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⟨852⟩ ATOMIC ABSORPTION SPECTROSCOPY

INTRODUCTION

Atomic absorption (AA) spectroscopy is an analytical method that supports qualification and/or quantification of elements. In this use, the AA method supports procedures that measure the absorbance of radiation at a characteristic wavelength by a vapor composed of ground state atoms. A typical instrument consists of a primary energy source that produces the spectrum of the element under examination, a monochromator, and a suitable detector.

For discussion of the theory and principles of measurements, see *Atomic Absorption Spectroscopy—Theory and Practice* ⟨1852⟩, a resource that may be helpful but is not mandatory.

QUALIFICATION OF ATOMIC ABSORPTION SPECTROPHOTOMETERS

Qualification of an atomic absorption spectrophotometer (AAS) can be divided into three elements: installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ); see also *Analytical Instrument Qualification* ⟨1058⟩.

Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location.

Operational Qualification

In OQ, an instrument's performance is characterized using standards of known spectral properties to verify that the system operates within target specifications (see *Table 1* and *Table 2*). The purpose of OQ is to demonstrate that instrument performance is suitable. OQ is a check of the key operational parameters performed following installation and following repairs and/or maintenance.

The OQ tests in the following sections are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package.

Table 1. OQ Test and Acceptance Criteria for Flame AAS

Test	Procedure	Acceptance Criteria
Sensitivity	Aspirate a 0.3-µg/mL zinc standard and record the absorbance.	±20% of AU[a] specified by instrument manufacturer
Linearity	Aspirate a blank and 0.05-, 0.075-, 0.10-, 0.25-, and 0.50-µg/mL zinc standards. Generate a calibration curve and record the correlation coefficient (*R*).	Correlation coefficient NLT 0.995
Precision	Assay 5 separate replicates of the 0.10-µg/mL zinc standard versus the standard curve generated for the *Linearity* test. Calculate the %RSD of the 5 results in µg/mL.	%RSD[b] NMT 3%

[a] AU = absorption unit.

[b] %RSD = % relative standard deviation.

Table 2. OQ Test and Acceptance Criteria for Graphite Furnace AAS

Test	Procedure	Acceptance Criteria
Sensitivity	Prepare a copper standard and measure the characteristic mass as described by the manufacturer.	±20% of copper characteristic mass as specified by the manufacturer

Test	Procedure	Acceptance Criteria
Linearity	Generate a calibration curve from a blank and 25-, 50-, 75-, and 100-µg/L copper standards. Inject each standard in triplicate and record the %RSD.	Correlation coefficient NLT 0.995
		%RSD of triplicate injections of each copper standard NMT 5% (not including the blank)
Precision	Assay 5 separate replicates of the 50-µg/L copper standard versus the standard curve generated for the *Linearity* test. Inject each replicate in triplicate.	%RSD of 5 replicates NMT 3%
		%RSD of triplicate injections NMT 5%

Performance Qualification

PQ determines that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement.

Depending on typical use, the specifications for PQ may be different from the manufacturer's specifications. For validated methods, specific PQ tests, also known as system suitability tests, can be used in lieu of PQ requirements.

Specific procedures, acceptance criteria, and time intervals for characterizing AA spectrophotometer performance depend on the instrument and intended application. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from test sample spectra using validated AA procedures.

Change to read:

PROCEDURE

Evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in AA analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents. For some analyses, diligence must be exercised to prevent the adsorption of analytes onto the surface of a vessel, particularly in ultra-trace analyses. Contamination of the sample solutions from metal and ions present in the container also can lead to inaccurate results.

For the analysis of a ubiquitous element, it is often necessary to use the purist grade of reagent or solvent available. Check all solutions (diluents, matrix modifier solutions, ionization suppression solutions, reactants, and others) for elemental contamination before they are used in an analysis.

Standard Solution

Prepare as directed in the individual monograph. [NOTE—Commercially available single- or multi-element standard solutions, traceable to the National Institute of Standards and Technology or to an equivalent national metrology organization, can be used in the preparation of standard solutions.] Standard solutions, especially those used for ultra-trace analyses, may have limited shelf life. ▲Working standard solutions for ultra-trace analyses▲ (USP 1-Aug-2022) should be retained for not more than 24 h unless stability is demonstrated experimentally.

The method of standard additions also can be used. This method involves adding a known concentration of the analyte element to the sample at no fewer than 2 concentration levels against an unspiked sample preparation. The instrument response is plotted against the concentration of the added analyte element, and a linear regression line is drawn through the data points. The absolute value of the *x*-intercept multiplied by any dilution factor is the concentration of the analyte in the sample.

Sample Solution

Prepare as directed in the individual monograph.

A variety of digestion techniques may be indicated to dissolve the sample. These may include hot-plate and microwave-assisted digestions, including open-vessel and closed-vessel approaches. Note that open-vessel digestion generally is not recommended for the analysis of volatile metals, e.g., selenium and mercury.

Analysis

Follow the procedure as directed in the individual monograph for the instrumental parameters.

The instrument must be standardized for quantification at the time of use. ▲For a Category I assay, the absorbances of standard solutions that bracket the target concentration of the sample solutions are determined. For a Category II quantitative assay, the absorbances of standard solutions that bracket the specification limit concentration of the sample solutions are determined. For a Category II limit test or a Category IV identification test, only a single standard measurement may be required per the specific monograph. For a multiple standard curve, the detector response is plotted as a function of the analyte concentration to generate the standard plot.▲ (USP 1-Aug-2022) Regression analysis of the standard plot should be used to evaluate the linearity of detector response, and individual monographs may set criteria for the residual error of the regression line.

To demonstrate the stability of the system's initial standardization, the analyst must reassay a solution used in the initial standard curve as a check standard at appropriate intervals throughout the analysis of the sample set. Unless otherwise indicated in the individual monograph, the reassayed standard should agree with its expected value to within ±3% for an assay or ±20% for an impurity analysis.

▲When the method of standard additions is applied, a separate system suitability standard is prepared at a concentration within the analytical range of the method. The concentration of the system suitability standard is determined using the method of standard additions in the same manner as the test solutions. The measured concentration of the system suitability standard should agree with its expected value within ±3% for an assay or ±20% for an impurity analysis.▲ (USP 1-Aug-2022)

Sample concentrations are calculated versus the working curve generated by plotting the detector response versus the concentration of the analyte in the standard solutions.

Change to read:

VALIDATION AND VERIFICATION

Validation

Validation is required when an AA method is intended for use as an alternative to the official procedure for testing an official article.

The objective of an AA procedure validation 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 or limit tests (Category II), and identification tests (Category IV). [Note—For definition of different categories, see *Validation of Compendial Procedures* ⟨1225⟩.] Depending on the category of the test, analytical procedure validation for AA may require the testing of linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized methods and to the method of standard additions.

General information chapter ⟨1225⟩ provides definitions and general guidance on 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 to demonstrate suitability for the intended use.

- **Accuracy:** For Category I assays or Category II tests, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of elements. It is also an acceptable practice to compare assay results obtained using the AA procedure under validation to those of an established analytical procedure. In standard addition methods, accuracy assessments are based on the final intercept concentration, not the recovery calculated from the individual standard additions.

  Validation criteria: 95.0%–105.0% mean recovery for the drug substance assay and the drug product assay, and 70.0%–150.0% mean recovery for the impurity analysis. These criteria apply throughout the intended range.

- **Precision**

  Repeatability: The analytical procedure should be assessed ▲using 6 replicate measurements (▲ (USP 1-Aug-2022) measuring the concentrations of 6 independently prepared sample solutions▲)▲ (USP 1-Aug-2022) at 100% of the assay test concentration. Alternatively, ▲3 concentration levels with 3 independently prepared samples for each level▲ (USP 1-Aug-2022) can be used. The 3 concentrations should be close enough that the repeatability is constant across the concentration range. If this is done, the repeatability at the 3 concentrations is pooled for comparison to the acceptance criteria. If validating a procedure by the method of standard additions, the precision criterion applies to the final experimental result, not the accuracy of the individual standard addition levels.

  Validation criteria: The relative standard deviation is not more than 5.0% for the drug substance assay, not more than 5.0% for the drug product assay, and not more than 20% for the impurity analysis.

  Intermediate precision: The effect of random events on the analytical precision of the procedure should be established. Typical variables include performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts. As a minimum, the analytical procedure should be assessed by performing the repeatability test ▲(6 replicate measurements)▲ (USP 1-Aug-2022) in any combination of at least two of the conditions previously mentioned (totaling 12 measurements).

  Validation criteria: The relative standard deviation is not more than 8.0% for the drug substance assay, not more than 8.0% for the drug product assay, and not more than 25.0% for the impurity analysis ▲based on the 12 measurements.▲ (USP 1-Aug-2022)

- **Specificity:** The procedure must be able to unequivocally assess each analyte element in the presence of components that may be expected to be present, including any matrix components.

  Validation criteria: Demonstrated by meeting the accuracy requirement.

- **Quantitation limit:** The limit of quantitation (QL) can be estimated by calculating the standard deviation of no fewer than 6 replicate measurements of a blank solution, divided by the slope of a standard curve, and multiplying by 10. If validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used (see ⟨1225⟩).

  A measurement of a test solution prepared from a representative sample matrix spiked at the estimated QL concentration must be performed to confirm accuracy. If validating a procedure using the method of standard additions, the validation criterion applies to the final experimental result, not the spike recovery of the individual standard addition levels.

  Validation criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.

- **Linearity:** A response curve between the analyte concentration and absorbance is prepared from no fewer than 5 standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve is then evaluated using appropriate statistical methods, such as a least-squares regression.

For experiments that do not yield a linear relationship between analyte concentration and AA response, appropriate statistical methods must be applied to describe the analytical response.

Validation criteria: Correlation coefficient (*R*), not less than 0.995 for Category I assays and not less than 0.99 for Category II quantitative tests.

- **Range:** Range is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. Range is demonstrated by meeting the linearity, precision, and accuracy requirements.

  Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.
- **Robustness:** The reliability of an analytical measurement is demonstrated by deliberate changes to experimental parameters. For AA this can include but is not limited to sample preparation steps and heating programs, including atomization hold time or atomization temperature. Exercise caution when changing fuel and oxidant gas flows and burner hardware, because this could potentially create a flash-back condition.

Verification

US current good manufacturing practices regulations [21 CFR §211.194(a)(2)] indicate that users of the analytical procedures, as described in *USP–NF*, are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of an AA procedure verification is to demonstrate that the procedure, as prescribed in a specific monograph, can be executed by the user with suitable accuracy, specificity, linearity, and precision using the instruments, analysts, and sample matrices available. According to *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*.

▲▲ (USP 1-Aug-2022)

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<852> ATOMIC ABSORPTION SPECTROSCOPY	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

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