⟨735⟩ X-RAY FLUORESCENCE SPECTROMETRY

INTRODUCTION

X-ray fluorescence (XRF) spectrometry is an instrumental method based on the measurement of characteristic X-ray photons caused by the excitation of atomic inner-shell electrons by a primary X-ray source. The XRF method can be used for both qualitative and quantitative analysis of liquids, powders, and solid materials. The X-rays produced by an X-ray tube include characteristic lines that correspond to the anode material and a continuum known as Bremsstrahlung radiation. Both types of X-rays can be used to excite atoms and thus induce X-rays. XRF instrumentation can be divided into one of two categories: Wavelength Dispersive X-ray Fluorescence (WDXRF) and Energy Dispersive X-ray Fluorescence (EDXRF). The main factor distinguishing these technologies is the method used to separate the spectrum emitted by the atoms in the sample. The energy of the X-ray photon is characteristic for a given electron transition in an atom and is *qualitative* in nature. The *intensity* of the emitted radiation is indicative of the number of atoms in the sample and constitutes the *quantitative* nature of the method.

QUALIFICATION OF XRF SPECTROMETERS

Installation Qualification

See also *USP* general information chapter *Analytical Instrument Qualification* ⟨1058⟩.

Operational Qualification

The purpose of operational qualification (OQ) is to verify that the system operates within target tolerances using appropriate samples with known spectral properties. OQ is a check of the key operating parameters and should be performed following installation, repairs, or major maintenance that can affect the performance of the instruments. Note that all calibration samples must be handled with cotton or nitrile gloves and must be stored in sealed plastic containers. Alternatively, they may be fixed in the instrument.

The OQ tests and specifications in the following sections are typical examples only (see *Tables 1* and *3*). Other tests and standards can be used to establish tolerances for these purposes. The instrument vendor often makes samples and test parameters available as part of the IQ package.

Table 1. EDXRF OQ Specifications

Test	Procedure	Acceptance Criteria
Peak position	Acquire a spectrum of the Al–Cu energy calibration sample.	The spectrum should have at least 3000 counts at the top of both the Al Kα and the Cu Kα peaks. The energy corresponding to the peaks of Al Kα and Cu Kα should differ less than 0.1% from the tabulated values.
Detector resolution	Calculate the resolution (full width, half maximum) at the same energy and at the same count rate that was used at IQ.	The resolution value should not change more than 10% from the value determined at IQ.
Count rate	Measure the count rate of Al and Cu Kα lines from the energy calibration sample.	<10% change from initial measurements at IQ, for each peak

The Al–Cu energy calibration sample is a disc of Al–Cu alloy (EN AW-AlCu6BiPb; Alloy 2011, ASTM B211) that has been selected for use in EDXRF spectrometers. This alloy is generally available, is resistant to corrosion, and provides adequate intensities for both Al Kα and Cu Kα. These characteristic lines cover the typical energies used for XRF analysis. Also, sufficient information can be obtained from spectra recorded on this material to assess detector resolution. A more complete compositional specification for this calibration sample is given in *Table 2*.

Table 2. Specification of the Concentration of the Alloying Elements in Al–Cu Alloy (the Remaining Balance Is Aluminum)

Element	Concentration Limits (in % by Weight)
Cu	5–6
Zn	0.3 maximum
Fe	0.7 maximum
Bi	0.2–0.6
Pb	0.2–0.6
Si	0.4 maximum

When the energy range of the analytical lines for which the spectrometer will be used includes energies above 50 keV, a pure W metal (99.5% minimum) should be used instead of the Al–Cu alloy. This metal is stable, is generally available, and provides well-defined characteristic lines at 8.40 keV (L-lines) and 59.3 keV (K-lines).

Table 3. WDXRF OQ Specifications

Test	Procedure	Acceptance Criteria
Peak angle	Perform according to the manufacturer's procedure. Repeat for each crystal.	The angle corresponding to the peak maximum should differ less than 0.10 degree 2θ of the angle measured at IQ.
Detector resolution	Full width half maximum at specified wavelengths and at the same measurement conditions at the time of IQ. Repeat for each detector available.	NMT 20% change
Count rate	Measure count rate from the specified monitor specimen at a specified wavelength at the same measurement conditions at the time of IQ. Repeat for each detector available.	<10% change from initial measurements at IQ

Use Inconel 625 (Special Metals Corporation, New Hartford, NY) as a sample for WDXRF spectrometers. Other designations for this alloy are UNS N06625, DIN 2.4856, ASTM B443, ASME SB-443, and AMS 5599. This is a nickel-based alloy that includes chromium, molybdenum, and niobium as the most important alloying elements. A more complete compositional specification is given in *Table 4*.

Table 4. Elemental Concentrations in Inconel 625

Element	Concentration Limits (in % by Weight)
Ni	58.0 minimum
Cr	20.0–23.0
Fe	5.0 maximum
Mo	8.0–10.0
Nb[a]	3.15–4.15

[a] This could include Ta.

A polished piece of Inconel 625 should never require resurfacing when stored and used appropriately.

The radiation characteristics of nickel can be detected by all detectors that are used in sequential WDXRF, whether they are flow proportional, sealed gas, or scintillation detectors. In combination with Mo K (and L) radiation, the tests regarding peak position and detector response of WDXRF spectrometers can be completed readily. *Table 5* includes the typical wavelengths or energies that are used for XRF analysis.

Table 5. Energies and Wavelengths[a] for Al, Ni, Cu, Mo, and W

	Al	Ni	Cu	Mo	W
$K\alpha_1$ Line transition	$K–L_{2,3}$	$K–L_{2,3}$	$K–L_{2,3}$	$K–L_3$	$K–L_3$
$K\alpha_1$ Energy (eV)	1487	7473	8041	17479	59310
$K\alpha_1$ Wavelength (Å)	8.340	1.659	1.542	0.709	0.209
$L\alpha_1$ Line transition	N/A[b]	N/A	N/A	$L_3–M_5$	$L_3–M_5$
$L\alpha_1$ Energy (eV)	N/A	N/A	N/A	2293.2	8398.2
$L\alpha_1$ Wavelength (Å)	N/A	N/A	N/A	5.4066	1.47632

[a] From: Deslattes RD, Kessler EG, Indelicato P, et al. X-ray transition energies: new approach to a comprehensive evaluation. *Rev Mod Phys*. 2003;75(1):35–99. For Al, Ni, and Cu, the $K\alpha_1$ and $K\alpha_2$ energies from *Table V* were averaged with 2:1 weighting. Wavelength conversion uses the *hc*/*E* value from page 94. Values for Mo and W are taken from *Table VI*.
[b] N/A = not applicable.

Performance Qualification

The purpose of performance qualification (PQ) is to determine that the instrument is capable of meeting the user's requirements for all critical-to-quality measurements.

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

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

PROCEDURE

General Recommendations

Analysts should check the suitability of all reagents and materials for contamination before using depending on the method used. The analysis of a ubiquitous element often requires the use of the purest grade of reagent or material available. Sample holders and support windows should be appropriate for the analysis and the instrument configuration. Analysts should evaluate the cleaning of equipment used to prepare samples for XRF analysis in order to avoid cross-contamination of samples.

SAMPLES

Liquids: Liquid samples can be introduced directly to the XRF spectrometer provided that the solution consists of a single phase and has sufficiently low volatility. Analysis of liquid samples requires use of a special liquid sample holder and a commercially available support window composed of a suitable polymer film. Alternatively, liquid samples can be transferred onto the surface of a disk and dried before analysis. The experiment typically is conducted using a purge gas. The liquid sample can be spiked directly with solution standards at appropriate concentrations to facilitate accuracy, precision, and specificity tests as required for method validation.

Powders: Prepared powders may be measured directly in a liquid sample holder. Alternatively, they may be pressed into pellets. If the powder has poor self-binding properties, it may require a binder such as a wax or ethyl cellulose.

Powders also can be prepared for XRF analysis by fusing the sample material into a glass using a flux, typically sodium tetraborate, lithium tetraborate, and lithium metaborate. Because the temperatures required to melt the flux and dissolve the sample are relatively high (800°–1300°), this procedure is not suitable for the analysis of volatile elements such as mercury and arsenic.

Powder samples can be mixed with appropriate quantities of a certified reference material to facilitate accuracy, precision, and specificity tests as required for method validation. Alternatively, powder samples can be spiked with appropriate quantities of solution-based standards and then dried, ground if necessary, and thoroughly mixed before analysis. Standard additions can be used in instances when physical or chemical properties of the powder may introduce an analyte response bias.

Standards

Appropriate reference materials that are traceable to the National Institute of Standards and Technology, or equivalent, can be used in the preparation of XRF standards.

Analysis

For the instrumental parameters (if applicable) follow the procedure in the individual monograph. Because of differences in manufacturers' equipment configurations, analysts can use the manufacturer's suggested default conditions. At the time of use, the instrument must be standardized for the intended use. Analysts should use calibration standards to bracket the expected range of typical analyte concentrations. When they perform an analysis at or near the detection limit, analysts cannot always use a bracketing standard, which is an acceptable strategy for limit tests. Analysts should use regression analysis of the standard plot 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, at appropriate intervals throughout their tests on the sample set analysts must re-assay the calibration standard used in the initial standard curve as a check standard. The use of an independently prepared standard also is acceptable. Unless otherwise indicated in the individual monograph, the re-assayed standard should agree with its expected value to within ±2% for *Assay* or ±20% for an impurity analysis.

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

VALIDATION AND VERIFICATION

Current Good Manufacturing Practice regulations [21 CFR 211.194(a)(2)] indicate that users of analytical methods described in *USP–NF* are not required to validate the accuracy and reliability of these methods but rather must verify their suitability under actual conditions of use. In this context, and according to these regulations, validation is required when an XRF procedure is used to test a nonofficial article and when this procedure is used as an alternative to the official procedure for testing an official article (see *USP–NF General Notices, 6.30 Alternative and Harmonized Methods and Procedures*). On the other hand, verification must be performed the first time an official article is tested using a *USP* procedure (for informational purposes only, refer to *Verification of Compendial Procedures* ⟨1226⟩).

Validation

The objective of an XRF method validation is to demonstrate that the measurement procedure 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). Depending on the category of the test (see *Table 2* in *Validation of Compendial Procedures* ⟨1225⟩), the analytical method validation process for XRF requires the testing of linearity, range, accuracy, specificity, precision, quantitation limit, and robustness.

Performance characteristics that demonstrate the suitability of an XRF method are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in ⟨1225⟩. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the method. The samples for validation should be independent of the calibration set.

ACCURACY

For Category I assays or Category II tests, analysts can determine accuracy by conducting recovery studies using the appropriate matrix spiked with known concentrations of elements. An appropriate certified standard material provided by USP also can be used. It also is an acceptable practice to compare assay results obtained using the XRF method under validation to those from an established analytical method. When analysts use the method of standard additions, accuracy assessments are based on the final intercept concentration, not the recovery calculated from the individual standard additions.
Acceptance Criteria: 98.0%–102.0% recovery for drug substances and drug product assay, 70.0%–150.0% recovery for impurity analysis. These acceptance criteria should be met throughout the validated range.

PRECISION

Repeatability: Analysts should assess the analytical method by measuring the concentrations of six separate standards at 100% of the assay test concentration. Alternatively, they can measure the concentrations of three replicates of three separate samples at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. In this case, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

Acceptance Criteria: The relative standard deviation is NMT 1.0% for drug substance assay, NMT 2.0% for drug product assay, and NMT 20.0% for impurity analysis.
Intermediate Precision: Analysts should establish the effect of random events on the method's analytical precision. Typical variables include performing the analysis on different days, using different instrumentation, or having two or more analysts perform the method. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Acceptance Criteria: The relative standard deviation is NMT 1.0% for drug substance assay, NMT 3.0% for drug product assay, and NMT 25.0% for impurity analysis.

SPECIFICITY

The procedure must unequivocally assess each analyte element in the presence of components that may be expected to be present, including any matrix components.
Acceptance Criteria: Demonstrated by meeting the *Accuracy* requirement.

QUANTITATION LIMIT

The limit of quantitation (LOQ) can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank and multiplying by 10. Other suitable approaches may be used (see ⟨1225⟩). A measurement of a test sample prepared from a representative sample matrix and spiked so that the concentration is similar to the estimated LOQ concentration must be performed to confirm accuracy.
Acceptance Criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.

LINEARITY

Analysts should demonstrate a linear relationship between the analyte concentration and corrected XRF response by preparing no fewer than five standards at concentrations that encompass the anticipated concentration of the test sample. The standard curve then should be evaluated using appropriate statistical methods such as a least squares regression. The correlation coefficient (R), y-intercept, and slope of the regression line must be determined.

For experiments that do not have a linear relationship between analyte concentration and XRF response, appropriate statistical methods must be applied to describe the analytical response.
Acceptance Criteria: R is NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

The range is the interval between the upper and lower concentration (amounts) of analyte in the sample (including the upper and lower 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 and accuracy requirement.
Acceptance Criteria: For 100.0% centered acceptance criteria: 80.0%–120.0%. For non-centered acceptance criteria: 10% below the lower limit of the specification to 10% above the upper limit of the specification. For content uniformity: 70.0–130.0%. For Category II the range requirements are 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters. For XRF this can include measuring the stability of the analyte under specified storage conditions.

Acceptance Criteria: The measurement of a standard or sample response following a change in experimental parameters should differ from the same standard measured using established parameters by NMT ±2.0% for a drug product assay and NMT ±20.0% for an impurity analysis.

Verification

The objective of an XRF method verification is to demonstrate that the procedure as prescribed in a specific monograph is being executed with suitable accuracy, sensitivity, and precision. According to ⟨1226⟩, if the verification of the compendial procedure according to 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 *USP–NF General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Although complete revalidation of a compendial XRF method is not required, verification of compendial XRF methods should at minimum include the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated under *Validation* (above).