X-Ray Fluorescence Spectrometry〈*G1-10-191*〉(蛍光 X 線分析法〈*G1-10-191*〉)

X-ray fluorescence (XRF) spectrometry is a method used to measure the X-rays (fluorescent X-rays) emitted when the electrons from outer shell transition to the vacancy left by the ejection of inner shell electrons of atoms upon excitation by X-rays irradiated from a primary X-ray source. Since fluorescent X-rays are generated by electron transitions that are specific to each element, qualitative identification of elements can be achieved by measuring the energy of the emitted X-rays. As the intensity of the emitted X-ray correlates with the number of atoms in the sample, this method can also be used for quantitative analysis. In addition, semi-quantitative analyses, such as the fundamental parameter method, can also be applied. This method can be used to measure elemental impurities for quality control of active pharmaceutical ingredients, excipients and drugs.

1. Equipment

An XRF spectrometer basically consists of an X-ray generator unit, a sample chamber, a spectroscopic, detecting, and counting unit, an instrument control unit, and a data processing unit. The components of each unit vary depending on the spectroscopic method used. The spectroscopic methods are largely classified into a wavelength-dispersive XRF (WD-XRF) spectrometer, focusing on wavelength of X-rays, and an energy-dispersive XRF (ED-XRF) spectrometer, focusing on their energy.

With the WD-XRF spectrometer, fluorescent X-rays are dispersed using a spectroscopic element, and desired fluorescent X-rays are selectively counted by a detector that converts them into the electronic signals proportional to their intensity.

With the ED-XRF spectrometer, fluorescent X-rays are directly detected by a detector, generating amplified electric pulses proportional to the energy of each X-ray. As the detector captures fluorescent X-rays from all elements, separates the spectra based on their energy, and counts the electric pulses, the method enables faster measurements and is widely used in various screening analyses.

2. Sample Preparation

2.1. Liquid Samples

The sample is placed in a resin container with a polymer film measurement window and measured directly. Care should be taken to prevent the sample from becoming volatile, turbid or phase separated. The polymer film used for the measurement window should be transmissive to X-rays and solvent resistant. If the analyte element concentration is low, the sample is dropped onto a polymer film or filter paper and allowed to dry to enable measurement.

2.2. Powder Samples

The sample can be directly measured using a container for X-ray analysis made of a thin polymer film transmissive of X-rays. The sample is placed in a container designed for powder samples and the container is gently tapped to pack the sample. After tapping, additional sample can be added to the container, if necessary. For powder samples, the packing density and the flatness of the measurement surface can influence the quantitative results, the attention should be paid to the particle sizes and the packing method. The test and control samples must be prepared in the same volume. If homogeneous samples are not prepared by pulverization or mixing, the sample can be melted and homogenized with a melting agent (flux), such as lithium tetraborate [Flux (Glass Bead) Method]. The temperatures required to melt the flux, usually 800 – 1300℃, are not suitable for volatile elements such as mercury and arsenic. In addition, samples made by compressing powder and forming it into a flat plate are often used. When forming the sample into a flat plate, the sample is compressed as is, or a binder such as wax or ethyl cellulose can be used.

2.3. Solid Samples

A solid sample is placed on the measurement window of the spectrometer to ensure complete coverage. The sample needs to be of uniform shape and flat surface to give a reproducible analysis, whereas the sample can be measured ‘as is’ if the sample depth is sufficient.

2.4. Matrix Effects

Due to absorption of the incident X-ray by the matrix or interference of the fluorescent X-rays from the matrix itself, the fluorescent X-rays intensity of the analyte element may not be linear with concentration. These phenomena are referred to as matrix effects. The presence of non-target elements and their concentrations, the composition of the sample matrix, and the particle sizes of the sample are known to contribute to matrix effects. The effect of the particle sizes of the sample is specifically referred to as particle size effect. These matrix effects must be considered when preparing a calibration curve for quantitative analysis.

3. Measurements

3.1. Measuring Conditions

The manufacturer’s user manual for operation and specifications of the instrument should be followed. The measurements may be performed under vacuum, or under nitrogen or helium atmosphere to improve the sensitivity.

3.2. Reference Materials

The reference materials required for calibration curves, system suitability tests or control of equipment performance are prepared from certified reference materials. Reference materials with a high carbon content may be more representative for pharmaceutical applications.

3.3. Calibration

A calibration model that is fit for purpose should be selected. Sample amount, sample density, and material properties can be corrected using scatter lines. Calibration models include the fundamental parameter method, calibration based on experimental results, Compton scattering/Rayleigh scattering normalization, multiple linear regression, etc. The fundamental parameter method theoretically calculates the intensity of fluorescent X-rays emitted from the samples using fundamental physical parameters and equipment parameters and is used for quantitative calculations and correction factors calculations.

3.4. System Suitability Test

When using this method, the system suitability test should be conducted beforehand to ensure that the performance of the measurement system is suitable. This test may also be performed to verify the calibration of the measurement system.

Acceptance criteria: The difference between the measured concentration of the standard sample containing the analyte elements within the measurement concentration range and the actual concentration meets the following criteria:

- Not more than 5% for assays
- Not more than 20% for identification or purity tests

When using concentrations determined from reference analytical methods such as atomic absorption spectrometry, the accuracy of fluorescent X-rays calibration should be aligned to that of the method used. Therefore, in such cases, an acceptance criterion of not more than 10% for assays can be set.

3.5. Analysis

The sample are measured with the same parameters as used for calibration of the equipment.

4. Control of Equipment Performance

The procedures, acceptance criteria and verification intervals for the control of equipment performance depend on the equipment and its intended application. Demonstrating stable performance of the equipment over the long term ensures the reliability of measurements.

4.1. X-axis and Y-axis

The X-axis (energy or peak angle) and Y-axis (intensity) should be verified at least on equipment installation and thereafter at appropriate intervals defined according to the user's quality system procedures. In X-axis verification, the attention should be paid to the peak angles for WD-XRF spectrometers and the peak positions for ED-XRF spectrometers. In Y-axis verification, the attention should be on the count rate.

4.2. Detector Resolution

Calculate the resolution (a half width) at the energy used during calibration of the equipment.

Acceptance criteria: The difference from the resolution obtained during equipment calibration is not more than 20% for assays and not more than 25% for identification or purity tests.

5. Validation Requirements

The purpose of XRF spectrometry validation is to demonstrate whether that the measurement procedure is fit for purpose. The validation requirements for the elemental impurities test are described in Elemental Impurities *<2.66>*. Appropriate validation should also be conducted for the other tests.

6. References

1) European Pharmacopoeia 11.0 (2023), 2.2.37. X-Ray Fluorescence Spectrometry.
2) US Pharmacopeia (2024), <735> X-Ray Fluorescence Spectrometry.
3) US Pharmacopeia (2024), <1735> X-Ray Fluorescence Spectrometry—Theory and Practice.