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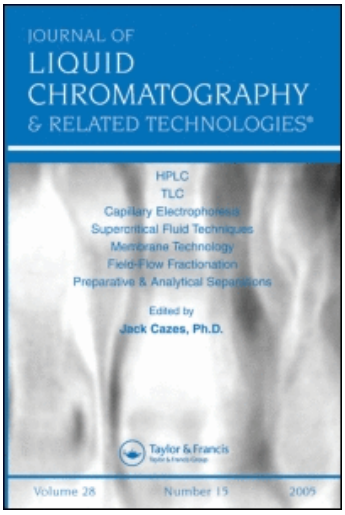

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Chromatographic Method Validation: A Review of Current Practices and Procedures. II. Guidelines for Primary Validation Parameters.

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CHROMATOGRAPHIC METHOD VALIDATION: A REVIEW OF CURRENT PRACTICES AND PROCEDURES. II. GUIDELINES FOR PRIMARY VALIDATION PARAMETERS.

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ABSTRACT

Validation of analytical methodologies is an important aspect of their development/utilization and is widely required in support of product registration applications. In this manuscript, definitions, procedures and acceptance criteria which appear in the pharmaceutical literature are summarized for the more commonly encountered validation parameters. Parameters examined include accuracy, precision, specificity, linearity and sensitivity limits.

INTRODUCTION

Chromatographic methods are used for the quantitative and qualitative analysis of environmental and pharmaceutical samples. The object of the analysis is to generate reliable, accurate and interpretable information about the sample. In order to ensure that the analytical method fulfills this objective, it undergoes an evaluation loosely termed validation. In the first part of this series,[1] accuracy, precision, specificity, linearity and sensitivity were identified as validation parameters which were most frequently cited in general

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manuscripts related to the validation of chromatographic assays in primarily pharmaceutical applications. In this manuscript, these primary validation parameters are examined in greater detail to develop their working definition, to establish specific measurement protocols and to summarize recommended acceptance criteria.

Characteristics of an Effective Validation Parameter

An investigator's ability to understand, evaluate and utilize a validation parameter is influenced by the parameter's description. Descriptors of validation parameters include its definition, its scope (applicability), recommended assessment procedures and its acceptance criteria. An effective validation parameter is one where the user knows what the parameter is, when to use it, how to perform its evaluation and what criteria to use to complete the validation assessment. For a validation parameter to be effectively understood and utilized it must possess the following minimal characteristics:

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Definition

The parameter must be defined in a clear, concise and unambiguous manner. Alternate definitions of the parameter should contain a core set of universally acceptable concepts or phrases. As necessary, the definition should be quantitative and mathematically rigorous.

Scope

The acceptability and applicability of the parameter in common situations should be clearly established.

Procedures

The procedures for performing the validation must be presented in a complete, well defined, practical and understandable format. Procedures should be outlined with sufficient detail so that all important experimental variables can be set to defined values. While it is most advantageous for the procedures to be as broadly applicable as possible, exceptions should be clearly and completely stated.

Acceptance Criteria

Once the validation is complete, an investigator must be able to interpret

the results. Acceptance criteria must be available which allows the researcher to unambiuously determine, by comparing method performance data to the criteria, whether the method under evaluation is performing in a valid manner.

To facilitate the evaluation, acceptance criteria should be universally applicable, numerically and mathematically explicit, complete and achievable. Additionally, acceptance criteria should be referenceable in that they should be traceable, through appropriate literature citations, to a rigorous scientific evaluation of their development and justification.

Given these criteria, several validation parameters will be considered with respect to their published descriptions. Trends of conceptual commonality within the literature will be established via nesting of similar literature citations.

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Accuracy

Definition

Generally one expects a properly working procedure to produce the expected results when it is performed in a standardized manner. Thus a procedure is validated for accuracy by performing it in a standardized manner and comparing the observed results with the expected behavior. For an analytical method, the accuracy is most commonly defined as follows:

The closeness of agreement between the value found by the method and the value which is accepted either as a conventional true value or a reference value.[2,3,4,5,6,8,19,20,21,24-27,30]

Accuracy can also be defined as the difference between a result and a true or known value.[12,15] The concept of acceptable limits, recognizing that the comparison between observed and true behavior must be statistically based given the inherent variation in the observed behavior, is addressed by several authors.[10]

Procedures

Several procedures appear in the literature for the determination of method accuracy. A commonly referenced procedure involves the fortification of a test solution with a known amount of the analyte of interest. Accuracy is assessed by "applying the analytical method to samples or mixtures of sample

matrix components to which known amounts of the analyte have been added both above and below the normal levels expected in the samples."[3,7,8,11-13,17,18,26,30,36] Method accuracy is the agreement between the difference in the measured analyte concentrations of the fortified and unfortified samples and the known amount of analyte added to the fortified sample. If the solution being fortified is a placebo (an artificially prepared simulation of the sample's matrix alone), the fortification procedure is termed spiking. The method of standard additions is the fortification of a sample which already contains the analyte at its normal level.

In a variation of fortification, Cardone and associates propose a relative response curve method wherein the placebo and standard blank are both spiked at several analyte levels encompassing the method's linear range.[22] Both sets of data are subjected to separate linear regression analyses and the determination of accuracy is performed by comparing the slopes and intercepts of the respective best fit lines.

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Other procedures suggested for assessing method accuracy include collaboration, in which data obtained from the candidate method is compared to data generated with a widely accepted (e.g., validated, compendial, standard) method.[5,11,15,35,34,36] In theory, "the best way to determine system bias (accuracy) is to use some definitive method, based on some unique property of the analyte, which eliminates or corrects for every possible source of error."[36] Analysis of reference materials, prepared externally by an approved vendor[7,19,25,36] or internally via spiking,[4,7,12,15,21,29,34] is another procedure for assessing accuracy. Its application is limited by both the availability and stability of the reference materials and the degree of certainty with which the analyte's true concentration in the reference material is known. For chromatographic assays a mass balance approach has been recommended wherein the sample is injected into the chromatographic system both with and without the column and the total peak response in both configurations is compared.[17]

Procedure Guidelines

The following procedural guidelines appeared in the validation literature:

* Accuracy requires six replicate assays.[6,19,27]

* Accuracy is determined over the range from 80% of the lowest expected assay value to 120% of the highest expected assay value[2,4,9,17,26] with triplicate measurements[4] or at five levels.[17]

* Recovery studies should be run at 75% to 125% of label claim.[30]

* Use six samples of drug in the matrix spanning 50 to 150% of the expected content.[6]

* The appropriate standard addition level is 20% of the target analyte level.[8]

* Use replicates,[12,15] minimum of five samples[29] or at least six degrees of freedom[7] at 3 concentrations within the analytical range (extremes and midpoint of expected[7] or near quantitation limit, center of range and upper bound of standard curve).[29]

Acceptance Criteria

For trace level analyses, the following criteria are pertinent:

* Below 100 ppb, 60 to 110% recovery is acceptable; above 100 ppb, 80 to 100% recovery is acceptable.[9,27]

* Below 1 ppm, 70 to 120% recoveries ar acceptable.[19]

* Impurities present at 0.1 to 10% should produce data within ± 5% of actual.[17]

General criteria for pharmaceutical samples included:

* The average recovery of spikes should be 98% to 102% of the theoretical value.[14,17]

* The recovery of the drug (as % of theory) must be within ± 4S of the theoretical value where S is the system (or method) precision.[6,8]

* For standard additions, the plot of assay response versus amount added should have a slope of 0.95 or greater and an intercept equal to the initial concentration.[26,34]

* For spiking, the plot of recovered versus known spike should have a correlation coefficient of 1.00, a slope of 1.00 and an intercept of 0.00.[11,34]

* For the relative response curve method, analyte/matrix interaction effects are absent if the intercepts of the matrix and standard plots are

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statistically equal to zero. Proportional systematic error is absent if the ratio of the slopes of the response curves for the matrix and standard is statistically equivalent to one.[22]

In biological samples, method accuracy for discovery phase investigations should be ± 20% of actual, with recoveries of ± 10% being necessary in pre-clinical and clinical studies.[27] Alternatively, it is recommended that the mean recovery value should be within ± 15% of actual except at the quantitation limit where ± 20% is acceptable.[29]

Precision

Definition

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A properly performed validated procedure will produce consistent results reflecting those sources of variation inherent in the procedure's steps. Thus precision reflects a procedure's ability to reproduce the same, but not necessarily the correct or expected, result each time it is correctly preformed. In the pharmaceutical literature, precision is commonly defined as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple analyses of the same homogeneous samples under the prescribed assay conditions.[3,4,5,9,11,18,21,24,25,27,30,33,36] This definition clearly establishes that method precision is sample and procedure specific and emphasizes the role that a uniform sample and a standard procedure protocol have in establishing precision. Since precision has statistical connotations, it can be defined as the distribution of individual test results around their mean.[6,12,15,19,25]

Precision is frequently subdivided into three flavors as a function of the number of locations at which the procedure is performed and the time span over which the precision data is collected. Repeatability (intra-assay or within day precision) reflects the variation in replicate procedures performed within a short time period (same analytical run) with the same operational conditions (operator, instrument, reagents, operating conditions). Intermediate (day to day) precision is related to analyses performed on different days by different analysts on different instruments with different reagents at the same operating facility. Reproducibility (intra-laboratory precision) is related to the procedure being performed at two or more laboratories in, for example, a collaborative study.

Precision can be categorized in terms of its source within a procedure. System precision is related only to the operation of an analytical instrument or

the performance of the analytical test while method precision is related to all aspects of a procedure including sampling and sample preparation.

Procedure Guidelines

Precision is assessed by repetitively performing the procedure in a prescribed manner with a prescribed sample and statistically evaluating the resulting data. Important issues related to the precision determination include the number of replicates required and the type of sample to be tested. Considering the issue of replicates, recommendations in the literature are somewhat discordant. For the determination of repeatability, the following recommendations are noted:

* Five replicates for release or stability assays[4]

* At least six to ten replicates.[4,8,31]

* Duplicate measurements made on ten samples at each of three different analyte levels.[12,15]

* Five replicates at three levels (limit of quantitation, mid-range and upper calibration bound).[29]

* Replicate samples at analyte levels of 80 to 120% of expected for dosage forms and drug substance tests.[2]

* Sufficient data should be generated to ensure more than thirty degrees of freedom.[11]

For intermediate precision, the repeatability experiments should be performed on 2,[4,20,21] 3 to 5[30] or at least 10[12,15] separate days. To assess reproducibility, the repeatability experiments have to be performed in at least two laboratories.[6]

The issue of sample type is of key importance, particularly if material sampling is an important procedure component. Central to this issue is the use of authentic versus artificially prepared samples. While several authors recommend the use of authentic samples,[5,13,20] it is recognized that sample instability or the inability to produce a sufficiently homogeneous authentic sample limits their use. If artificially prepared samples are used, several authors suggest that they be prepared to mimic either freshly prepared or degraded product.[5,21,29,30] Alternatively, several authors argue that authentic or artificially prepared product is not required for the precision evaluation,

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suggesting the use of standard solutions[6,18] or any sample which will produce a similar response when the method is applied to it.[25]

Acceptance Criteria:

The following acceptance criteria for precision were found in the literature:

* The desired precision of stability indicating methods should not be more than ± 1.0 % RSD.[16]

* System precision should be ≤ 1% RSD (or higher for low level impurities).[24]

* System precision ≤ 1.5% RSD; method precision ≤ 2.0% RSD.[18]

* % RSD ≤ 2.0%.[9,20,27,30]

* The required discrimination ability must be ≥ the quantity 1.96 σ/n.[26]

* The repeatability is generally 1/2 to 1/3 of the reproducibility.[19]

* For biological samples, a CV of 10% should be acceptable as the minimum precision.[12,15]

* For biological samples a CV of ±15% is appropriate except at the quantitation limit where ±20% is acceptable.[29]

* For discovery samples, a ±20% RSD is acceptable. For pre-clinical and clinical samples, ±10% is more appropriate.[21]

Several authors link precision criteria with the procedure's acceptance range. For example, for acceptance ranges of 95 to 105% and 90 to 110% respectively, the recommended precision is 2% or 4% RSD.[8] A more detailed breakdown of precision versus the procedure's acceptance range is summarized in Table 1.

Specificity

The purpose of performing an analytical determination is to assign an accurate value to some chemical property of a sample. This process is

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Table 1

Recommended Precision Acceptance Criteria

Acceptance Range (% of Claim	Maximum Allowable % RSD: Single Determinations Method % RSD	Single Determinations System % RSD	Multiple Determinations Method % RSD	Multiple Determinations System % RSD
98.5-101.5	0.58	0.41	0.82	0.58
97-103	1.2	0.82	1.6	1.2
95-105	1.9	1.4	2.7	1.9
90-110	3.9	2.8	5.5	3.9
90-115	4.8	3.4	6.9	4.8
90-125	6.8	4.8	9.6	6.8
85-155	5.8	4.1	-	-
75-125	9.7	6.9	-	-
50-150	19.4	13.7	-	-

This table is based on 99% confidence levels, assuming that half of the variance of a method is attributable to system error. (From reference 6)

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facilitated if the determination's response to that specific chemical property can be distinguished from its response to any other sample property. In general, specificity relates to the ability of a method to measure only what it is intended to measure[19] even though the sample may contain a sea of excipients and related compounds.[10] More specifically, specificity is commonly defined as the ability of an assay to assess unequivocally, with a requisite level of accuracy and precision, the analyte of interest in the presence of compounds which might be expected to be in the sample, which for pharmaceutical samples might include inactive excipients, degradation products, synthesis impurities and precursors, container extractables and analytical artifacts.[3,5,9,11,18,21,24,25,30]

Specificity is a measure of the method's sensitivity to potential sample-related interferents[27] and for chromatographic procedures reflects the system's ability to resolve all other sample components that will give a detector response[6,8] from the peak of interest. The specificity determination ensures that the signal measured in the method is not influenced by interfering species or, at least, that the contribution of such substances has been removed.[32]

Procedures

The most commonly cited specificity evaluation procedure is the analysis of a placebo, wherein the sample matrix without the analyte is analyzed and the resulting system response is examined for the presence of responses which interfere or overlap with that of the analyte of interest.[3,6,11,15,17,19-21,25,26,28,29] If the sample matrix is variable either in terms of absolute composition or source of its component raw materials (for example, in the case of a manufactured product), it has been recommended that specificity be established with six independent sources of the sample matrix.[6,18] The method of standard additions can be applied to specificity evaluations.[3,8,13,18,25,27] In this approach, samples (in the matrix) and standards (no matrix) prepared at equivalent analyte concentration levels can be analyzed or, alternatively, both samples and standards can be fortified with equivalent levels of the analyte and re-analyzed. In either case, specificity is the degree of agreement between the sample and standard responses. One author recommends that five different spike levels, encompassing the method's linear range, be used to assess specificity.[13]

Additional procedures for specificity include:

* Peak re-analysis, wherein the peak of interest is collected and re-analyzed by different chromatographic conditions or with methods that are sensitive to analyte structure.[6,18]

* Collaboration in which the sample is quantitatively analyzed using two or more detection/separation strategies and the results compared.[19]

* Use of information-rich detectors (e.g., mass spectrometric[16,21] or multiple wavelength UV[4,6,16,18,21] to assess peak purity.

Procedure Guidelines

A specificity guideline whose use facilitates an effective validation evaluation involves degradation products. Clearly if the assay supports product expiry dating via stability studies, its response must be unaffected by any degradation products (from either the active ingredient or formulation matrix) which could be generated over the entire course of the study. Ideally, the most effective test articles for the specificity evaluation would be stability samples retrieved throughout the study's duration. The duration of most stability studies makes this ideal situation untenable. Thus the analyst is faced with artificially producing degraded samples via accelerated methods. Two issues are encountered; degradation mechanism and the extent of degradation. While it is

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recommended that the selectivity of a method be established by forcing drug degradation using acid, alkali, oxidizing agents, temperature and intense light,[4,8,16,20,30] it is clear that the analyst should use methods which are consistent with the product's decomposition under normal manufacturing, storage and use conditions. Since these conditions are application specific, it is impossible to provide general decomposition guidelines; however, the analyst must be able to defend his choice of accelerated decomposition conditions based on a sound scientific understanding of the product's decomposition mechanism under typical use conditions.

Once conditions have been established for producing artificially degraded samples, the issue of how much degradation is adequate must be addressed. The key to a realistic specificity evaluation is to perform the assessment on samples which one might reasonably encounter at extremes in the product's utilization environment. Specificity evaluations performed on samples which have degraded significantly more or less than those which might encountered in worst case applications serve no useful purpose. For active ingredient assays on products whose stability is dictated by the typical pharmaceutical limit of 90% of label claim, peak purity should be performed on stressed samples exhibiting a demonstrable degradation of 10 to 15%.[16] If other product properties limit stability (e.g., the accumulation of a degradate, solution color or solution pH), the accelerated decomposition conditions used to produce specificity samples must result in a sample whose behavior is slightly beyond these product limits.

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Acceptance Criteria

Published acceptance criteria include:

1. Placebo, Visual Examination. For chromatographic procedures, there should be baseline separation between the peak of interest and all other analytical responses.[4,6,8] One author suggests that the nearest peak maximum should be separated from the designated analyte peak by at least one full width at half height.[14]

2. Peak Re-analysis. If the peak is collected and re-analyzed on another chromatographic system, it should produce a single response.[6]

3. Low Resolution Mass Spectrometric Detection.[14] The intensities of four diagnostic ions (including the molecular ion) must be measured in the sample and a standard. The relative abundances of all diagnostic ions (expressed as a percentage of the intensity of the base peak) must be the same in the sample and the standard within a margin of ± 10% (EI mode) or ± 20% (CI mode).

4. Peak Purity (Multiple wavelength UV detection). Wavelength ratio techniques should show that the spectrum of the analyte peak matches that of a reference standard.[6] The maximum absorption wavelength of the analyte in the sample must be the same as that of the standard reference material within the resolution of the detector (±2 nm). The spectrum of the analyte in the sample should not be visually different from that of the standard for parts of the spectrum with a relative absorbance larger than 10%.[14] If the peak absorbance ratios at two (or more) wavelengths determined for treated, spiked and non-treated samples are within ± 5%, the chromatographic peak is considered to be pure.[16]

Linearity

Definition. Most analytical procedures do not produce output which is an absolute indication of the sample property being measured. Rather, instrument output must be mathematically transformed into sample property units. In chromatography, peak parameters are related to analyte concentration via standardization procedures. This relationship is then used to convert a sample's peak parameter to its apparent analyte concentration. A linearity assessment establishes the nature of the peak parameter to standard analyte concentration relationship. The linearity assessment determines the procedure's ability to obtain test results which are proportional to the concentration of the analyte in the sample within a given range either directly[5,8,13,17,20,21,27] or via a well defined mathematical transformation.[3,11,24,25,27]

A procedure's range is linked to its linearity. The range is the interval between the lower and upper analyte concentration for which it has been demonstrated that the analytical procedure has a suitable level of accuracy, precision and linearity.[3,5,24,27,30] The range is validated by verifying that the procedure provides acceptable accuracy, precision and linearity at the extremes of the range as well as within the range.[5]

Procedure Guidelines

Establishing the appropriate concentration range is the major issue associated with the linearity assessment. Generally, the appropriate range is application specific. Recommendations noted in the literature for the range include:

* the range of expected concentrations.[3,15,27,29,30]

* 80% of the lowest expected level to 120% of the highest expected level.[2,4,9]

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* 50% to 150% of the expected working range.[6,8,11,13,18]

* 25% to 125% of the target range specified.[30]

* 10% to 200% of the expected range.[13,20]

* For impurity tests and dissolution studies, several orders of magnitude.[4,13]

Specific guidelines for linearity ranges, as provided by Carr and Wahlich,[17] are contained in Table 2.

In determining the range, the analyst must balance the requirements of scientific rigor with practical constraints. A method validated for linearity need only produce accurate values in the concentration range in which it is intended to be used.[17] The range selected for validation should not be unrealistically wide, as this may lead to rejection of a method which is really quite suitable for the intended purpose.[25]

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Acceptance Criteria

Acceptance criteria provided by various researchers include:

* Data should be plotted to look for dubious points and to visually establish the calibration range.[15]

* The correlation coefficient of the best linear least squares regression model should be between 0.98 and 1.00[8] or greater than 0.999 with the slope and intercept reported.[4]

* The value of n in the equation $y = mX^n + b$ should be between 0.9 and 1.1 and the maximum allowable relative error is 1%.[6]

* Taking the regression line as a mean, a RSD calculated for the data should not be greater than 2.0%.[17]

* The intercept of the regression line should not be significantly different from zero[18,22] or, more specifically, the percentage of the intercept relative to the 100% analyte level should be ± 2%.[17]

* A response factor plot is used to identify concentrations where true proportionality is not observed.[30]

Table 2

Recommended Validation Ranges for Linearity Studies

Purpose of Analysis	Typical Product Range (%)	Recommended Validation Range (%)
Release Specification Assay	95 to 105	80 to 120
Check Specification Assay	90 to 110	80 to 120
Content Uniformity Test	75 to 125	70 to 130
Asssay for a Preservative in a Stability Study	50 to 110	40 to 120
Assay for a Degradant in a Stability Study	0 to 10	0 to 20

From reference 17.

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While the correlation coefficient is commonly cited as a test of linearity, its use is not universally accepted (for example, references 10 and 28). These authors suggest a more rigorous statistical evaluation of linearity, including a test of significance for the b_2 term in the equation[22] $y = b_0 + b_1X + b_2x^2$ and the utilization of the residual sum of squares.[17]

In closing, this author notes that there is no unwritten rule that states the relationship between instrumental response and analyte concentration must be directly linear for a procedure to be valid. Rather, the requirement is that the relationship between method response and analyte concentration be rigorously defined over the expected analyte range. The desire to have a linear relationship reflects the practical consideration that a linear relationship can be accurately described with fewer standards than a non-linear relationship and the subjective expectation that a linear relationship is more rugged than a more complicated one.

Limits of Sensitivity

Definitions

Sensitivity is the ability of a method to reliably respond in a consistently recognizable manner to decreasingly smaller amounts of analyte. Frequently utilized measures of sensitivity are the limit of detection (LOD) and limit of

quantitation (LOQ). While similar in concept, their utility is application specific. For example, the LOD is suggested by the USP for qualitative limits tests while LOQ is specified for quantitative impurity determinations.[3] The LOD is usually required for impurity tests, assays for dissolution test samples, limit tests and "absence of" tests.[26] The LOD is the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated.[3,4,5,21,27,30] In practice, it is the lowest concentration of analyte which can be distinguished from the blank with a stated degree of confidence.[11.12.15.17] It is generally the lowest concentration of analyte that is detected at the most sensitive instrument setting[25] and is that point in the response range that a measured value is greater than the uncertainty associated with it.[18] For chromatographic procedures, it is the lowest amount of analyte which can be detected above the baseline detector noise.[20,32]

Alternatively, LOQ is the lowest amount of analyte which can be reproducibly quantitated above the baseline noise.[20] Quantitation implies that the measurement possess a specified accuracy and precision.[4-6,10,16,17,18,21,24,25,27,30,32] LOQ has been variously defined as that quantity of analyte which has a signal to noise ratio of at least 10 and a precision of less than 10% or which has a signal to noise ratio greater than 20 and a precision of 5% or less.[30] In some applications, LOQ is defined as the smallest concentration included in the standard curve.[26]

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Procedures

LOD can be determined either directly or from other validation data. Its direct measurement involves an analysis of the method's peak to peak baseline noise[4,11,13,20,26] or an analysis of the variation in the method's blank response[3,15] In either case, LOD is calculated as either 2 or 3 times the variation in measured response, where the factors are associated with the 95 and 99% confidence intervals for a normal distribution. Practically, LOD can be measured by the serial dilution of samples until the peak can no longer be observed.[13,15] LOD can be estimated as the value of the linear calibration curve's y-intercept.[11,12,15] Considering method precision, LOD has been defined as the concentration equal to 3.29 times the injection to injection standard deviation[35] or as that concentration at which the system precision (CV) reaches 20%.[15]

Similarly LOQ can be determined via the precision of replicate blank analyses (ten times the %RSD of the replicates)[3,4,27,35] or by analyzing successively diluted samples until the requisite levels of accuracy and precision are achieved.[20,21] Several authors have suggested procedures for estimating

LOQ based on an analysis of the method's baseline response.[17,26] Such procedures generally require that the chromatogram resulting from a blank injection be examined over a range of twenty peak widths and that the noise be measured as either the largest peak to peak fluctuation or as the largest deviation (positive or negative) from the mean response. The LOQ is then calculated as the product of ten times the measured deviation and the calibration curve slope. The LOQ can also be determined as the lowest analyte concentration for which duplicate injections results in a %RSD $\leq$ 2%.[20]

Regardless of the method employed, it is commonly recommended that the calculated LOQ be confirmed by injecting samples prepared to contain the analyte at or near the LOQ.[3,17,27]

Acceptance Criteria

In general, there exists no specific criteria for what value LOD and LOQ must have. Rather, the requirements are generally stated in terms of the relationship between LOD/LOQ and the concentration of the analyte in the samples to be analyzed. For routine applications involving LOD, it is desired that test samples contain 2 to 3 times the minimum amount detectable.[27] Alternatively, a factor of 5 or 10 is recommended between the LOD and the specification value for an analyte level.[13]

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In routine applications it has been recommended that LOQ be within the working linear concentration range[30] and that a specification limit should be no lower than twice the LOQ.[6] For clinical applications, the LOQ should be at least 10% of the minimum effective concentration.[21]

A Survey of Procedures Used

To determine what type of validation protocols were typically being performed in industry, C.S. Clarke of Bristol-Myers Squibb surveyed twenty major research based pharmaceutical companies in the UK. Portions of the results of this survey[37] are shown in Table 3 and document parameters used in the evaluation of specific validation parameters as well as acceptance criteria.

CONCLUDING COMMENTS

The purpose of this manuscript is to provide the reader with a general sense of current procedures used by active investigators or recommended by

Table 3

Criteria for Performing Method Validation Experiments; Median Responses from a Survey of UK Pharmaceutical Manufacturers

Parameter	Number of Samples	Range (1)	Tolerance
		Bulk Drug Assays	
Accuracy	6	50-150	±2%
Repeatability	6	-	±2%
Reproducibility	6	-	±2%
Linearity	6	20-150	r^2>0.999, Intercept
LOD & LOQ		- - - - - - Not Applicable - - - - - -	
		Bulk Drug Impurity Assays	
Accuracy	5	50-150	±20%
Repeatability	6	-	±2%
Reproducibility	6	-	±5%
Linearity	6	20-150	r^2>0.999, Intercept
LOD		3 times the signal-to-noise ratio	
LOQ		10 times the signal-to-noise ratio	
		Finished Product, Active Ingredient Assays	
Accuracy	6	75-125	±2%
Repeatability	6	-	±2%
Reproducibility	6	-	±5%
Linearity	6	25-150	r^2>0.999, Intercept
		Finished Product, Degradant Assays	
Accuracy	6	50-150	±10%
Repeatability	6	-	±2%
Reproducibility	5	-	±3%
Linearity	6	0-2%(2)	Intercept
LOD		3 times the signal-to-noise ratio	
LOQ		10 times the signal-to-noise ratio	

(1) Range is represented as 95% of label claim

(2) As the percent of the active drug level in the formulation.

Intercept criteria is that the 95% confidence interval for the intercept include 0.

From Reference 37.

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industrial, academic and governmental experts, to assess the validity of chromatographic analytical methods with respect to several common parameters. While specific details are provided as appropriate, space limitations make it impossible to completely describe the exact application for which the details were appropriate.

Thus in order to gain a greater understanding of how specific details can be applied to a particular validation situation, readers are directed to the references supplied herein. Such an in-depth analysis is particularly necessary when the details seem to be mutually discordant.

In closing, the following key points are offered.

1. The validation strategy is specific for a given application and is influenced by the purpose of the analytical measurement, the analytical procedure used, the nature of the analyte, the concentration of the analyte and the nature of the test sample (matrix).

2. Validation is the systematic comparison of measured performance and pre-determined acceptance criteria. It is absolutely essential that these criteria be clearly established as part of a formal validation plan prior to the initiation of the validation study.

3. Acceptance criteria for validation are not always available from a decision-making third party. In instances where acceptance criteria must be established by the validation team, two concepts are pertinent.

Firstly, the criterion established must be both clearly relevant and applicable to the assay's intended use and scientifically defensible. Secondly, if you set the rules, you had better well follow them.

4. The burden of proof with respect to establishing a method's validity rests with the user/developer.

5. Validation builds quality into the method, ensuring that the method works when needed with no unexpected results.

6. Validation is the insurance policy that assures our customers that our products contain what they should and are capable of doing what they were intended to do.

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