Validation Viewpoint

Validation of Impurity Methods, Part II

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This column is the second installment in a two-part series that reviews International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and U.S. Food and Drug Administration (FDA) impurity method validation guidelines. In the first column, the authors discussed background information such as validation policy and laboratory controls that pertain to validation. In the second column, they address specifics of ICH and FDA guidelines about impurity method validation components such as specificity, linearity, and reproducibility.

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Validation Viewpoint Editors

This column is the second in a two-part series about impurity method validation guidelines provided by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the U.S. Food and Drug Administration (FDA). ICH and FDA have written many guidance documents for scientists in the pharmaceutical industry to facilitate successful and consistent performance of method validation work and therefore provide the highest level of protection to patients who rely upon high-quality medicines. Documents from ICH are available on its web site at http://www.ich.org/, and FDA documents can be found on its web site at http://www.fda.gov/cder/guidance/index.htm.

Part I of this series included an overview of both FDA and ICH guidelines — including topics such as method validation background, validation policy, laboratory controls, types of analytical procedures to be validated, classification of impurities, validation documentation, and reporting impurity content of active pharmaceutical ingredient (also known as drug substance, bulk drug substance, or bulk active) batches (1). Part II of this series addresses specific validation components such as specificity, linearity, and reproducibility. The ICH and FDA guidelines contain a great deal of detailed information about method validation, and readers should refer to them to gain a full understanding of this subject (2–5). Method validation discussed in this series pertains to validation of high performance liquid chromatography (HPLC) methods for assessing organic impurity levels in active pharmaceutical ingredients. To ensure that the data from impurity methods are reliable (precise and accurate), pharmaceutical companies are expected to validate impurity methods for the active pharmaceutical ingredient and latter-stage key synthetic intermediates.

Validation of Impurity Methods

The common aspects of assay validation include specificity, accuracy, precision, limit of detection, limit of quantitation, linearity, range, and robustness. In addition, we recommend that analysts examine sample-solution stability and establish an appropriate system-suitability test to verify the proper functioning of the HPLC system (2–5). These validation aspects are discussed below.

Specificity: ICH defines specificity as the ability to unequivocally assess an analyte in the presence of components that can be expected to be present (2). Impurity methods must be specific to ensure that levels of all impurities in the active pharmaceutical ingredient are measured accurately. For an impurity method to be acceptable, the impurity and active pharmaceutical ingredient peaks should be well resolved from each other. Impurities are those organic impurities discussed above that would be expected to be present in active pharmaceutical ingredients; they could include starting materials, intermediates, by-products, and degradants. Because method specificity must be satisfied by the conditions chosen in the separation, it makes the most sense to examine method specificity first, before moving to other validation criteria. If the current chosen chromatographic conditions fail to satisfy method specificity requirements, then the analytical method will require further optimization. After the method has been optimized to satisfy method specificity, then analysts can address the remaining validation characteristics with confidence.

We will devote most of our attention in this column to specificity testing relative to the other method validation aspects. Specificity testing probably is the most complex but also the most interesting part of impurity method validation. The goal is to design an analytical method that separates all impurities from each other and from the active pharmaceutical ingredient peak.

Thus, the separation challenge comes from the impurity challenge. During method development and before investigational new drug (IND) application filing, analytical researchers first must identify active pharmaceutical ingredient samples that contain impurities that are expected to be in the toxicology and clinical active pharmaceutical ingredient batches. The impurity challenge depends, in part, upon the chemical process used to synthesize the active pharmaceutical ingredient. By working closely with colleagues in the process chemistry department, analysts usually can obtain representative samples containing impurities to be expected in the active pharmaceutical ingredient. Degradants can be obtained from forced degradation studies or from actual stability samples (6,7). The analytical method can be developed with representative samples.

Two main challenges exist at this first stage: to ensure impurities are resolved from each other and to ensure impurities are resolved from the active pharmaceutical ingredient. Peak resolution can be verified by inspecting the chromatograms. Resolution from the active pharmaceutical ingredient can be verified by inspecting the chromatograms to ensure the peaks are Gaussian and analyzing peaks for homogeneity by photodiode-array detection, liquid chromatography–mass spectrometry (LC–MS), and chromatographic methods of alternate selectivity.

Photodiode-array detection can check for the presence of coeluted substances with differing chromophores. Peak-purity analysis software is available as part of standard chromatographic software packages provided by several vendors. This technique has limitations, however. Firstly, if coeluted substances have the same chromophores, then it is more difficult for the peak-purity software to detect peak inhomogeneity. Secondly, if impurities coeluted with the active pharmaceutical ingredient are present at 0.1–0.5% levels, it will be difficult for the peak-purity software to detect the subtle active pharmaceutical ingredient peak chromophoric changes, even if the impurity chromophores differ from the active pharmaceutical ingredients. This problem is a concern, because impurities greater than or equal to 0.10% that are not qualified for safety by toxicology testing (that is, not present in the toxicology batch) typically are not permissible in clinical active pharmaceutical ingredient batches unless their structures and toxicities are known.

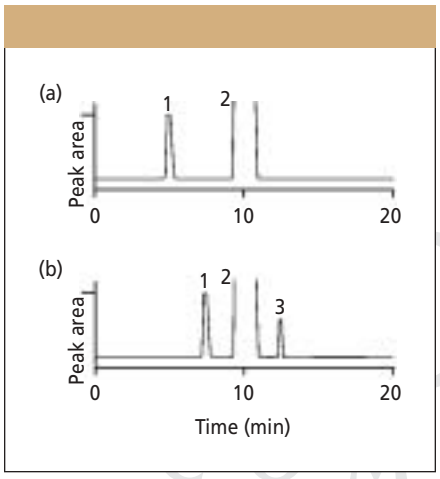

Figure 1: Hypothetical example of improved resolution achieved by changing mobile-phase selectivity under (a) methanol–water and (b) tetrahydrofuran–water conditions. Peaks: 1 = 0.5% impurity, 2 = active pharmaceutical ingredient, 3 = 0.3% impurity.

LC–MS is a powerful tool for peak-purity analysis. Selected-ion monitoring by LC–MS enables the detection of single substances as they are eluted, based upon their mass-to-charge ratio and fragmentation pattern. This tool gives researchers a greater ability to detect peak inhomogeneity. For example, very low–level impurities coeluted with the active pharmaceutical ingredient peak (with different masses or fragmentation patterns) can be detected in this manner. However, impurities with identical masses and similar fragmentation patterns, such as some stereoisomers, cannot be detected this way. Furthermore, the method must be amenable to MS detection. Mobile phases that contain nonvolatile additives such as phosphoric, sulfuric, and perchloric acids or nonvolatile ion-pairing agents typically are not amenable to LC–MS; however, newer LC–MS designs can accommodate nonvolatile additives. If the separation depends upon the use of these additives, then the use of LC–MS is difficult. Nevertheless, it is worth the effort to have LC–MS methods for active pharmaceutical ingredient impurity analyses because this technique can provide a wealth of information — primarily impurity identification, a very important part of the drug development process.

Finally, using methods of alternate selectivity is a valuable, yet traditional, approach to ensuring method specificity. This approach is based upon the changing of peak elution order concomitant with method selectivity alteration. To illustrate, we will discuss a hypothetical example: The preferred analytical method is 20 min long and uses an isocratic elution of a C18 HPLC column with 50:50 (v/v) methanol–water. Only one impurity (0.5%) is observed by this technique, and it is eluted at a retention time of 5 min, but the active pharmaceutical ingredient is eluted at a retention time of 10 min and accounts for 99.5% of the integrated chromatogram peak area. We observe no other impurities, and all impurities are eluted within the run time (see Figure 1).

To determine if any impurities are coeluted with the active pharmaceutical ingredient, we can change the method selectivity. Changing the elution conditions from 50:50 (v/v) methanol–water to 30:70 (v/v) tetrahydrofuran–water can accomplish this change. As shown in Figure 1, using the tetrahydrofuran–water conditions, the active pharmaceutical ingredient peak still is eluted at 10 min retention time, but the 0.5% impurity now shifts to 7.5 min and a new impurity, present at a level of 0.3%, is observed at 12.5 min. Furthermore, the active pharmaceutical ingredient peak now represents only 99.2% of the integrated chromatogram peak area. This strongly suggests that the 0.3% impurity was coeluted with the active pharmaceutical ingredient peak when using the water–methanol conditions. If the 0.3% impurity was an active pharmaceutical ingredient stereoisomer with the same MS fragmentation pattern, then this traditional approach would have been the best one to solve this problem.

Other approaches to changing selectivity include changing columns (for example, to different reversed-phase, ion-exchange, or normal-phase medias) and using entirely different techniques orthogonal to HPLC (such as capillary electrophoresis, gas chromatography, or thin-layer chromatography); however, each of these approaches has its strengths and limitations.

After analysts have established method specificity for the active pharmaceutical ingredient and impurities, they must examine method specificity to ensure that degradants also are well resolved. Success with this part of validation is necessary to ensure a method will be stability indicating. Typically, during stability testing, the active pharmaceutical ingredient will degrade partially under the more stressful (forced degradation) conditions. ICH has no specific guidelines for conducting forced degradations (8). The industry standard is to degrade the active pharmaceutical ingredient by 10–30% using conditions of acid and base hydrolysis, oxidation, dry heat, and light exposure. The interesting part of

this research is designing conditions that will degrade the active pharmaceutical ingredient only partially. This research is where analysts have the opportunity to use chemical knowledge as it relates to the active pharmaceutical ingredient structure to achieve these degradations. After active pharmaceutical ingredient partial degradation, the samples should be quenched, if necessary, to stop further reaction and analyzed to ensure all impurity and degradant peaks are resolved and all degradants are resolved from each other and the active pharmaceutical ingredient (using peak-purity analysis approaches, as described above).

With success in validation of method specificity, researchers can be confident that the chromatographic separation conditions have been achieved. Subsequently, other aspects of the chromatographic method should be validated.

Accuracy: Accuracy is defined as the measure of how close an experimental value is to the true value (2). ICH recommends that accuracy be assessed on samples spiked with known amounts of impurities (3). Users simply spike increasing amounts of known impurities into an active pharmaceutical ingredient test sample and measure the closeness of the obtained result to that of the known amount of each impurity in the sample plus the added amount. This testing should be performed individually for each impurity in an ideal case.

Early in the drug development process, impurity and degradant standards sometimes are unavailable. In these cases, it is acceptable to compare impurity values from the procedure under validation with an alternative impurity assay such as an alternative impurity assay designed during the method specificity testing as described above (3). Workers simply obtain an impurity content value for each impurity from one procedure and compare it with those obtained from a second, well-characterized procedure.

When authentic impurity standards are unavailable, it is acceptable to use the active pharmaceutical ingredient response factor when measuring impurity levels (3). In these cases, the accuracy of the impurity measurement relies upon the closeness of the impurity response factor to that of the active pharmaceutical ingredient. Finally, the method should specify how the individual or total impurities should be determined; for example, by weight-to-weight assay (versus external, authentic impurity standards or the active pharmaceutical ingredient diluted to a concentration close to that of the expected individual impurity levels) or by area percentage. In all cases, impurity levels should be expressed with respect to the major analyte.

Precision: The precision of an analytical procedure is defined as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (2). The precision of an analytical procedure usually is expressed as the variance, standard deviation, or coefficient of variation of a series of measurements. Three types of precision are relevant to impurity methods: repeatability, intermediate precision, and reproducibility.

Repeatability expresses the precision under the same operation conditions during a short interval of time. Repeatability also is called *intraassay precision.* Assay attributes examined in this regard include impurity peak retention time and area. Repeatability is broken down into injection repeatability and analysis repeatability. Injection repeatability is a measure of the precision of the analytical instrument to measure impurity levels in the same sample. Analysis repeatability is a measure of the precision of impurity measurements generated by an analyst working with one analytical system and analyzing several preparations of the same analyte.

Intermediate precision formerly was considered a part of ruggedness. Intermediate precision is a measure of the reliability of the method in an environment other than that used during method development. The objective is to ensure that the method will provide the same results when similar samples are analyzed after the method development phase is complete (3). This attribute typically is examined by having multiple analysts perform analyses of the same sample on different days and different instruments. Finally, *reproducibility* is assessed by an interlaboratory trial (3).

Limit of detection: The limit of detection of an analytical procedure is defined by ICH as the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value (2). ICH and U.S. Pharmacopeia–National Formulary (USP–NF) recommend several approaches to detection limit determination but indicate that other approaches could be acceptable (3,9). These approaches are based upon visual inspection, signal-to-noise ratio (S/N), and the standard deviation of the response and the slope.

Using the approach based upon visual inspection, the detection limit is determined by analyzing samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be detected reliably (3). This approach typically is used for noninstrumental methods. In the second approach, based upon S/N, the detection limit is determined by analyzing samples containing known low concentrations of analyte and samples containing blank solutions and then measuring the signal in the former and the level of noise in the latter. Typically, the limit of detection is considered to be the sample concentration at which the S/N is between 3 and 2:1. This convenient, commonly used method is for determining the detection limit for HPLC impurity methods. A third method of determining the detection limit is based upon the standard deviation of the response and the slope (3). The ICH guidance provides a good description of this third approach (3).

Limit of quantitation: The limit of quantitation of an analytical procedure is defined by ICH as the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy (2). The ICH guideline states that for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection and quantitation limits should be commensurate with the level at which the impurities must be controlled (3). Similar to the case with detection limit determination, ICH and USP–NF recommend several approaches to quantitation limit determination, but again indicate that other approaches can be acceptable (3). As above, these approaches are based upon visual inspection, S/N, and the standard deviation of the response and the slope.

Using the approach based upon visual inspection, the detection limit is determined by analyzing samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision (3). Using the second approach, based upon S/N, the quantitation limit is determined by analyzing samples containing known low concentrations of analyte and samples containing blank solutions and then measuring the signal in the former and the level of noise in the latter. Typically, the sample concentration at which the S/N is 10:1 is considered the limit of detection. This method for determining HPLC impurity method

limits of quantitation is common and convenient. A third method of determining the quantitation limit is based upon the standard deviation of the response (3). The ICH guidance also provides a good description of this third technique (3).

Linearity: The linearity of an analytical procedure is defined by ICH as its ability within a given range to obtain test results that are directly proportional to the concentration or amount of analyte in a sample (2). ICH recommends that the linearity should be determined at reasonably spaced concentrations across the range (see the Range section below) of the analytical procedure; it recommends a minimum of five concentrations (3). For active pharmaceutical ingredient impurity methods, the linearity for each impurity should be made with impurity standards in the range of the specification limit (again, see the Range section below). In the absence of authentic impurity standards, the drug substance can be substituted, and workers can make the assumption that the HPLC detector response for the active pharmaceutical ingredient is the same for the impurities (3). When analysts obtain additional information about the detector responses to specific impurities, they should include how to quantify or calculate the levels of these impurities in the HPLC method.

Users should make a plot of the detector response versus the analyte concentration and evaluate linearity by visual inspection of the plot. They should use statistical methods to evaluate the linearity such as calculation of a regression line by the method of least squares (3). ICH recommends that workers should obtain a regression or correlation coefficient (r) that is greater than or equal to 0.999 (3). The correlation coefficient, y intercept, slope of the regression line, and residual sum of squares also should be determined (3).

Range: The range of an analytical procedure normally is derived from the linearity studies, and ICH defines the range as the interval between the upper and lower concentration or amounts of analyte in a sample, including these concentrations, for which the analytical procedure has demonstrated a suitable level of precision, accuracy, and linearity (2,4). For an impurity, the range should be determined from the reporting level to 120% of the specification (3).

Robustness: The ICH guideline defines robustness as the measure of an analytical procedure's capacity to remain unaffected by small, but deliberate variations in method parameters, and provide an indication of its reliability during normal usage (2). If users know that quantifications can be affected by certain changes in test method conditions, they should include a precautionary statement in the test method report (3). For example, if test samples must be protected from laboratory light, the test method report should specify using amber HPLC vials.

During the robustness portion of method validation performance, various HPLC method parameters are varied (3). These parameters can include

- mobile-phase pH,
- mobile-phase composition,
- column selection (different lots or suppliers),
- column temperature, and
- mobile-phase flow rate.

System-suitability testing: Although it is not part of a formal validation, a system-suitability test should be conducted to ensure the entire chromatographic system is functioning properly before performing an analysis (3). Conducting this test verifies that the data obtained from the analysis are acceptable. The system-suitability test checks the proper establishment of a method on a properly functioning HPLC system. Analysts can measure certain chromatographic performance parameters such as resolution and tailing factor and establish specifications so they can determine when a method and HPLC system are functioning properly (3). This test provides greater confidence in the data obtained from each analysis.

Sample-solution stability: Chromatographic analyses typically are performed by using autosamplers and overnight runs. As such, it is important to verify that the sample is stable in the solution prescribed by the method for periods encompassing the expected analysis duration period. This stability is particularly important if samples are found or suspected to undergo hydrolysis, photolysis, or adhesion to the sample vial. Workers should test appropriate sample-stability storage times to ensure that proper results are obtained from an assay (3).

Conclusion

We discussed ICH and FDA guidelines related to the background information about impurity method validation in Part I of this series. In Part II, the final part of this series, we reviewed and discussed ICH and FDA guidelines about validation components, including validation topics such

as specificity, selectivity, linearity, range, accuracy, precision, recovery, limits of detection and quantitation, robustness, system suitability, and sample-solution stability. More-detailed information pertaining to ICH and FDA guidelines for method validation can be found on the organizations' respective web sites.

Editors' Note

The views and opinions expressed in this "Validation Viewpoints" column are those of the authors — John D. Orr, Ira S. Krull, and Michael E. Swartz — and do not necessarily reflect the views and opinions of Eisai Research Institute (nor of its parent company, Eisai Co., Ltd., nor of any of its subsidiaries), Northeastern University, or Waters Corp.

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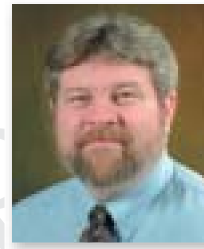

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The columnists regret that time constraints prevent them from responding to individual reader queries. However, readers are welcome to submit specific questions and problems, which the columnists may address in future columns. Direct correspondence about this column to "Validation Viewpoint," LCGC*, 859 Willamette Street, Eugene, OR 97401, e-mail lcgcedit@lcgcmag.com.*