

Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-May-2018
Document Type: General Chapter
DocId: GUID-1121F1D0-AFE7-4506-94CF-9112714BE4EB_2_en-US
DOI: https://doi.org/10.31003/USPNF_M99525_02_01
DOI Ref: 5t5yl

⟨736⟩ MASS SPECTROMETRY

INTRODUCTION

Mass spectrometry (MS) is an analytical technique based on the measurement of the mass-to-charge ratio of ionic species related to the analyte under investigation. MS can be used to determine the molecular mass and elemental composition of an analyte as well as provide an in-depth structural elucidation of the analyte.

In addition to being recognized as a powerful structure-elucidation tool, MS is also extensively used for quantitative measurements. For additional information, see general information chapter *Applications of Mass Spectrometry* ⟨1736⟩, which provides a detailed discussion of MS.

Currently available MS instrumentation offers a wide range of capabilities for qualitative and quantitative analysis, which results in a wide range of potential MS experimental approaches for a given measurement need. Because of the diversity of approaches, this chapter does not present specific procedures but instead provides experimental and system suitability information for MS procedures.

QUALITATIVE ANALYSIS

MS is a sensitive and highly specific technique for the identification of analytes. Identification or verification of structure (i.e., comparison against an authentic standard) by MS is particularly powerful when used in conjunction with a separation technique such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). Additional degrees of specificity can be obtained by the use of tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS). Also, tighter tolerances on mass accuracy are also afforded using high-resolution mass spectrometry (HRMS).

Experimental Parameters

The following MS experimental parameters should be defined for a qualitative (e.g., identification) procedure.

MASS RESOLUTION

Unit mass resolution is sufficient for most identification tests. When higher resolution is required, the resolution is specified in the procedure, and demonstration of adequate resolution is included in the system suitability tests for the procedure.

MASS ACCURACY

A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. A mass accuracy or agreement of ±0.05% from a standard should be sufficient for the identification of large molecules (above 2000 m/z) when multiply charged ions are employed for the identification test.

When higher mass accuracy is required, the required mass accuracy is specified in the procedure. A demonstration of the mass accuracy then is included in the system suitability tests for the procedure or as part of the instrument's established performance qualification (PQ) procedure discussed later in this chapter.

MASS RANGE

The mass range to be scanned is also presented in the procedure. The mass range must encompass all ions used as part of the identification confirmation.

System Suitability

The system suitability for the MS procedure should include demonstration of adequate performance for the following experimental attributes.

MASS RESOLUTION

A demonstration of the appropriate resolution is included in the system suitability tests for a procedure. The performance test in the instrument's established PQ, executed daily or prior to the time of use, may suffice. When resolutions greater than unit mass are required for a procedure, the system suitability test includes demonstration of adequate resolution along with acceptance criteria.

MASS ACCURACY

A demonstration of the mass accuracy is included in the system suitability tests for the procedure or is part of the instrument's established PQ procedure. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. If higher mass accuracy is required for a given procedure, the appropriate acceptance criteria are specified.

Interpretation Directions

An identification or verification experiment involves comparison of the compound of interest to an authentic standard (e.g., a USP Reference Standard). For this form of identification or verification, the standard and the sample are run under identical conditions and should have results that are within acceptable experimental error for the procedure. Applications of MS for identification purposes in a compendial monograph may incorporate the mass spectral data alone or may combine this spectral identification with the chromatographic retention time if more specificity is dictated by the particular monograph identification needs.

The procedure should also provide specific instructions that define a successful mass spectral match. If the procedure represents the only spectroscopic identification test or if no other identification tests (e.g., peptide mapping or amino acid analysis) provide structural information, the spectrum of the standard should closely resemble that of the sample and a minimum of three structurally relevant ions, preferably one of which is an ion representing the molecular mass of the analyte, should be used for the comparison. In the case where only the ion representing the intact molecule is produced, the accurate mass or MS/MS spectrum of the molecular ion may be used to strengthen the identification. If other structurally relevant identification tests are also conducted, the comparison can be conducted with fewer ions as long as one of the ions represents the molecular mass of the analyte. For example, the MS identification test for a protein may examine only an ion that represents the molecular mass if several other tests are conducted to confirm the protein structure.

QUANTITATIVE ANALYSIS

The sensitivity and specificity of MS also make it a suitable analytical tool for the quantification of analytes. Quantification is particularly powerful when used in conjunction with a separation technique such as GC or HPLC. Further degrees of specificity can be obtained by the use of MS/MS or HRMS.

Experimental Parameters

The following experimental parameters should be defined within a quantitative (e.g., identification) MS procedure.

MASS RESOLUTION

Unit mass resolution is sufficient for most quantitative tests. When higher resolution is required, the resolution is specified in the procedure, and demonstration of the mass accuracy is included in the system suitability tests for the procedure.

MASS ACCURACY

The mass accuracies listed in the previous qualitative section should be sufficient for most quantitative applications. When higher mass accuracy is required, it is specified in the procedure. A demonstration of the mass accuracy is included in the system suitability tests for the procedure or as part of the instrument's established PQ procedure.

MASS SELECTION

The masses to be monitored (e.g., mass range, individual masses, or MS/MS transitions) are presented in the procedure.

System Suitability

The system suitability for the MS procedure should include demonstration of adequate performance for the following experimental attributes, as appropriate for the procedure.

MASS RESOLUTION

A demonstration of the appropriate resolution is included in the system suitability tests for the procedure or is part of the instrument's established PQ procedure. When resolutions greater than unit mass are required for a procedure, the system suitability test includes demonstration of adequate resolution along with acceptance criteria.

MASS ACCURACY

A demonstration of the mass accuracy is included in the system suitability tests for the procedure or is part of the instrument's established PQ procedure. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. If higher mass accuracy is required for a given procedure, the appropriate acceptance criteria are specified.

PRECISION

The system suitability includes a demonstration of adequate precision. See *Table 1* for maximum limits for precision. Typically, system suitability limits are set to tighter limits than those employed to ensure adequate precision for the validation experiments. (See also the section on *Validation and Verification of Mass Spectrometry Analytical Procedures*.)

LINEARITY

The system suitability includes a demonstration of adequate linearity. See *Table 1* for appropriate linearity limits. (See also the section on *Validation and Verification of Mass Spectrometry Analytical Procedures*.)

ACCURACY

In certain situations, quality control (or check) samples may also be appropriate for inclusion in the procedure to ensure the quality of the measurement. Typically, these quality control samples are of known analyte concentration and are prepared identically to the test samples. If used, quality control (or check) samples are also prepared as a verification of time-of-application method accuracy. The procedure specifies the number or analysis order of quality control (or check) samples needed. Acceptance criteria for calibration and quality control

(or check) sample results should be aligned with the validation requirements as required by the application type (i.e., Category I or II) as outlined in *Table 1*.

QUANTITATION LIMIT

In certain applications (e.g., limits tests), it may be necessary to include demonstration of the ability to detect the analyte at a prescribed level. For these applications, the procedure specifies the limit and success criteria (e.g., signal-to-noise ratio).

QUALIFICATION OF MASS SPECTROMETRY INSTRUMENTS

Qualification of an MS instrument can be divided into three elements: installation qualification (IQ), operational qualification (OQ), and PQ. For additional information, see *Analytical Instrument Qualification* ⟨1058⟩, which may be a helpful, but not mandatory resource.

Installation Qualification

IQ provides evidence that the hardware and software are installed to accommodate safe and effective use of the instrument at the desired location.

Operational Qualification

In OQ, an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable for a given application. Because so many different approaches are available for measuring MS spectra, OQ using standards with known spectral properties is recommended. Because of the diversity of MS instrumentation, interfaces, and experimental approaches, MS instruments should be qualified against target specifications for the intended application, not simply the specifications supplied by the manufacturer.

Performance Qualification

PQ helps to determine that the instrument is capable of meeting the user's requirements for all critical-to-quality measures. PQ documentation should describe the following:

- the definition of the specific performance criteria and detailed test procedures, including test samples and instrument parameters;
- the elements that will be measured to evaluate the criteria and the predefined specifications;
- the test interval, which may be daily or time-of-use measurements;
- the use of bracketing samples or groups of samples; and
- corrective actions that will be implemented if the spectrometer does not pass the specifications.

Periodic PQ should include a subset of the OQ tests to ensure that the instrument as supplied is performing at a level that produces data that are suitable for their intended use. Depending on typical use, the specifications for PQ may be higher or lower than the manufacturer's installation specifications. Method-specific PQ tests, also known as system suitability tests, may be used in lieu of PQ requirements for validated procedures.

Because of the diversity of MS instrumental configurations and experimental designs, a standard sample or experiment for all PQ assessments may not be available. Thus, method-specific PQ tests or system suitability tests often are needed. The PQ experimental design should be sufficiently robust to ensure proper instrument performance for the intended application, including the specifications associated with the measurement. At minimum, PQ experiments should include the following.

- For qualitative applications, the PQ experiment includes a check of the mass accuracy of the instrumentation. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications.
- For quantitative applications, the PQ experiment includes checks of mass accuracy and precision. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. The success criteria for precision is established via consideration of the instrument and method capability, and provides sufficient controls relative to the specification for the measurement in question.

Characterizing Instrument Performance

Specific procedures, acceptance criteria, and time intervals for characterizing MS spectrometer performance depend on the instrument and its intended applications. Many MS applications use previously validated experiments that relate MS spectra to a chemical property of interest. Analysts typically demonstrate stable instrument performance over extended periods of time. This practice provides some assurance that reliable measurements can be taken from sample spectra using previously validated MS experiments.

VALIDATION AND VERIFICATION OF MASS SPECTROMETRY ANALYTICAL PROCEDURES

Validation is required only when an MS procedure is an alternative to the official procedure for testing an official article.

The objective of validating an MS procedure is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities (Category II), and identification tests (Category IV). [Note—For additional information on the different category definitions, see *Validation of Compendial Procedures* ⟨1225⟩.] Depending on the category of the test, analytical procedure validation requires the testing of linearity, range, accuracy, specificity, precision, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized methods and to the method of standard additions.

Chapter ⟨1225⟩ provides definitions and general guidance about analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the

minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

Measurement Categories for Mass Spectrometry Analytical Procedures

The required validation performance characteristics of an MS analytical procedure, assuming the typical Category I USP specifications of 98.0%–102.0% for drug substances and 95.0%–105.0% for drug products, are listed in *Table 1*. The actual validation performance characteristics would be dependent upon the specifications in place and should provide sufficient evidence that the measurement capability is sufficient for those specifications. A procedure validation protocol must specify the required validation experiments and validation criteria. These criteria are determined according to the intended purpose of the analytical procedure.

Table 1. Analytical Measurement Requirements

Analytical Performance Characteristics	Category I	Category II Quantitative
Specificity	Ensured by use of a reference standard when possible and demonstrable lack of interference from other components	
Linearity	Correlation coefficient *(R)* NLT 0.995	Correlation coefficient *(R)* NLT 0.99
Range	For 100.0% centered acceptance criteria: 80.0%–120.0%. For noncentered acceptance criteria: 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity: 70.0%–130.0%	50%–120%
Accuracy	98.0%–102.0% (drug substance) 95.0%–105.0% (drug product)	80.0%–120.0%
Repeatability	NMT 1.0% (drug substance) NMT 2.0% (drug product)	NMT 20.0%
Intermediate precision	NMT 1.0% (drug substance) NMT 3.0% (drug product)	NMT 25.0%
Quantitation limit	—	The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.
Robustness	The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters.	

Analytical Procedure Validation

The objective of analytical procedure validation is to demonstrate that the analytical procedure is suitable for its intended purpose by conducting experiments and obtaining results that meet predefined acceptance criteria. MS analytical procedures can include quantitative tests for major component and impurities content, limit tests for the presence of impurities, quantification of a component in a product or formulation, or identification tests.

VALIDATION PARAMETERS

Performance characteristics that demonstrate the suitability of an analytical procedure are similar to those required for any analytical procedure. For additional information on the applicable general principles, see ⟨1225⟩. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the analytical procedure.

The performance characteristics that are required as part of a validation for each of the analytical procedure categories are given in *Table 1*.

Specificity: The purpose of a specificity test is to demonstrate that measurements of the intended analyte signals are free of interference from components and impurities in the test material. Specificity tests can be conducted to compare spectra of components and impurities that are known from synthetic processes, formulations, and test preparations. Specificity is also to be demonstrated for any materials added as part of the procedure (e.g., specificity versus isotope-labeled internal standards).

For an identification MS analytical procedure (Category I and II), validation experiments may include multidimensional MS experiments to validate correct assignments of an ion's structure or origin.

Linearity: A linear relationship is exhibited between the analyte concentration and instrument response. This is demonstrated by measuring analyte responses from NLT five standard solutions at concentrations that encompass the anticipated concentration range of analyte(s) in the test solution. For Category I, standard solutions can be prepared from reference materials in appropriate solvents. For Category II (MS analytical procedures that are used to quantitate impurities), linearity samples are prepared by spiking suitable test samples that contain low amounts of analyte or by spiking matrix samples at concentrations of the expected range. The standard curve then is constructed using appropriate statistical analytical procedures such as a least-squares regression. The correlation coefficient (*R*), *y*-intercept, slope of the regression line, and residual root mean square are then determined. Absolute values determined for these factors are appropriate for the procedure being validated.

Range: The range between the low and high concentrations of analyte is given by the quantitative MS analytical procedure. This typically is based on test article specifications in the *USP* monograph. It is the range within which the analytical procedure can demonstrate an acceptable degree of linearity, accuracy, and precision and can be obtained from an evaluation of that analytical procedure.

Recommended ranges for various MS analytical procedures are as follows.

- For Category I—assay of a drug substance (or a finished product): 80%–120% of the test concentration;
- For Category I—content uniformity: a minimum of 70%–130% of the test concentration;
- For Category II—determination of an impurity: 50%–120% of the acceptance criteria.

Accuracy: The accuracy of a quantitative MS analytical procedure is determined across the required analytical range. Typically, three levels of concentrations are evaluated using triplicate preparations at each level.

Preparation of accuracy samples: For drug substance assays (Category I), accuracy is determined by analyzing a reference standard of known purity. For drug product assays (Category I), a composite sample of reference standard and other components in a pharmaceutical finished product should be used for analytical procedure validation. The assay results are compared to the theoretical value of the reference standard to estimate errors or percent recovery. For the quantitation of impurities (Category II), the accuracy of the analytical procedure can be determined by conducting studies with drug substances or products spiked with known concentrations of the analyte under test.

Assay results from the analytical procedure being validated may be compared to those of an established alternative analytical procedure.

Precision:

Repeatability: The analytical procedure is assessed by measuring the concentrations of three replicates of separate standard solutions at three different concentrations that encompass the analytical range. Alternatively, the concentrations of six separate standard solutions at 100% of the test concentration can be measured. The relative standard deviation from the replicate measurements is then evaluated to determine if the solutions meet the acceptance criteria.

Intermediate precision: The effect of random events on the analytical precision of the analytical procedure is to be established. Typical variables include performing the analysis on different days, using different instruments that are suitable as specified in the analytical procedure, or having the analytical procedure performed by two or more analysts.

Quantitation limit: The quantitation limit is validated by measuring six replicates of test samples spiked with analyte at 50% of specification.

From these replicates, analysts are then able to determine accuracy and precision. Examples of specifications for Category II quantitative determinations are that the measured concentration is within 70%–130% of the spike concentration and the relative standard deviation is NMT 15%.

Robustness: The reliability of an analytical measurement is demonstrated with deliberate changes to critical experimental parameters. These can include measuring the stability of the analyte under specified storage, chromatographic, or ionization conditions.

Analytical Procedure Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* do not need to validate procedures that are provided in a monograph. Instead, they must simply verify the suitability of the procedures under actual conditions of use.

The objective of an MS procedure verification is to demonstrate that the procedure as prescribed in a specific monograph can be executed by the user with suitable accuracy, specificity, and precision using the instruments, analysts, and sample matrices available. According to the general information chapter *Verification of Compendial Procedures* ⟨1226⟩, if the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures* .

Verification of a compendial MS procedure includes at minimum the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in the *Validation and Verification of Mass Spectrometry Analytical Procedures* section in this chapter.

Topic/Question	Contact	Expert Committee
<736> MASS SPECTROMETRY	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 39(5)

Current DocID: GUID-1121F1D0-AFE7-4506-94CF-9112714BE4EB_2_en-US
Previous DocID: GUID-1121F1D0-AFE7-4506-94CF-9112714BE4EB_1_en-US
DOI: https://doi.org/10.31003/USPNF_M99525_02_01
DOI ref: 5t5yl