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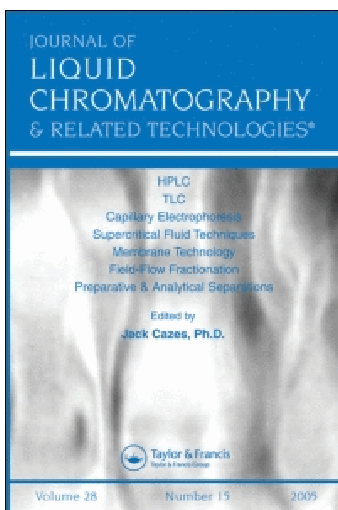


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# Guidelines for the Design, Implementation, and Interpretation of Validations for Chromatographic Methods used to Quantitate Leachables/Extractables in Pharmaceutical Solutions

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# Guidelines for the Design, Implementation, and Interpretation of Validations for Chromatographic Methods used to Quantitate Leachables/Extractables in Pharmaceutical Solutions

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## ABSTRACT

This paper deals with validation of chromatographic methods for quantitation of leachables and extractables in pharmaceutical solutions. Guidelines are presented for designing, implementing, and interpreting validations.

*Key Words:* Validation; Chromatographic methods; Design; Implementation; Interpretation; Leachables; Extractables; Pharmaceutical solutions.

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## INTRODUCTION

An important consideration in the development and registration of pharmaceutical products that are packaged in, or contact (during production, storage or use), plastic materials is a consideration of the possibility that components of the plastic may leach into the product during contact. Such compounds are termed leachables, if they have been demonstrated to actually accumulate in the product, or extractables, if they are known components of the plastic material that may (or may not) accumulate in the product.

While numerous direct and indirect methods exist that allow one to assess the levels to which such leachables or extractables accumulate in the product due to contact with the plastic, the most direct method is to test actual product samples, typically as part of the product development or stability evaluation processes, for their levels of the leachables/extractables. Clearly, the success of such a strategy rests on the utilization of valid test methods, especially if the resultant data is to be used for product registration purposes.

As leached substances typically are present in pharmaceutical products (or their associated simulating solutions) at trace levels, and as the pharmaceutical product matrix is typically quite complex (containing the pharmaceutical active ingredient, formulation components, degradation products, impurities, and contaminants), the measurement of leached substances in pharmaceutical products is a difficult analytical challenge. It is reasonable to expect, therefore, that chromatographic methods developed for such a purpose may have performance characteristics that are less rigorous than those for methods that are used for other pharmaceutical applications (e.g., quantitation of the amounts of the active ingredient). Thus, an effective validation of a method for quantifying leachables includes not only an appropriate testing strategy but also rigorous but achievable acceptance criteria.

The purpose of this manuscript is to discuss a complete process by which a chromatographic method can be validated for the purpose of leachables/extractables quantitation in a pharmaceutical product solution. This complete process includes experimental design, design implementation, and data analysis. While the process is based on a chromatographic method applicable over the concentration range of 100–1000 ppb (such as liquid chromatography–mass spectrometry, LC/MS), the experimental design can be expanded (or contracted) to encompass different analytical ranges as necessary.

## PREREQUISITES TO THE METHOD VALIDATION STUDY

Prior to the initiation of the method validation endeavor, it is necessary that the following activities be completed and/or the following documents generated.

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(a) A list of targeted leachables/extractables, specific for the plastic material and pharmaceutical product, must be generated and justified.
(b) An analytical method that was developed and evaluated for the specific application at hand. While the guidelines enumerated in this manuscript provide no direction as to how such a development or evaluation study should be performed, it should be clear that the evaluation should be sufficiently comprehensive that there is little, if any, possibility that the method validation activity could produce results that do not meet the established acceptance criteria if the validation is competently executed.
(c) A draft procedure specification must be available for the method to be validated and such a specification should be included in the validation protocol. The draft procedure specification should include a system suitability assessment, complete with performance requirements. It is required that all analytical runs performed in the validation meet the specified system suitability requirements.
(d) A complete inventory of chemicals, reagents, and other materials required to complete the validation should be compiled and said materials procured.

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## GENERAL DISCUSSION

The following is a general discussion of the salient aspects of a method validation endeavor. Each aspect is treated in greater detail in latter sections of this manuscript.

1. The validation is initiated with a validation protocol. The validation protocol includes the following:
   (a) *Purpose of Study*: In this section, the target compounds, plastic material contacted, pharmaceutical product, and general analytical method type (e.g., LC/MS) are specified.
   (b) *Background*: This section establishes the nature of the developed product, delineates the validation parameters to be assessed, references the method development and evaluation studies, and lists the product's formulation information.
   (c) *Test and Control Articles*: This section outlines the production of stock solutions, test solutions, standard solutions, internal standard solutions, and the preparation of sample and standard diluents.
   (d) *Experimental Design*: This section describes the methods by which each validation parameter will be assessed, and provides

general guidance for the construction of the analytical run sequences.

(e) *Evaluation of Data*: This section establishes the validation's acceptance criteria, primarily in the context of the acceptance criteria master table. It provides a general reference to the steps to be taken should an acceptance criterion failure be obtained. It may provide general guidance in terms of the presentation of the validation data.

(f) *References*: This section contains all references relevant to the validation study. This section should include, in addition to the development/evaluation report(s), instrument operating, calibration and maintenance specifications, and data analysis/handling/archiving specifications.

(g) *Attachment*: The draft procedure specification (typically obtained from the development or evaluation report, with few, if any, modifications) is attached to the validation protocol.

2. An assay for the quantitation of plastic-related leachables/extractables in drug products can be considered to be equivalent, in intent, to an assay for the quantitation of impurities. Such a classification of an assay is considered in the compendial literature (e.g., USP and ICH guidelines[1–3]). Specific assay elements that must be validated for this class of assay includes: accuracy, precision, specificity, quantitation limit, linearity and range, and ruggedness. Other characteristics of the method, such as limit of detection and the stability of standard and sample preparations, may also be addressed in the validation if such method characteristics are not addressed in method development/evaluation studies. Robustness must be addressed during the development and evaluation of the method and thus is not relevant to the validation. The ability of the validated system to meet specified and justified system suitability criteria must be demonstrated throughout the method validation.
3. The entire method validation activity can be completed in two extended analytical runs, performed by two independent analysts using two different analytical columns (consistent with the method's draft procedure specification) and two different preparations of the mobile phase (as appropriate). As the creation of the test formulation (spiked and unspiked) is not an intrinsic part of the method under validation, but rather is necessary only for performing the validation, it is not necessary for sample creation to be repeated by both analysts. However, if the method calls for a specific sample preparation process (e.g., dilution, solvent/solvent

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extraction) prior to instrumental analysis, then each analyst should perform the entire sample preparation process independently.

4. A standard range encompassing an order of magnitude in analyte concentration (e.g., 100–1000 ppb) is utilized in this manuscript and can be properly characterized via the use of four calibration standards and a standard blank. The sample range of 100–1000 ppb can be properly characterized via three test samples spiked to appropriate analyte levels, and a sample blank. Other standard ranges can be envisioned depending on the specific application, but in general it is adequate that the standard range encompass not more than (NMT) a single order of magnitude in analyte concentration.
5. For practical purposes, it is most desirable that standards be prepared in an aqueous diluent. However, it is occasionally the case that the product formulation influences the performance of the analytical method to such a great extent that matrix matching is appropriate and standards must be prepared in the product formulation.
6. Internal standards are often included in chromatographic methods. Our experience with direct injection LC/MS leachables/extractables assays is that internal standards that are not very close chemical matches of the analytes are not effective in terms of correcting for sample-to-sample assay variations. While use of isotopically labeled analog internal standards (e.g., deuterated form of the analyte as the internal standard) may be effective in such instances, the isotopically labeled internal standards are not universally available. Our experience with direct injection LC/UV is that internal standards provide no benefit in terms of assay performance. On the other hand, internal standards are frequently useful in accounting for analyte recovery issues associated with assays that involve sample preparation steps (e.g., extraction and concentration). Additionally, internal standards may be useful in terms of appropriately identifying suspect or compromised injections.
7. For the purpose of assessing all validation parameters except precision and ruggedness, three replicates shall be considered appropriate. The determination of precision at one analyte level shall include six replicates. The determination of ruggedness at one analyte level shall include 12 total replicates, six obtained from analyst 1 and six obtained from analyst 2.
8. The randomization strategy used in this manuscript represents a trade-off between the general expectation for complete randomization and the practical benefits of approach standardization. Thus,

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while samples and standards of a given concentration are generally injected sequentially, the various concentrations themselves are not arranged in descending or ascending order.

9. An analytical run shall last for no longer than 24 hr, where this time reflects the practical trade-off between efficiency (longer runs = greater cost efficiency) and assay performance (response instability and/or stability of samples and standards). It is expected that samples and standards are stable over the course of such an analytical run. This 24 hr is based on the filling of the autosampler vials immediately prior to the start of the analytical run, and immediately after the samples are prepared in their final injected form. This 24 hr is measured from the first injection of an appropriate concentration standard during the system suitability assessment, to the last injection of the same standard in the last calibration bracket.
10. The measurement of leachables/extractables at concentrations of 1000 ppb or less in concentrated drug-containing formulations is an instance of trace analysis. It is thus reasonable to expect that method performance at trace levels is characterized by a greater variability and lesser rigor than is method performance at higher concentrations typically encountered in pharmaceutical applications (e.g., assay for active ingredient). Therefore, performance expectations for leachables assays will be less rigorous than those typically encountered in other pharmaceutical applications.
11. It is a general rule of thumb in analytical chemistry that as the analyte concentration goes up, assay performance improves, and thus, acceptance criteria should become more stringent. This rule of thumb is not appropriate for trace analysis, and for certain types of detection (e.g., mass spectrometry), is actually counter to experience. Thus, acceptance criteria for the validation of leachables/extractables assays (specifically accuracy and precision) are the same across all concentration levels.
12. It is generally accepted that the most preferable calibration model is a linear one. However, it is our experience, especially with MS detection, that some curvature in the calibration model is observed over an order of magnitude concentration range. Thus, the calibration model to be used in leachables/extractables assessments is a quadratic model:

$$\text{Response} = \text{slope 1} \times (\text{concentration})^2 + \text{slope 2} \times (\text{concentration}) + \text{intercept}$$

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It is noted that such a quadratic model will simplify to a linear model, if in fact the experimental data is fit well by the linear model.

13. Blanks for the calibration standards and validation samples may produce a small analytical response that is indistinguishable from that of the target analyte. This response may be a true response, arising from low levels of the analyte intrinsically present in the standard or sample, or it may be a false response, arising from an interferant in the sample or a change in the background signal. Regardless of the nature and origin of the blank response, it must be considered in the assessment of linearity, accuracy, and specificity. Thus, all calibration curves must be constructed with the standard blank as a data point. Additionally, analytical accuracy should be calculated only after the response from the formulation blank is appropriately accounted for (e.g., blank subtraction). At trace levels and in complicated pharmaceutical products, it is difficult to achieve absolute specificity (complete absence of an non-analyte peak). Rather, a practical requirement for specificity is one that considers an allowable limit for the response in a sample blank. As noted in Table 3, a proposed requirement is that the magnitude of the signal in the sample blank must be less than 30% of the magnitude of the response to a sample spiked to the lowest analyte concentration in the method's range.
14. The actual levels of ingredients in products contacted by plastic materials may vary somewhat from the labeled levels. This variation can arise due to allowed variations in composition during production and/or due to post-production changes, such as ingredient decomposition or water vapor loss. In order to assess the effect that different levels of active ingredients have on assay performance, accuracy and precision must be measured in samples formulated at a range of concentrations, centered around the product's 100% labeled composition. For leachables/extractables assessments, this range is typically 90%–110% of label.
15. If the product under development is to be sterilized by autoclaving, it is possible that heat-related degradation products may be present in actual product test units. Such degradation products may have a direct or indirect effect on assay performance, and thus, the validation should be performed with a formulation test matrix that has been heat-stressed in some manner. As it is not practically possible to mimic a product's heat exposure history precisely, the formulation test matrix shall be autoclaved at a nominal temperature of 121°C for 30 min.

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16. It is observed that the level of 100 ppb is significant to the method's validation, as it is this level that is the method's desired limit of quantitation (LOQ). As such, it reflects a trade-off between the analytical capability of the method and the observation that, in general, the impact of a leachable becomes negligible below a certain concentration.

## MASTER VALIDATION ANALYTICAL RUN SEQUENCES

As the method validation exercise involves a defined number of experiments performed in a clear and typically inter-related manner, it is possible to specify up front the exact analytical sequences to be used. Such analytical sequences should be constructed so that all the data relevant to the validation is collected in a time and cost efficient manner. As noted previously, the entire method validation activity can be completed in two extended analytical runs, performed by two independent analysts (consistent with the method's draft procedure specification), using two different analytical columns and two different preparations of the mobile phase. Such sequences are contained in Tables 1 and 2. Consistent execution of the study, per the exact sequence requirements noted in Tables 1 and 2, greatly facilitates the generation and interpretation of the validation data. However, as the means by which some performance parameters can be assessed (e.g., sample/standard stability) can vary somewhat from one validation study to the next, Tables 1 and 2 should be viewed as strong recommendations but not requirements.

## ACCEPTANCE CRITERIA MASTER TABLE

The cornerstone of the method validation process is the comparison of measured performance properties vs. pre-established acceptance criteria. It follows, therefore, that an important function of the validation protocol is the documentation of the acceptance criteria. This is accomplished via the acceptance criteria master table, which appears in the validation protocol in the evaluation of data section. Table 3 represents the acceptance criteria master table and includes the acceptance criteria that are appropriate for the validation of a leachables/extractables method.

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***Table 1.*** First validation injection sequence, analyst 1.

| | | Purpose | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | System suitability | | | | | Validation | | | | | | | |
| Injection | Sample ID | Linearity | Precision | $S/N$[a] | $R$[b] | Response stability | STD linearity | Sample linearity | Accuracy precision | Specificity | Ruggedness | Sample stability | STD stability | LOQ |
| 1 | STD High | | | | | | | Conditioning injection only | | | | | | |
| 2 | STD High | | | | | | | Conditioning injection only | | | | | | |
| 3 | STD High | | | | | | | Conditioning injection only | | | | | | |
| 4 | STD Mid | | X | | | | | | | | | | X (initial) | |
| 5 | STD Mid | | X | | | | | | | | | | | |
| 6 | STD Mid | | X | | | | X | | | | | | | |
| 7 | STD Mid | X | X | | | | X | | | | | | | |
| 8 | STD Mid | X | X | | X | | X | | | | | | | |
| 9 | STD High | X | | | | X | X | | | | | | | |
| 10 | STD High | X | | | | X | X | | | | | | | |
| 11 | STD Low | X | | | | | X | | | | | | | |
| 12 | STD Low | X | | X | | | X | | | | | | | X |
| 13 | STD Blk | X | | | | | X | | | | | | | |
| 14 | STD Blk | X | | | | | X | | | | | | | |
| 15 | STD Blk | | | | | | X | | | | | | | |
| 16 | STD Low | | | | | | X | | | | | | | |
| 17 | STD High | | | | | | X | | | | | | | |
| 18 | STD Int | | | | | | X | | | | | | | |
| 19 | STD Int | | | | | | X | | | | | | | |
| 20 | STD Int | | | | | | X | | | | | | | |

(*continued*)

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**Table 1.** Continued.

| | | Purpose | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | System suitability | | | | | Validation | | | | | | | | |
| Injection | Sample ID | Linearity | Precision | $S/N$[a] | $R$[b] | Response stability | STD linearity | Sample linearity | Accuracy precision | Specificity | Ruggedness | Sample stability | STD stability | LOQ |
| 21 | STD High | | | | | X | | | Bracket 1 | | | | | |
| 22 | STD Med | | | | | | | | Bracket 1 | | | | | |
| 23 | STD Low | | | | | | | | Bracket 1 | | | | | |
| 24 | STD Blk | | | | | | | | Bracket 1 | | | | | |
| 25 | TA-100-Med | | | | | | | X | X | | X | X (initial) | | |
| 26 | TA-100-Med | | | | | | | X | X | | X | | | |
| 27 | TA-100-Med | | | | | | | X | X | | X | | | |
| 28 | TA-100-Med | | | | | | | | X | | X | | | |
| 29 | TA-100-Med | | | | | | | | X | | X | | | |
| 30 | TA-100-Med | | | | | | | | X | | X | | | |
| 31 | TA-Blk | | | | | | | X | X | | | | | |
| 32 | TA-Blk | | | | | | | X | X | | | | | |
| 33 | TA-Blk | | | | | | | X | X | X | | | | |
| 34 | TA-90-Med | | | | | | | | X | | | | | |
| 35 | TA-90-Med | | | | | | | | X | | | | | |
| 36 | TA-90-Med | | | | | | | | X | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 37 | STD High | X | | Bracket 1,2 | | | |
| 38 | STD Med | | | Bracket 1,2 | | | X |
| 39 | STD Low | | | Bracket 1,2 | | | |
| 40 | STD Blk | | | Bracket 1,2 | | | |
| 41 | TA-100-Low | | X | X | | | |
| 42 | TA-100-Low | | X | X | | | |
| 43 | TA-100-Low | | X | X | X | | |
| 44 | TA-100-High | | X | X | | | |
| 45 | TA-100-High | | X | X | | | |
| 46 | TA-100-High | | X | X | | | |
| 47 | TA-110-Med | | | X | | | |
| 48 | TA-110-Med | | | X | | | |
| 49 | TA-110-Med | | | X | | | |
| 50 | TA-100-Med | | | X | | X | |
| 51 | STD High | X | | Bracket 2 | | | |
| 52 | STD Med | | | Bracket 2 | | | X |
| 53 | STD Low | | | Bracket 2 | | | |
| 54 | STD Blk | | | Bracket 2 | | | |

[a] $S/N$ = signal to noise ratio. This is a measure of sensitivity.
[b] $R$ = resolution. It is noted that other chromatographic performance parameters, such as tailing, capacity factor, etc., can be added to the system suitability requirements and evaluated from this injection.

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*Table 2.* Second validation injection sequence, analyst 2.

| | | Purpose | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | System suitability | | | | | Validation | | | | | | | |
| Injection | Sample ID | Linearity | Precision | $S/N$[a] | $R$[b] | Response stability | STD linearity | Sample linearity | Accuracy precision | Specificity | Ruggedness | Sample stability | STD stability | LOQ |
| 1 | STD High | | | | | | | Conditioning injection only | | | | | | |
| 2 | STD High | | | | | | | Conditioning injection only | | | | | | |
| 3 | STD High | | | | | | | Conditioning injection only | | | | | | |
| 4 | STD Mid | | X | | | | | | | | | | X | |
| 5 | STD Mid | | X | | | | | | | | | | | |
| 6 | STD Mid | | X | | | | | | | | | | | |
| 7 | STD Mid | X | X | | | | | | | | | | | |
| 8 | STD Mid | X | X | | X | | | | | | | | | |
| 9 | STD High | X | | | | X | | | | | | | | |
| 10 | STD High | X | | | | X | | | | | | | | |
| 11 | STD Low | X | | | | | | | | | | | | |
| 12 | STD Low | X | | X | | | | | | | | | | |
| 13 | STD Blk | X | | | | | | | | | | | | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 14 | STD Blk | X | | | | | |
| 15 | STD High | | X | Bracket 1 | | | |
| 16 | STD Med | | | Bracket 1 | | | |
| 17 | STD Low | | | Bracket 1 | | | |
| 18 | STD Blk | | | Bracket 1 | | | |
| 19 | TA-Blk | | | X | X | | |
| 20 | TA-100-Med | | | X | X | | |
| 21 | TA-100-Med | | | X | X | | |
| 22 | TA-100-Med | | | X | X | | |
| 23 | TA-100-Med | | | X | X | | |
| 24 | TA-100-Med | | | X | X | | |
| 25 | TA-100-Med | | | X | X | X (final) | |
| 26 | STD Med-f | | | | | | X |
| 27 | STD High | | X | Bracket 1 | | | |
| 28 | STD Med | | | Bracket 1 | | | X (final) |
| 29 | STD Low | | | Bracket 1 | | | |
| 30 | STD Blk | | | Bracket 1 | | | |

[a] $S/N$ = signal to noise ratio. This is a measure of sensitivity.
[b] $R$ = resolution. It is noted that other chromatographic performance parameters, such as tailing, capacity factor, etc., can be added to the system suitability requirements and evaluated from this injection.

*Table 3.* Acceptance criteria master table, assay parameters and acceptance criteria.

| Parameter | Method of evaluation | Acceptance criteria |
|---|---|---|
| Specificity | Injection of matrix blank (TA-Blk) and test solution TA-100-Low | No co-eluting peaks in TA-Blk with peak area 20% of peak area of TA-100-Low (for each analyte) |
| Accuracy | Recovery of analyte from test solutions | 100 $\pm$ 30%, rsd $\leq$ 10%, all levels |
| Precision | | |
| Repeatability | six replicate determinations of TA-100-Med | % rsd $\leq$ 10% |
| Interim | two analysts on separate columns or separate days | % rsd $\leq$ 15% (pooled results) |
| Linearity (quadratic model) | Accuracy samples | $r^2 \geq 0.98$. % $y$-intercept $\leq$ 10% of TA-100-Med; provide plot, residual sum of squares |
| | Standards | Same criteria, % $y$-intercept $\leq$ 10% of STD Med |
| Range | Base on actual concentration of test solutions determined against the reference standard | Must encompass the minimum reportable value of 100 ppb and the high calibration level of 1000 ppb |
| Sensitivity, LOQ | Estimate from $S/N = 10$, confirm accuracy and precision | LOQ NMT 100 ppb; % rsd $\leq$ 10%, mean recovery 70–130% (see accuracy) |
| Ruggedness | Multiple systems/analysts (interim precision) | All results within the criteria above (interim precision) at the 300 ppb level |
| Standard and sample stability | Injections of standard and samples over an analytical run (not less than 24 hr) | ASF $\leq$ 20% for 24-hr result. Calculate % of initial at times less than 24 hr and examine results at less than 24 hr for consistency in trend (discontinuous changes) |

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## DETAILED DISCUSSIONS OF INDIVIDUAL SECTIONS OF THE VALIDATION PROTOCOL

### Preparation of Stock Solutions, Working Test Solutions, and Working Standards

Three solutions that are fundamental to method validation are the stock solutions, the working standards, and the working test solutions. The preparation of these and other required solutions is as follows.

#### Stock and Working Stock Solutions

The stock solution(s) are the concentrated solutions containing one or more of the method's analytes that are used to produce all standards and test samples. In general, the stocks are prepared at an analyte concentration of 1000 ppm in a matrix in which they are soluble at this concentration. While it is desirable to prepare a single stock that contains all the analytes, this is not always possible and sometimes a method requires the preparation of individual stocks for each analyte. As the amount of available reference material may be limited, the stocks must be prepared in proportions that minimize material usage without sacrificing preparation accuracy. The minimum quantities used to prepare stocks at the 1000 ppm levels are 25 mg of material dissolved into a final volume of 25 mL of solvent.

The directions for the preparation of the stock solutions are documented in the validation protocol in a tabular format, an example of which is provided in Table 4.

In order to make standard and samples in the appropriate concentration range (100–1000 ppb) with reasonable dilutions and preparation volumes, a working stock solution is needed. The working stock solution can be prepared

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***Table 4.*** Preparation of stock solutions.

| Solution name | Component(s) | Amount needed (g) | Dilution volume (mL)[a] | Approximate concentration (mg/L, ppm) |
|---|---|---|---|---|
| Stock | Leachable A | 0.025 | | 1,000 |
| | Leachable B | 0.025 | 25 | 1,000 |
| | Leachable C | 0.025 | | 1,000 |
| | Leachable D | 0.025 | | 1,000 |

[a]Diluent is an appropriate solvent (e.g., methanol).

at the 10 ppm level by diluting the stock 1–100 (e.g., 2 mL stock to a final volume of 200 mL) with an appropriate diluent. This working stock solution will be used to prepare the working standards and working test solutions.

### Working Standards

The working standards are produced to assess standard linearity and to calibrate the system response for concentration determinations. In routine use of the assay, four working standards are prepared and used, Std Blk, Std Low, Std Med, and Std High. A fifth working standard, STD Int, is prepared for and used only in the assessment of standard linearity during method validation.

The directions for the preparation of the working standards are documented in the validation protocol in a tabular format, an example of which is provided in Table 5. If the standards are matrix matched to the samples, the table for the preparation of working standards may be more complicated than the example shown in Table 5.

### Working Formulation Solution

The working formulation solution is the diluent that is used to prepare the working test solutions. It is directly related to the product code under investigation and accurately reflects that product's composition. Whenever possible, the working formulation solution should be prepared from raw materials obtained from manufacturing plant inventories.

***Table 5.*** Preparation of working standards.

| Working standard | Volume of working stock[a] (mL) | Volume of internal standard stock[b] (mL) | Final dilution volume[c] (mL) | Approximate concentration (ppb) |
|---|---|---|---|---|
| Std Blk | 0 | 1 | 100 | 0 |
| Std Low | 1 | 1 | 100 | 100 |
| Std Med | 3 | 1 | 100 | 300 |
| Std Int | 6 | 1 | 100 | 600 |
| Std High | 10 | 1 | 100 | 1,000 |

[a]The working stock is prepared at a target concentration of 10 ppm.
[b]For cases where an internal standard is used. This volume assumes a target of 1000 ppb internal standard from a working stock of 100 ppm.
[c]The appropriate diluent is specified in the validation protocol.

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Because of the dilutions involved in the preparation of the working test solutions, the working formulation solution cannot be prepared at the nominal levels of product ingredients. Rather, a proportionally concentrated working formulation solution is prepared. While a twofold concentration factor is convenient (and is the factor upon which Table 6 is based), it is occasionally the case that a twofold concentration cannot be achieved due to solubility issues. In such instances, an appropriate concentration factor must be established. In all cases, a recipe for the product of such a concentrated working formulation solution must be contained in the validation protocol.

If the product under development is sterilized by autoclaving, it is possible that heat-related degradation products may be present in actual product test units, and thus, the validation should be performed with a formulation test matrix that has been heat-stressed. As it is not practically possible to mimic a product's heat exposure history precisely, the working formulation solution shall be autoclaved at a nominal temperature of 121°C for 30 min.

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## Working Test Solutions

The working test solutions are produced for assessing virtually every validation performance characteristic other than standard linearity. For the purpose of assay validation, working test solutions are prepared at four concentration levels, TA-Blk (0 ppb), TA-Low (typically 100 ppb), TA-Med (typically 300 ppb), and TA-High (typically 1000 ppb).

The directions for the preparation of the working test solutions are documented in the validation protocol in a tabular format, an example of which is provided in Table 6. As the actual levels of ingredients in products contacted by plastic materials may vary somewhat from the labeled levels, accuracy and precision must be measured in samples formulated over a range of concentrations around the product's 100% labeled composition. For leachables/extractables assessments, this range is 90–110% of label. The effect of formulation composition on analytical performance is evaluated only at the Med concentration level. The working test solutions prepared at the 90% and 110% formulation levels are not used to assess sample linearity, as to do so would give uneven weighting to the Med concentration level.

## Internal Standard Solution

If the method being validated requires the utilization of one or more internal standards, the validation protocol must provide detailed information

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***Table 6.*** Preparation of working test solutions.

| Test solution name | Target formulation level | Volume of working stock solution[a] (mL) | Volume of working formulation solution (mL) | Volume of internal standard stock[b] (mL) | Dilution volume (mL) | Approximate concentration of each leachable (ppb) |
|---|---|---|---|---|---|---|
| TA-90-Med | 90% of Nominal | 4 | 45 | 1 | 100 | 300 |
| TA-100-Low | Nominal | 1 | 50 | 1 | 100 | 100 |
| TA-100-Med | Nominal | 4 | 50 | 1 | 100 | 300 |
| TA-100-High | Nominal | 10 | 50 | 1 | 100 | 1,000 |
| TA-110-Med | 110% of Nominal | 4 | 55 | 1 | 100 | 300 |
| TA-Blk | Nominal | 0 | 50 | 1 | 100 | 0 |

[a]The working stock is prepared at a target concentration of 10 ppm.
[b]For cases where an internal standard is used. This volume assumes a target of 1000 ppb internal standard from a working stock of 100 ppm.

related to the preparation of the internal standard solutions that may include a stock and a working solution.

As noted in Tables 5 and 6, the internal standard solution that is added to the working standards and samples has a concentration of 100 ppm. By adding this solution in a proportion of 1–100 (volume of internal standard solution to final sample volume), the working standards and samples contain 1000 ppb of the internal standard.

## Standard Linearity

### *Experimental Design*

Working standards are prepared at five concentration levels [Blank, Low (at the LOQ), Medium, Intermediate, and High] and injected sequentially in triplicate. While standards are not injected in either concentration ascending or descending order, the order is not completely random, as the replicate injections for each standard are typically sequential. These injections are made by the primary analyst (analyst 1) and, as shown in Table 1, include the injections made for the system suitability assessment of analysis run 1. Standard linearity is assessed as a validation parameter only once in a validation study but is included, in an abbreviated format, in system suitability testing for all test runs.

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### *Injections to Use from Validation Sequence (Tables 1 and 2)*

Table 1, Analyst #1: Injections 6 through 20.

Table 2, Analyst #2: Not applicable.

### *Calculations*

The resultant response vs. prepared standard target concentrations are subjected to regression analysis using a quadratic model of the form:

$$\text{Response} = \text{slope 1} \times (\text{concentration})^2 + \text{slope 2} \times (\text{concentration}) + \text{intercept}$$

The analysis must be based on individual data points and not the means for a standard concentration level. As the standard blank is included in the analysis, measured responses are not corrected for any blank response. The responses used may be peak area, peak height, or peak response ratio (analyte vs. internal standard), as specified in the method's procedure specification.

Characteristics of the resulting best-fit regression line that must be obtained and reported include: slope 1, slope 2, $y$-intercept, % $y$-intercept,

residual sum of squares. A calibration plot, exhibiting the entire set of data points and the best-fit regression line, must be generated and reported.

The % $y$-intercept is calculated as:

$$\% \; y\text{-intercept} = \frac{y\text{-intercept} \times 100\%}{\text{mean peak response, injections 6–8 of STD Med}}$$

*Acceptance Criteria*

The acceptance criteria for standard linearity are: coefficient of determination ($r^2$) greater than or equal to 0.98; % $y$-intercept less than or equal to 10%.

While residual sum of squares, slopes, and intercept are calculated and reported, these are characteristics of the calibration model that have no corresponding acceptance criteria.

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*Presentation of Data*

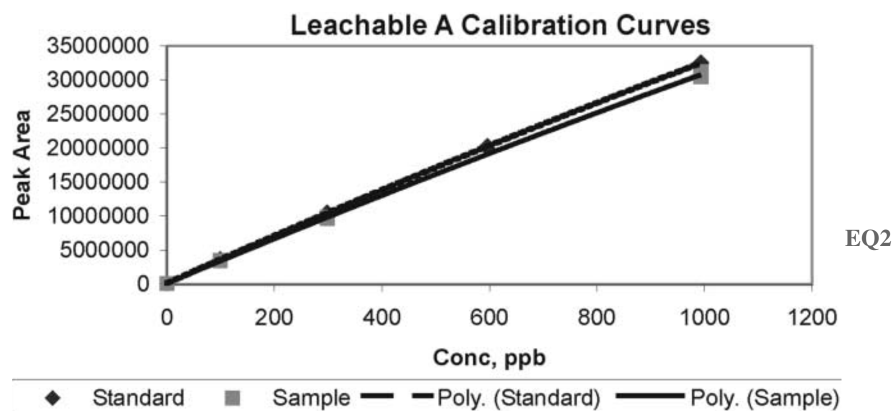
The curve fit parameters are summarized in tabular format as is illustrated in Table 7. When both standard and sample linearity are assessed in method validation, it is useful to tabulate the sample and standard curve fit data in the same table, as such a presentation readily highlights any sample to standard analytical bias (e.g., as differences in slopes or intercepts).

The calibration curve is presented in graphical format as shown in Figure 1. When both standard and sample linearity is assessed in method validation, it is useful to plot the sample and standard calibration curves on the same diagram as such a presentation readily highlights any

***Table 7.*** Standard linearity.[a]

| Calculation | Standard result (analyst 1) | Sample result (analyst 1) |
|---|---|---|
| Slope 1 ($x^2$) | −0.1537 | 0.1113 |
| Slope 2 ($x$) | 4694 | 4304 |
| $y$-intercept | −6019 | 15450 |
| % $y$-intercept | −0.43[a] | 1.15[b] |
| Coefficient of determination ($r^2$) | 0.9995 | 0.9996 |
| Residual sum of squares | 1.858E+10 | 1.657E+10 |

[a]Versus the mean Std Med response.
[b]Versus the mean TA-100-Med response.




EQ2

*Figure 1.* Calibration plot, linearity of samples and standards, leachable A. A quadratic function was used. *Note*: Quadratic and poly(nomial) are the same function.

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sample to standard analytical bias (e.g., as clearly visible differences in the best-fit lines).

## Sample Linearity

*Experimental Design*

Working test solutions at the 100% formulation level are prepared at four concentration levels [Blank, Low (at the required LOQ), Medium, and High], and injected in triplicate throughout the course of an analytical run. While it is noted that the Med working sample is injected a total of six times for the purpose of assessing precision, only the first three injections are used to assess sample linearity to avoid any weighting-related bias. It is also noted that while working test solutions are prepared in 90% and 110% of the formulation matrices at the middle concentration level, these solutions are not used in the sample linearity assessment to avoid any weighting-related bias. While the working test solutions are not injected sequentially or in either concentration, ascending or descending order, the order is not completely random, as the replicate injections for each standard are sequential. These injections are made by the primary analyst (analyst 1).

*Injections to Use from Validation Sequence (Tables 1 and 2)*

Table 1, Analyst #1: Injections 25 through 27, 31 through 33, 41 through 46.

Table 2, Analyst #2: Not applicable.

*Calculations, Acceptance Criteria, and Presentation of Data*

The process and procedures for evaluating and presenting sample linearity data are analogous to those enumerated previously for standard linearity.

## Accuracy and Precision

*Experimental Design*

Working test solutions are prepared in triplicate or injected three times into the chromatographic system. The measured concentrations of the leachables in the each working test solution are calculated from the measured working test solution's response in a manner consistent with the method's procedure specification. The responses used may be peak area, peak height, or peak response ratio (analyte vs. internal standard), as specified in the procedure specification. As the working test solutions are prepared by spiking the working formulation solution, if the working formulation solution produces an apparent leachables response, this response must be appropriately accounted for (e.g., blank subtraction). The magnitude of this blank response is obtained as the mean response for working test sample TA-Blk. The measured concentrations for the working test solutions are compared to the theoretical preparation concentrations (concentration target) for these solutions to assess analytical accuracy. Precision is assessed as the % relative standard deviation (% R.S.D.) of the measured concentrations for the replicate preparations or injections of a working test solution.

Most of the accuracy and precision data are obtained in the first validation run (analyst 1). However, as analyst 2 performs the equivalent of an accuracy assessment for sample TA-100-Med, this information is also used to assess accuracy and precision (as well as ruggedness).

*Injections to Use from Validation Sequence (Tables 1 and 2)*

Table 1, Analyst #1: Injections 25 through 36, 41 through 50.

Table 2, Analyst #2: Injections 19 through 25.

*Calculation*

The following calculations are performed.

1. Net solution response:

$$\text{Net solution response} = \text{measured response for working test solution} - \text{mean measured response, TA-Blk.}$$

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2. Leachables concentration in the working test solution: Put the net solution response into the standard calibration curve to obtain the leachable concentration in each injection or preparation.
3. % Recovery: For each injection or preparation, calculate % recovery as:

$$\% \text{ recovery} = \frac{\text{measured concentration}}{\text{concentration target}} \times 100\%$$

   For all injections or preparations for a particular working test solution, calculate the mean % recovery.
4. Precision: The precision is calculated as the % R.S.D. of the replicate measured concentrations obtained for each working test solution using $n - 1$ statistics.

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*Acceptance Criteria*

Accuracy: % recovery for all individual injections/preparations: 70–130%.

Precision: % R.S.D. NMT 10%.

*Presentation of Data*

The accuracy and precision data may be reported in a tabular format as shown in Table 8. As all the data relevant for ruggedness is contained in the accuracy and precision table, ruggedness results are also reported in the same table.

Ruggedness

*Experimental Design*

Working test solution TA-100-Med is prepared or injected six times into the chromatographic system in two different analytical runs by two different analysts. At least one injection of TA-Blk is made in each run. The measured concentration of the leachables in the each preparation/injection is calculated from the measured working test solution response in a manner consistent with the method's draft procedure specification. Ruggedness is calculated as the population % R.S.D. of the concentration data from both analyst 1 and analyst 2 ($n = 12$).

The ruggedness assessment is completed in two extended analytical runs, performed by two independent analysts using two different analytical columns (consistent with the method's draft procedure specification) and two different preparations of the mobile phase (if applicable). As the creation of the test

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*Table 8.* Accuracy, precision and ruggedness (Leachable A).

| Test solution | Theoretical concentration (ppb) | Experimental concentration (ppb) | | | Recovery (%) | | Mean recovery (%) | | R.S.D. (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Analyst 1 | Analyst 2 | Overall mean | Analyst 1 | Analyst 2 | Analyst 1 | Analyst 2 | Analyst 1 | Analyst 2 | Ruggedness[a] |
| TA-Blk | 0[c] | — | —[b] | — | — | — | — | — | — | — | — |
| TA-90-Med | 298 | 296 | —[b] | — | 99.3 | — | 98.5 | — | 0.7 | — | — |
| | | 292 | — | | 98.1 | — | | | | | |
| | | 292 | — | | 98.1 | — | | | | | |
| TA-100-Low | 99 | 96.7 | —[b] | — | 97.4 | — | 96.6 | — | 0.7 | — | — |
| | | 95.5 | — | | 96.2 | — | | | | | |
| | | 95.6 | — | | 96.2 | — | | | | | |
| TA-100-Med | 298 | 284 | 298 | 290 | 95.2 | 100 | 94.0 | 100 | 1.6 | 2.2 | 3.9 |
| | | 284 | 305 | | 95.3 | 102 | | | | | |
| | | 278 | 298 | | 93.2 | 99.9 | | | | | |
| | | 284 | 287 | | 95.2 | 96.4 | | | | | |
| | | 279 | 304 | | 93.5 | 102 | | | | | |
| | | 273 | 303 | | 91.5 | 102 | | | | | |
| TA-100-High | 993 | 969 | —[b] | — | 97.6 | — | 96.0 | — | 1.4 | — | — |
| | | 950 | — | | 95.6 | — | | | | | |
| | | 942 | — | | 94.9 | — | | | | | |
| TA-110-Med | 298 | 282 | —[b] | — | 94.6 | — | 95.7 | — | 1.1 | — | — |
| | | 288 | — | | 96.7 | — | | | | | |
| | | 285 | — | | 95.7 | — | | | | | |

[a]The Ruggedness portion of the validation combines the results of TA-100-Med for analysts 1 and 2. Twelve replicates total.
[b]These samples are analyzed by analyst 2.
[c]While a peak was observed at the retention time and $m/z$ of Leachable A, its area was too small to calculate its concentration.

formulation (spiked and unspiked) is not an intrinsic part of the method under validation but rather is necessary only for performing the validation, it is not appropriate for that creation to be repeated by both analysts. However, if the method calls for a specific sample preparation process (e.g., dilution, solvent/solvent extraction) prior to instrumental analysis, then each analyst should perform the sample preparation process independently.

*Injections to Use from Validation Sequence (Tables 1 and 2)*

Table 1, Analyst #1: Injections 25 through 33.

Table 2, Analyst #2: Injections 19 through 25.

*Calculation*

The following calculations are performed by both analysts.

1. Net solution response:

   Net solution response = measured response for working test solution − mean measured response, TA-blk.

2. Leachables concentration in the working test solution: Put the net solution response into the standard calibration curve to obtain the leachable concentration in each injection or preparation.
3. Recovery: For each injection or preparation, calculate % recovery as:

$$\% \text{ recovery} = \frac{\text{measured concentration}}{\text{concentration target}} \times 100\%$$

   For all injections or preparations for a particular working test solution, calculate the mean % recovery.
4. Ruggedness: The ruggedness calculation is performed as the % R.S.D. of the calculated concentration for all preparations/injections of working test solution TA-100-Med made by both analysts ($n = 12$).

*Acceptance Criteria*

% R.S.D. NMT 15%.

*Presentation of Data*

The ruggedness data is typically reported in a tabular format along with the accuracy and precision data as shown in Table 8.

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Detectability, LOQ

*Experimental Design*

The level of 100 ppb is significant to the method's validation as it reflects the desired LOQ. This level is dictated not solely by the analytical capability of methods used in leachables applications, but also by the observation that, in general, the product impact of leachables/extractables at levels below 100 ppb is small.

While numerous methods can be used to calculate LOQ, a chromatographically derived method is used for leachables/extractables investigations. This method involves the direct measurement of the signal vs. noise obtained from a chromatogram of the test sample spiked to the lowest analyte concentration level (TA-100-Low).

*Injections to Use from Validation Sequence (Tables 1 and 2)*

Table 1, Analyst #1: Injection 12.

Table 2, Analyst #2: Not applicable (unless required by system suitability, injection 12).

*Calculations*

See Fig. 2 for the proper measurement of the signal and noise. The LOQ is calculated as:

$$\text{LOQ} = (\text{concentration of TA-100-Low}) \times 10 \times (\text{noise/signal})$$

*Acceptance Criteria*

LOQ NMT 100 ppb. Additionally, the accuracy and precision requirements for TA-100-Low must be met (% recovery of 70–130%, % R.S.D. NMT 10%).

*Presentation of Data*

The LOQ is typically reported in the body of the validation report.

Sample and Standard Stability

*Experimental Design*

During the implementation of an analytical method for quantitating leachables in a drug product, it is required that an analytical run shall last for no longer than 24 hr. This 24 hr is based on the filling of the autosampler vials with samples and standards immediately prior to the start of the analytical run, and immediately after the samples are prepared in their final injected form. This 24 hr is measured from the first injection of an appropriate

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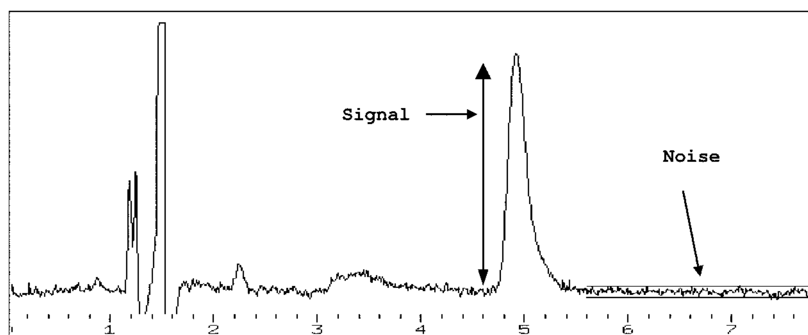


*Figure 2.* Signal-to-noise. Typical chromatogram illustrating the calculation of the signal and noise. The signal is measured from the apex of the peak to the middle of the straight line peak base. The noise is measured as the distance between straight lines constructed from the tops and bottoms of the baseline variation. The peak to peak baseline variation (noise) should be measured in a clean portion of the chromatogram and should include a fair representation of the inherent baseline variation.

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concentration standard during the system suitability assessment to the last injection of the same standard in the last calibration bracket. It must, therefore, be established in the validation exercise that the samples and standards, fully prepared and stored in autosampler vials, maintain their analyte concentration for a period of not less than 24 hr. This is accomplished by filling several autosampler vials with STD Med and TA-100-Med and then injecting the vials throughout one or both of the validation sequences.

The exact nature of the stability assessment may vary from one validation study to the next, depending primarily on the analysis time for each injection. If the analysis time is relatively short (10–15 min), completion of sequence 1 will not require 24 hr. In order to meet the 24-hr sample and standard storage requirement, three possibilities are available. The first two involve modification of sequence 1 and include, (a) running the assay with all injections having an analysis time longer than the 'normal' method, or (b) adding 'dummy' injections to the sequence (where the sole purpose of the dummy injections is to keep the method running until 24 hr of storage has occurred). The last possibility is that the stability samples be run as part of the second (analyst 2) sequence.

If the analysis time is relatively long (30 min or more), then the first sequence will typically last more than 24 hr and the stability assessment can be accomplished internal to the first sequence.

Two mechanisms can contribute to a change in the magnitude of the peak response during the course of an extended analytical run. One such mechanism is a chemical/physical change in the sample during its storage in autosampler vials while it awaits analysis. During storage, the analyte may degrade or become absorbed by the vial, decreasing its concentration and analytical signal. Alternatively, the sample solvent may evaporate, resulting in analyte concentration and an increase in signal.

The second mechanism is a change in detection sensitivity, which can occur if the performance of the analytical system changes appreciably over time. While such a change is undesirable, it is not germane to sample and standard stability. Thus, the contribution of changing analytical system response to the over-all change in peak response must be accounted for. This is accomplished by injecting a fresh preparation of Std Med near to the final injections (24 hr or more of storage) of 'old' portions of Std Med and TA-100-Med. For the purpose of validating methods for leachables/extractables, a fresh standard shall be defined as a fresh dilution of a previously prepared stock solution with a previously prepared diluent.

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*Injections to Use from Validation Sequence (Tables 1 and 2)*

For standard stability:

Table 1, Analyst #1: Injections 4 and 22 (initial), 38, 52.

Table 2, Analyst #2: Injections 4, 26 (fresh standard), 28 (final).

For sample stability:

Table 1, Analyst #1: Injections 25 (initial), 50.

Table 2, Analyst #2: Injections 4, 25 (final), 26 (fresh standard).

*Calculations*

For all time points except initial, calculate the % of initial:

$$\% \text{ of initial} = \frac{\text{response for specific injection}}{\text{response for initial injection}} \times 100\%$$

For the final time point, first calculate the response stability factor (RSF):

$$\text{RSF} = \frac{\text{initial response for standard}}{\text{response for the fresh standard}}$$

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Secondly, calculate the absolute stability factor (ASF):

$$\text{ASF} = \text{absolute value}\left(\frac{\text{BOR} - (\text{EOR} \times \text{rsf})}{\text{BOR}} \times 100\%\right)$$

where BOR = initial response (sample or standard) and EOR = last response (sample or standard).

For example, if the peak response for the first injection of a sample was 100, the peak response for the last injection of the sample was 90, the peak response for the first injection of a standard was 85, and the peak response for the fresh standard injection was 80, the ASF is calculated as follows:

$$\text{RSF} = \frac{\text{initial response for standard}}{\text{response for fresh standard}}$$

$$\text{RSF} = \frac{85}{80} = 1.06$$

$$\text{ASF} = \text{absolute value}\left(\frac{\text{BOR} - (\text{EOR} \times \text{rsf})}{\text{BOR}} \times 100\%\right)$$

$$\text{ASF} = \text{absolute value}\left(\frac{100 - (90 \times 1.06)}{100} \times 100\%\right)$$

$$\text{ASF} = \text{absolute value}\left(\frac{100 - 95.4}{100} \times 100\%\right)$$

$$\text{ASF} = 4.6\%$$

Note that the concentration of the initial and fresh standards should be as close as possible, or else a standard concentration correction is required.

*Acceptance Criteria*

For the final (24 hr or greater) time point, ASF must NMT 20%. This calculation includes a correction for the response change due to analytical system drift.

For the time points prior to the final time point, it is not possible to correct for system drift because no fresh standard is prepared or injected in close proximity to the 'old' sample or standard. In these cases, while it is desirable for % of initial to be in the range of 80–120%, this may not be the case even if the sample or standard is stable. Thus, the data from the intermediate time points should be examined for response trends, to see if there is a definite drift in response or whether the response vacillates around a particular value due to analytical variation.

*Presentation of Data*

The stability data can be presented in tabular form, as shown in Table 9.

*Table 9.* Standard/sample stability (Leachable A).

| Standard (Std Med) | | Sample (TA-100-Med) | |
|---|---|---|---|
| Time after preparation (hr) | % of initial | Time after preparation (hr) | % of Initial |
| 0:00:00 | 100 | 0:00:00 | 100 |
| 8:12:30 | 99 | 8:22:30 | 101 |
| 16:45:00 | 99 | 16:54:55 | 90 |
| 24:32:15 | 98 | 24:17:20 | 88 |
| Fresh 24:47:25 | 84 | | |
| | Absolute stability factor | | |
| Elapsed time: 24:32:15 | 16.6 | Elapsed time: 24:17:20 | 4.7 |

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## Range

In the event that all the validation acceptance criteria are met, the range of the method for leachables quantitation is defined as 100–1000 ppb.

## Specificity

*Experimental Design*

Sample TA-Blk and TA-100-Low are appropriate for assessing specificity. Overlay chromatograms are generated for these samples and are examined for instances of interference between peaks in the blank vs. known analyte peaks in the spiked sample. If such an interfering peak is observed, then the response of the interfering peak in the blank and the response of the analyte peak in the sample are obtained.

*Injections to Use from Validation Sequence (Tables 1 and 2)*

Table 2, Analyst #1: Injections 33 and 43.

Table 2, Analyst #2: Not applicable.

*Calculation*

If an interfering peak is observed, the % bias is calculated as:

$$\%\ \text{bias} = \frac{\text{response of interferant in blank}}{\text{response of analyte in spiked sample}} \times 100\%$$

*Acceptance Criteria*

The acceptance criteria for specificity are:

Tier 1 Assessment: No interfering peaks observed (assay is reported as absolutely specific).

Tier 2 Assessment: Interfering peak(s) observed. % bias NMT 30% (assay is reported as provisionally specific).

Validation Summary Table

The results of the entire validation exercise are summarized in the validation summary table, as shown in Table 10. A successful validation will have pass noted in the entire results column.

## SYSTEM SUITABILITY TESTING

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It is required that all analytical runs performed in the validation endeavor meet the specified system suitability requirements. This assures that the method, as implemented, is capable of producing data of sufficient quality for the desired application and remains capable throughout the entire analytical run.

The procedure specification should include a system suitability assessment, complete with performance requirements. It is the responsibility of validation runs to meet acceptance criteria, not to establish system suitability criteria. This is true because the validation exercise includes too few runs and too few method variations to effectively establish operating ranges of appropriate performance.

While a viable system suitability test may include the evaluation of a number of parameters, all system suitability assessments should consider the following performance parameters:

linearity of response,

magnitude of response (detectability),

reproducibility of response (precision),

long-term consistency of response (stability),

accuracy in standard preparation (secondary standard recovery), and

chromatographic capability (chromatographic parameters such as resolution, capacity, tailing factor).

A validation report must minimally include a statement that a complete system suitability assessment was performed for each analytical run, and

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*Table 10.* Validation summary table.

| Parameter | Criterion | Value obtained | | Result |
|---|---|---|---|---|
| | | Analyst 1 | Analyst 2 | |
| Specificity | Peak response in TA-Blk less than 20% of response in TA-100-Low (% bias NMT 20%) | 10.1% | N/A | Pass |
| Standard linearity | $r^2$ NMT 0.98 | 0.9965 | N/A | Pass |
| | % $y$-intercept NMT 25% | 5.2% | N/A | Pass |
| | Residual sum of squares, calibration plot | Reported | N/A | Pass |
| Sample linearity | $r^2$ NMT 0.98 | 0.9965 | N/A | Pass |
| | % $y$-intercept NMT 25% | 5.2% | N/A | Pass |
| | Residual sum of squares, calibration plot | Reported | N/A | Pass |
| Precision, repeatability | % rsd NMT 10%, | | | |
| | TA-100-Med ($n = 6$) | 1.1% | 0.87% | Pass |
| | TA-90-Med ($n = 3$) | 2.5% | N/A | Pass |
| | TA-110-Med ($n = 3$) | 3.7% | N/A | Pass |
| | TA-100-Low ($n = 3$) | 1.2% | N/A | Pass |
| | TA-100-High ($n = 3$) | 5.6% | N/A | Pass |
| Accuracy | % Recovery between 70% and 130%, | | | |
| | TA-100-Med ($n = 6$) | 99.5% | 96.2% | Pass |
| | TA-90-Med ($n = 3$) | 102% | N/A | Pass |
| | TA-110-Med ($n = 3$) | 107% | N/A | Pass |
| | TA-100-Low ($n = 3$) | 91.3% | N/A | Pass |
| | TA-100-High ($n = 3$) | 89.5% | N/A | Pass |
| Sensitivity | LOQ NMT 100 ppb | 50 ppb | N/A | Pass |
| Ruggedness | % RSD NMT 15%, pooled data, two runs | 6.7% | | Pass |
| Stability, Std | ASF NMT 20%, results for intermediate tests in trend | ASF = 14%, in trend | | Pass |
| Stability, sample | ASF NMT 20%, results for intermediate tests in trend | ASF = 5.8%, in trend | | Pass |

*Table 11.* System suitability results.

| Parameter | Criterion | Value obtained | | Result |
|---|---|---|---|---|
| | | Analyst 1 | Analyst 2 | |
| Linearity | $r^2$ NLT 0.98 | 0.9998 | 0.9999 | Pass |
| Precision | % RSD, 300 ppb NMT 10% | 1.1% | 0.87% | Pass |
| Efficiency | Baseline resolution of all analytes in STD Med | Confirmed | Confirmed | Pass |
| Sensitivity | $S/N$ NLT 10 at the 100 ppb level | 146 | 146 | Pass |
| Response stability | % RSD NMT 10%, STD 1000 injections for entire analytical run | 3.7% | 6.2% | Pass |
| Accuracy in preparation | % Recovery, secondary standard 90–110% | 99.5% | 98.1% | Pass |

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that each run passed all of the acceptance criteria. However, it is more useful to report the results of the system suitability assessments, as such information may be relevant in terms of re-assessing the acceptance criteria or for augmenting a method performance database. The system suitability results can be provided in a tabular format, as is shown in Table 11.

## FAILING AN ACCEPTANCE CRITERION

An analytical method undergoing validation should have been sufficiently optimized and characterized prior to validation, so that there is little possibility that the method validation activity could produce results that do not meet the established acceptance criteria if the validation is competently executed. However, it is occasionally the case that a validation design is flawed, that a method weakness was not surfaced during evaluation, or that a capable method and an effective validation design were poorly executed. If a specified acceptance criteria failed during validation testing, a failure investigation is to be conducted in a thorough, timely, and well-documented manner. If the investigation concludes that the acceptance criterion has been set incorrectly, it must be changed by means of a protocol amendment with appropriate justification and approvals.

Laboratory tests may be necessary to eliminate potential contributing factors (e.g., instrument malfunctions or sample preparation errors). Such testing may be performed to assign actual cause, but is investigative only and cannot replace failing validation results. Examples of such testing include testing of standards, reagents, original solution preparations, or autosampler vial contents. The planned testing and its intended purpose must be

***Table 12.*** Common considerations for analytical investigations.

- Discuss test method with analyst(s) to determine if the procedure was performed per procedure specification
- Re-review documentation verifying system suitability, all calculations and examining all raw data (e.g., chromatograms) for anomalous or suspect information
- Calculations of applicable standards/reagents correct
- Linearity of standards, if applicable, verified throughout run
- Baseline verified for consistency
- Visually inspected for proper integration and consistency between samples and standards
- Retention times for injected samples and standards consistent
- No system failure due to high pressure, power failure, etc.
- Correct mobile phase used and not expired
- Control sample results acceptable
- Correct column used
- Column heater at correct temperature
- Correct flow rate and wavelength used
- Confirm instrument performance
- Instrument calibration ranges correct and instrument was within expiration date
- Equipment/instrument checked for malfunctions
- Correct instrumentation and parameter selection/settings used
- Detector zeroed
- System visually inspected for leaks
- No bubbles in mobile phase inlet line
- Vials properly capped
- Verify sample vial has been punctured by autosampler needle
- Verify quality of reference standards, solvents, and reagents
- Correct reagents or standards used with none expired
- Verify solutions were prepared accurately
- Clean glassware used
- Correct volumetric glassware used
- Correct dilution factor(s) used
- Correct weight/volume of samples and standards used
- Verify sample is within standard range
- Detector output and $A/D$ input range were not exceeded

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documented prior to analysis. If an actual cause can be assigned, original results may be invalidated. The failed experiment may be rerun with any appropriate changes made to the analytical procedure.

The study director must document the investigation and the investigation must be reviewed at the appropriate level of management. The documentation must include at a minimum:

***Table 13.*** Retesting guidelines and typical retesting schemes.

| Parameter | Original intent | Retesting scheme |
|---|---|---|
| Specificity | Show adequate specificity | Retesting typically not appropriate, unless cause can be assigned to inappropriate preparation of samples. Evaluate method parameters and acceptance criteria |
| Accuracy | Show accuracy at various levels across range | Retest all replicates at the failed level(s) with two analysts. If consistently biased results are obtained, sample preparation errors may be the cause. Re-prepare the samples and re-analyze (one analyst) |
| System precision | Show precision of instrument | Retest all replicate measurements of single sample preparation on two separate systems |
| Method precision | Show precision across sample preparations | Two analysts prepare and test all replicates |
| Intermediate precision | Show precision across days, instruments, and analysts | Repeat prescribed performance tests with two analysts or instruments not used in original experiment |
| Linearity | Show linearity across range | Independently repeat linearity test (all levels and replicates) with two analysts |
| LOD | Determine level of detection | See method precision |
| LOQ | Determine level of quantitation | See accuracy and/or method precision |
| Range | Determine range over which accuracy, precision and linearity can be shown | See accuracy, precision, and/or linearity above |

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A description of the failed acceptance criteria.

A summary of the analytical investigation including all parameters examined (examples of some assay parameters are listed in Table 12) and probable or assignable cause if found.

A review of earlier studies to determine if the problem has occurred previously.

A description of any retesting performed and its intended purpose (Table 13 contains general guidelines and typical retest schemes for failure of common validation criteria).

Results and conclusions of any retesting performed including any clarifications and changes required in the analytical procedure.

Assessment of the validation status of the method.

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## REVALIDATION OF A VALIDATED METHOD

Once the validation of an analytical method is completed and reported, the method is assumed to be valid until either it is: (a) applied under different circumstances (e.g., different product, different formulation), (b) found to be incapable of meeting system suitability criteria, (c) found to produce verifiably unacceptable results, or (d) modified in a significant manner. In such cases the analytical method must be revalidated. The method validation parameters to be included in the revalidation study will depend on the specifics of each situation, and the exclusion of specific parameters must be fully justified in a protocol and report.

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