CORE COMPONENTS OF ANALYTICAL METHOD VALIDATION FOR SMALL MOLECULES: AN OVERVIEW

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ABSTRACT

Analytical methods development and validation play significant roles in the drug discovery, development, manufacture of pharmaceuticals and estimation of small molecules. The official test methods that result from these processes are used by quality control laboratories to ensure the accuracy, precision, selectivity, sensitivity, reproducibility, stability, and performance of drug products. This must give a review and the information about various stages involved in development and validation of analytical methods for small molecules with emphasis on chromatographic techniques, a strategy for the validation of analytical methods for both methods developed in-house as well as standard methods, and a recommendation on the documentation that should be produced during, and on completion of, method validation as per ICH guide lines.

Keywords: Analytical, Validation, Quality control, Validation parameters, ICH, ISO, HPLC.

INTRODUCTION

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories. Analytical methods need to be validated, verified, or revalidated in the following instances:

- Before initial use in routine testing
- When transferred to another laboratory
- Whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or samples with a different matrix) and the change is outside the original scope of the method.

Method validation has received considerable attention in literature from industrial committees and regulatory agencies. This review outlines how method validation helps to achieve high quality data.

The Laboratory of the Government Chemist (LGC) developed a guide for internal method Validation1. It includes a discussion of related laboratory accreditation requirements. The United States Food and Drug Administration developed two industry guidelines: one for the validation of analytical methods2 and one for the validation of bioanalytical methods3. ICH published two guidelines for method validation. Q2A4 describes terminology and definitions for eight validation parameters that should be considered for validation. Q2B5 includes methodology but allows flexibility through the statement ”It is the responsibility of the applicant to choose the validation procedure and the protocol most suitable for their product”. IUPAC6 published “Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis”. EURACHEM7 published a detailed guide for method validation. This is the most detailed official guide for theory and practice of method validation. It has been primarily developed for ISO/IEC accredited laboratories but because of its completeness it is also a good source for (bio) pharmaceutical laboratories. Huber8 authored a validation reference book for the analytical laboratory with a chapter on method validation. AOAC9 has published a technical document for the verification of analytical methods for the ISO 17025 accreditation. Viswanathan and co-authors10 developed an overview for validation of bioanalytical methods.

This review gives a discussion on the importance of various elements of data quality in laboratories. It also explains why system suitability testing or the analyses of quality control charts are not enough to ensure valid analytical test results. Instrument qualification and method validation are equally important. Figure 1 illustrates the different components of data quality; analytical instrument qualification, analytical methods validation, and system suitability testing and analytical quality control through quality control samples.

Instrument qualification means that the specifications are defined, tested and confirmed so that the instrument is suitable for the methods to be validated. The analytical methods are then validated on qualified instruments to prove that the method works as intended. This is independent of any specific instrument. If we want to use the method with instruments from different vendors, the method should be validated on those instruments as well. A specific instrument is then combined with a specific method to run system suitability tests. System suitability parameters should be selected during method validation. Successful system suitability test runs ensure that the complete system meets the analyst’s expectations under the specific conditions of the tests.

The highest level of testing is the analysis of quality control samples. Standards or samples with known amounts are analyzed and the results compared with the known amounts. Method validation occurs between analytical instrument qualification and system suitability testing and is linked to all other quality elements. Methods should be validated using qualified instruments. During method validation, parameters and acceptance criteria for system suitability checks and quality control checks should be defined.
ANALYTICAL METHOD DEVELOPMENT

Analytical chemistry compacts with methods for identification, separation, and quantification of the chemical components of natural and synthetic components. The choice of analytical methodology is based on many considerations, such as chemical properties of the analyte and its concentration, sample matrix, the rapidity and cost of the analysis, type of measurements i.e., quantitative or qualitative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes in the sample. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

The following steps are common to most types of projects: a) Method development plan definition, b) Background information gathering, c) Laboratory method development, it includes various stages namely sample preparation, specific analytical method, detection and data processing and d) Generation of test procedure.

The most widely used methods for quantitative determination of drugs and metabolites in biological matrices such as blood, serum, plasma, or urine includes Gas chromatography (GC), High-performance liquid chromatography (HPLC), Thin layer chromatography (TLC), evaporative light-scattering detection (ELSD) combined GC and LC mass spectrometric (MS) procedures such as LC-MS, LC-MS-MS, GC-MS, and GC-MSMS. Defined peaks may be quantified directly or fractions containing the solutes can be collected for analysis by other means. However, the much wider use of light-scattering detectors in the last few years has changed the perspective greatly.

Liquid chromatography can be categorized on the basis of the mechanism of interaction of the solute with the stationary phase as: adsorption chromatography (liquid-solid chromatography), partition chromatography (liquid-liquid chromatography), ion-exchange chromatography (IEC), size exclusion chromatography (SEC) and affinity chromatography.

Early work in liquid chromatography was based on highly polar stationary phases, and nonpolar solvents served as mobile phases, this type of chromatography is now referred to normal-phase liquid chromatography (NPLC). Chromatography on bare silica is an example of normal-phase chromatography. In reversed-phase high performance liquid chromatography (RP-HPLC), the stationary phase is nonpolar, often a hydrocarbon, and the mobile phase is relatively polar. In RP-HPLC, the most polar component is eluted first, because it is relatively most soluble in the mobile phase.

HPLC-UV diode-array detection (DAD) and HPLC-MS techniques take advantage of chromatography as a separation method and DAD or MS as identification and quantification methods. The HPLC equipment consists of a high-pressure solvent delivery system, a sample auto injector, a separation column, a detector (UV or DAD) a computer to control the system and display results.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time, solvent consumption and analysis time as compared to the conventional HPLC.

Sample preparation

The purpose of sample preparation is to create a processed sample that leads to better analytical results compared with the initial sample. The prepared sample should be an aliquot relatively free of interferences that is compatible with the HPLC method and that will not damage the column. The main sample preparation techniques are liquid-liquid extraction (LLE) and solid-phase extraction (SPE). In these methods the analyte of interest was separated from sample matrix, so that as few potentially interfering species as possible are carried through to the analytical separation stage.

Detection

After the chromatographic separation, the analyte of interest is detected by using suitable detectors. Some commercial detectors used in LC are: ultraviolet (UV) detectors, fluorescence detectors, electrochemical detectors, refractive index (RI) detectors, Evaporative Light Scattering Detection (ELSD) and mass spectrometry (MS) detectors. The choice of detector depends on the sample and the purpose of the analysis.

The UV detectors are the most common HPLC detectors since they are robust, cheap, easy to handle, and since many solutes absorb light in this frequency range. The ordinary UV detector measures the absorbance at one single wavelength at the time. A diode-array detector (DAD) can measure several wavelengths at the same time, and since no parts are moved to change wavelength or to scan, there are no mechanical errors or drift with time.

DAD detectors have been proposed for various applications, such as preliminary identification of a steroidal glycoside in seed, peptide mapping, assay of sulfamethazine in animal tissues, or identification of pesticides in human biological fluids. HPLC with a mass spectrometer detector (LC-MS) showed superior sensitivity and selectivity compared to HPLC-UV methods.

Mass Spectrometry

Mass spectrometry (MS) is a powerful analytical tool that can supply both structural information about compounds and quantitative data relating to mass. Under optimum conditions, it can provide the molecular weight; the empirical formula and often the complete structure of an unknown compound in addition to giving a measure of the amount present. For some years it was necessary to volatilize the sample in the ion source of the instrument before ionizing it by electron-impact or chemical-ionization techniques, but recently methods have been developed for producing ions from materials in the condensed phase (e.g. by fast-atom bombardment techniques). There are several types of ion sources, which utilize different ionization techniques for creating charged species. Three popular ionization techniques are: electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and matrix-assisted laser desorption (MALDI). Electrospray is the most widely used ionization technique when performing LC-MS.

PARAMETERS AND TESTS

Analytical methods should be validated through laboratory tests “Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedures meet the requirements for the intended analytical applications.”

Unfortunately, some of the definitions vary between the different organizations. Therefore, laboratories should have a
Table 2 summarizes factors that should be the same, or reproducible. The parameters, as defined by the ICH and other organizations and authors, are summarized in Table 1 and are described briefly in the following paragraphs.51-53

The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. Possible parameters for a complete method validation are listed as: Precision, Accuracy, Linearity, Range, Ruggedness, Robustness, Limit of detection, Limit of quantitation.54 Selectivity and Specificity. This proposed procedure assumes that the instrument has been selected and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughput; and environmental, health and safety requirements.

**Precision**

Precision of an analytical procedure 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. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability expresses the precision under the same operating conditions over a short interval of time. Intermediate precision expresses variations within laboratories, such as different days, different analysts, different equipment, and so forth. Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

**Accuracy and Recovery**

The accuracy of an analytical procedure as the closeness of agreement between the conventional true value or an accepted reference value and the value found. Accuracy can also be described as the extent to which test results generated by the method and the true value agree.

**Typical variations affecting a method’s reproducibility**

Table 2 summarizes factors that should be the same, or different, for precision, intermediate precision and reproducibility.

**Linearity and Calibration Curve**

Linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components. Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from zero. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method. Figure 3 & 4 shows a comparison of the two graphical evaluations using HPLC.

Plotting the sensitivity (response/amount) gives clear indication of the linear range. Plotting the amount on a logarithmic scale has a significant advantage for wide linear ranges. Re = Line of constant response.

**Range**

The range of an analytical procedure as the interval from the upper to the lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. For assay tests, the ICH requires the minimum specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, or from 50 percent of the specification of each impurity, whichever is greater, to 120 percent of the specification.

**Ruggedness**

Ruggedness is not addressed in the ICH documents. Its definition has been replaced by reproducibility, which has the same meaning. Ruggedness is defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of the reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Ruggedness is determined by the analysis of aliquots from homogeneous lots in different laboratories.

**Robustness**

The robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of the procedure’s reliability during normal usage. Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method’s robustness, a number of method parameters, such as pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method’s robustness range.

**Limit of Detection**

The detection limit of an individual analytical procedure as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. (Figure: 5). The limit of detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of detection is frequently confused with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass.

**Limit of Quantitation**

The limit of quantitation (LOQ) of an individual analytical procedure as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities or degradation products.
The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. If the required precision of the method at the limit of quantitation has been specified, 5 or 6 samples with decreasing amounts of the analyte are injected six times. The amounts range from the known LOD as determined above to 20 times the LOD. (Figure: 6).

Selectivity
Selectivity is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix. Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and/or degradation products and/or impurities. Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and/or degradation products and/or impurities.

Peak Purity
In addition “diode array” detectors allow for the determination of the relative purity factor typically called: Peak Purity.

METHOD VALIDATION PROCESS
For an efficient validation process, it is of utmost importance to specify the right validation parameters and acceptance criteria. The more parameters, the more time it will take to validate. The more stringent the specifications or acceptance limits, the more often the equipment has to be recalibrated, and probably also prequalified, to meet the higher specifications at any one time. It is not always essential to validate every analytical performance parameter, but it is necessary to define which ones are required.

PURITY TESTS: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e., related substances test, heavy metals, residual solvents content, etc.

ASSAY (Content or Potency): To provide an exact result this allows an accurate statement on the content or potency of the analyte in a sample.

Stability
Chemical compounds can decompose prior to chromatographic investigations, for example, during the preparation of the sample solutions, extraction, cleanup, phase transfer or storage of prepared vials (in refrigerators or in an automatic sampler). Under these circumstances, method development should investigate the stability of the analytes and standards. It is a measure of the bias in assay results generated during a preselected time interval, for example, every hour up to 46 hours, using a single solution. (Figure: 7).

Validation Planning
Successful validation requires the cooperative efforts of several departments of an organization including regulatory affairs, quality assurance, quality control and analytical development. Therefore, it is important that the process follows a well-defined validation master plan for analytical methods. The plan documents a company’s approach and steps for method validation and serves two purposes. When implemented correctly, it ensures consistent and efficient execution of validation projects. In addition, it answers an inspector’s questions regarding the company’s approach for all aspects of analytical method validation. The master plan is...
Selecting Validation Parameters and Limits

Scope and Method Specifications

Revalidation is also required if the scope of the method has been changed or extended, for example, if the sample matrix or operating conditions change. Furthermore, revalidation is necessary if the method is to be used for different types of analytical procedures to be validated.

According to the ICH, accuracy, any type of precision and limits of detection and quantitation are not required if the analytical task is for identification purposes. For assays in USP Category 1, the major component or active ingredient to be measured is normally present at high concentrations; therefore, validation of limits of detection and quantitation is not necessary.

Testing for Performance Characteristics

Once tests and acceptance criteria have been defined, experiments for testing should be thoroughly prepared, executed and documented according to a validation protocol.

Preparation

Good preparation work is important for efficient experiment execution. Most important are the use of qualified material, qualified equipment, trained people and well documented procedures.

Any chemicals used to determine critical validation parameters, such as reagents and reference standards, should be available in sufficient quantities, accurately identified, sufficiently stable and checked for exact composition and purity according to specifications.

Any other materials and consumables, for example, chromatographic columns, should be new and qualified to meet the column’s performance criteria. This ensures that one set of consumables can be used for most experiments and avoid unpleasant surprises during method validation.

Analytical equipment should be clearly defined, well characterized, qualified or calibrated to make sure that it meets the functional and performance specifications as required by the analytical method. The selected equipment should have average performance rather than selecting best performing equipment. Otherwise there may be problems with intermediate precision and reproducibility studies to meet acceptance criteria with different equipment.

Operators should be sufficiently familiar with the technique and equipment. This will allow them to identify and diagnose unforeseen problems more easily and to run the entire process more efficiently.

Test Execution

There are no official guidelines for the sequence of validation testing. The optimal sequence may depend on the method itself, for a liquid chromatographic method; the following sequence has proven to be useful:

1. Specificity/selectivity
2. Repeatability of retention times and peak areas
3. Linearity, limit of quantitation, limit of detection, range
4. Accuracy at different concentrations
5. Intermediate precision
6. Reproducibility

The more time-consuming experiments, such as intermediate precision and reproducibility, are included towards the end. Some of the parameters, as listed under points 2-4, can be measured in combined experiments.

**Developing a Quality Control Plan for Routine Analysis**

The objective of analytical method validation is not only to ensure valid analytical data during initial use of the method but during its entire lifetime. Appropriate checks should be included in the routine sample analysis to verify that the method and the system perform as initially specified at the time of sample analysis. Checks do not need to cover all initial tests but should focus on the most critical performance characteristics, especially those that are most likely to change over time. Such check procedures for execution should be developed and documented as part of the method validation processes because information about the critical items is probably most readily available at this time.

Common on-going tests are system suitability tests (SST) and the analysis of quality control samples. For chromatographic methods system suitability tests are described in Pharmacopeias21, 25 and typically include resolution between two peaks, repeatability of peak areas, tailing factor, and number of theoretical plates. System suitability testing is recommended as a component of any analytical procedure, not just those that involve chromatographic techniques.

**Validation of Non-routine Methods for sample analysis**

Frequently, a specific method is used for only a few sample analyses. In this case, the validation may take much more time than the sample analysis and may be considered inefficient, because the cost per sample will increase significantly. The answer is quite simple: Any analysis is worthwhile only if the data are sufficiently accurate; otherwise, sample analysis is pointless. The suitability of an analysis method for its intended use is a prerequisite to obtaining accurate data; therefore, only validated methods should be used to acquire meaningful data. However, depending on the situation, the validation efforts can be reduced for non-routine methods. The CITAG/ EURACHEM guide25 includes a chapter on how to treat non-routine methods. The recommendation is to reduce the validation cost by using generic methods, for example, methods that are broadly applicable. A generic method could, for example, be based on capillary gas chromatography or on reversed phase gradient HPLC. With little or no modification, the method can be applied to a large number of samples. The performance parameters should have been validated on typical samples characterized by sample matrix, compound types and concentration range.

If, for example, a new compound with a similar structure in the same matrix is to be analyzed, the validation will require only a few key experiments. The documentation of such generic methods should be designed to easily accommodate small changes relating to individual steps, such as sample preparation, sample analysis or data evaluation.

The method’s operating procedure should define the checks that need to be carried out for a novel analyte in order to establish that the analysis is valid. Detailed documentation of all experimental parameters is important to ensure that the work can be repeated in precisely the same manner at any later date.

**CONCLUSION**

Recent development in pharmaceutical and biotechnological field generates a cumulative demand for analytical methods. Rapid and accurate quantification of the substrate and drug product is important in the process development. Improvements in analytical instrumentation leads to development of new techniques like isocratic and gradient RP-HPLC, which evolved as the primary techniques for the analysis of nonvolatile APIs and impurities. These analytical methods are critical elements of pharmaceutical development so it is very important to develop efficient and accurately validated analytical methods to develop safe and effective drugs.

Reproducible quality HPLC results can only be obtained if attention has been paid to the method development, validation and the system’s suitability to carry out the analysis.

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Table 1: Parameters for method validation with reference to ICH, USP and ISO 17025.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Precision</td>
<td>USP, ICH</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>ICH</td>
</tr>
<tr>
<td>Accuracy</td>
<td>USP, ICH, ISO 17025</td>
</tr>
<tr>
<td>Linearity</td>
<td>USP, ICH, ISO 17025</td>
</tr>
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<td>Range</td>
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<tr>
<td>Limit of detection</td>
<td>USP, ICH, ISO 17025</td>
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<tr>
<td>Limit of quantitation</td>
<td>USP, ICH, ISO 17025</td>
</tr>
<tr>
<td>Repeatability</td>
<td>ICH, ISO 17025</td>
</tr>
<tr>
<td>Reproducibility</td>
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<tr>
<td>Specificity</td>
<td>USP, ICH</td>
</tr>
<tr>
<td>Selectivity</td>
<td>ISO 17025</td>
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<tr>
<td>Robustness</td>
<td>USP, Included in ICH as method development activity, ISO 17025</td>
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<tr>
<td>Ruggedness</td>
<td>USP, defined as reproducibility in ICH</td>
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Table 2: Variables for measurements of precision, intermediate precision and reproducibility.

<table>
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<th>Precision</th>
<th>Intermediate Precision</th>
<th>Reproducibility</th>
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<tr>
<td>Instrument</td>
<td>same</td>
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<td>different</td>
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<tr>
<td>Batches of accessories e.g. chrom. columns</td>
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<td>different</td>
</tr>
<tr>
<td>Operators</td>
<td>same</td>
<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Sample matrices</td>
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</tr>
<tr>
<td>Concentration</td>
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<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Batches of material, e.g., reagents</td>
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<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Environmental conditions, e.g., temperature</td>
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<tr>
<td>Laboratory</td>
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Table 3: ICH Characteristics.

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<tr>
<th>Analytical Task</th>
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<th>Impurity Quantitative</th>
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<td></td>
<td>- interim precision no</td>
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<td>yes</td>
</tr>
<tr>
<td></td>
<td>- reproducibility no</td>
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<tr>
<td>Specificity</td>
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</tr>
<tr>
<td>Limit of detection</td>
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<td>no</td>
</tr>
<tr>
<td>Limit of quantitation</td>
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<tr>
<td>Linearity</td>
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<td>yes</td>
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<tr>
<td>Range</td>
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* may be required, depending on the nature of the specific test

Table 4: USP Characteristics.

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<th>Cat 2 quantitative</th>
<th>Cat 3 qualitative</th>
<th>Assay Cate 3</th>
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<td>Precision</td>
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<tr>
<td>Specificity</td>
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<td>Limit of detection</td>
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<tr>
<td>Limit of quantitation</td>
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<td>*</td>
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<tr>
<td>Linearity</td>
<td>yes</td>
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<tr>
<td>Range</td>
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<td>yes</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
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</table>

* may be required, depending on the nature of the specific test
Table 5: ICH validation characteristics.

<table>
<thead>
<tr>
<th>Analytical task</th>
<th>Identification</th>
<th>Quantitative</th>
<th>Limit tests</th>
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</thead>
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<td>Accuracy</td>
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<td>Precision</td>
<td></td>
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</tr>
<tr>
<td>Repeatability</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Intermediate precision</td>
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<tr>
<td>Reproducibility</td>
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<tr>
<td>Specificity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Limit of detection</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Linearity</td>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Range</td>
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Table 6: USP validation characteristics.

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<th>Analytical task</th>
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<th>Assay Category 2</th>
<th>Assay Category 3</th>
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<tr>
<td>Precision</td>
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<tr>
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<tr>
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<tr>
<td>Range</td>
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<td>Yes</td>
<td>*</td>
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<tr>
<td>Ruggedness</td>
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</table>

Category 1: Quantitation of major components

Category 2: Impurities

Category 3: Performance characteristics

* May be required, depending on the nature of the specific test

Figure 1: Components of analytical data quality.
Figure 2: Validation parameters of an Analytical method.

Figure 3: Graphical presentations of linearity plot using HPLC.

Figure 4: Graphical presentations of linearity plot using HPLC.
Figure 5: Definitions for linearity, range, LOQ, LOD.

Figure 6: Limit of detection and limit of quantitation via signal to noise.

Figure 7: Schematics of stability testing.