermore, it may simplify the characterization of such processes and their chemical profile.

Through the years, vast publications and general information have been presented to pharmaceutical industry specialists about the validation of analytical methods. Federal and international regulatory groups have published various guidelines to shed light on analytical method validation. No such emphasis has been given, or guidances described, however, the validation of in-process control methods. This article intends to establish a starting point for discussions about the validation of in-process methods.

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