

GUIDELINE FOR THE MICRO X-RAY FLUORESCENCE SPECTROSCOPY OF GLASS SAMPLES IN FORENSIC SCIENCE

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GENERAL REMARK

This guideline assumes prior knowledge in the forensic discipline. It is based on consensus among the relevant forensic experts and reflects the accepted practices at the time of writing. The requirements of the judicial systems are addressed in general terms only.

1. AIMS

This document describes use of micro X-ray fluorescence spectroscopy of glass samples in forensic science.

2. SCOPE

By application of the procedure described herein, a qualitative and semi-quantitative analysis of major, minor and partial trace elements of forensic glass samples can be obtained. The method can be used for comparative glass analysis in casework.

3. TERMS AND DEFINITIONS

For the purposes of this guideline, the relevant terms and definitions given in ENFSI documents, the ILAC G19 “Modules in Forensic Science Process”, as in standards like ISO 9000, ISO 17020 and 17025 apply. Also, refer to the main document Best Practice Manual for the Forensic Identification and Comparison of Glass (PGT-BPM-002).

4. INTRODUCTION

X-ray fluorescence spectroscopy (XRF) is an instrumental technique for elemental analysis. When a sample is hit by an incident X-ray beam, its electrons are excited and emit X-ray photons characteristic of the excited atom species that comprises the sample. With modern XRF instruments it is possible to detect the characteristic radiation of elements from $Z = 11$ (Na) to $Z = 92$ (U); for certain elements, these can be detected at several 10 ppm, if the measurement is done under vacuum and under ideal conditions.

Two basic detection methods exist: The wavelength dispersive (WD-XRF) and the energy dispersive (ED-XRF) method. This document deals only with the ED-XRF method, in particular ED-XRF equipped for micro-beam excitation (μ -EDXRF or in short μ -XRF), which is the most suitable for forensic purposes.

XRF is a fast and non-destructive technique that can provide qualitative and semi-quantitative information about the elemental composition of a sample. This may be used together with the determination of the refractive index (RI) to compare questioned and known control glass particles, when more sensitive methods such as laser ablation inductive coupled mass spectroscopy (LA-ICP/MS) is not available or applicable. Notable differences in elemental composition between the compared samples, by XRF analysis, can provide significant evidence that the samples have a different origin.

XRF excitation is to some extent complementary to the electron excitation by Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS), in which the detection of lighter elements is enhanced. In contrast however, heavier elements are better detected by XRF, due to the larger cross section of photons produced. While SEM/EDS is regarded as a surface sensitive method, the X-rays penetrate deeper into the sample (‘information depth’) which gives more information about the bulk composition.

5. METHODOLOGY

5.1 Instrumentation

Modern μ -XRF apparatus consist of an X-ray tube, X-ray optics (mono-/polycapillary and/or collimators), a motorized sample stage, a video (laser) positioning system, at least one energy-dispersive detector, a vacuum pump and a computer to operate the system and to process the data.

The excited X-rays produced by the X-ray tube (usually Mo, Rh, Ag or W anode) are directed onto the sample using the capillary optics or collimators. The sample is mounted on a stage, which can be moved in X, Y and Z directions. The video system provides an image enabling the operator to control the position of the sample, to bring the specimen to the correct height (focus) and to choose the positions on the sample for spot measurements, line scans or an element mapping. Modern spectrometers are equipped with a SDD (*Silicon Drift Detector*), but instruments with a liquid N₂ cooled Si-Li detector still exist.

The whole process takes place at low gas pressure (in vacuo), which requires a pump system to evacuate the chamber. Optionally, measurements can be performed in a helium atmosphere requiring a helium gas supply. Measurements are possible in air, but the sensitivity for light elements (such as Na) are severely diminished.

5.2 Limitations

The information depth from which the excited, characteristic X-rays can emerge without being re-absorbed, depends on the element under consideration as well as the glass composition and density. As can be seen in Figure 1 the elemental 'information depth' in a glass matrix is approximately:

- 10 μm for light elements such as Si
- 100 μm for metals such as Fe or Zn
- 1000 μm for heavy elements such as Cd, Sn or Ba

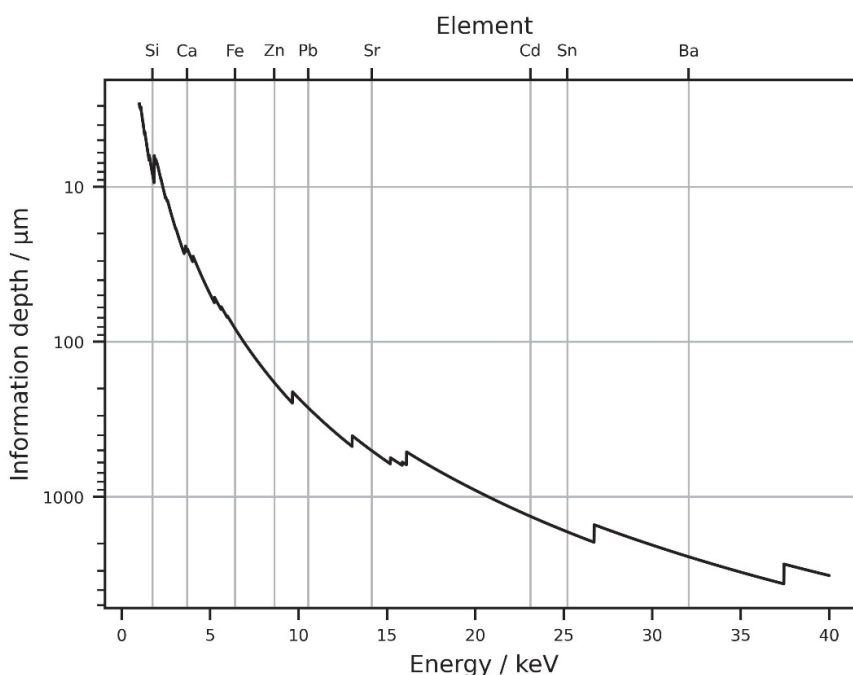


Figure 1: Information depth of different elements in a NIST1412 glass matrix (calculated for 1 % residual intensity emerging from the bulk after absorption).

For modern glasses no bulk inhomogeneities are observed but surface effects, such as the tin rich float side or a functional coating, may contribute to the spectrum, even from the beam being averted from the side of a particle when the thickness is below 1 mm.

Due to the excitation source, the characteristic lines of the anode material appear in the spectra and which can potentially overlap with the peaks of the elements comprising the glass sample; in particular minor or trace elements may be obscured.

6. SAMPLE PREPARATION

6.1 Cleaning

Any foreign material present on the glass fragments should be removed which should be retained and analysed, if necessary. Dirt, salt or any other insignificant material can be carefully removed with a scalpel and by washing with a piece of tissue or a cotton swab soaked into water, ethanol or any other suitable solvent. An ultrasonic bath can be used to agitate and remove more stubborn contaminants as required.

6.2 Embedding and Grinding/Polishing

Glass samples of suitable size and thickness may be ground and/or polished in order to achieve flat surfaces. Flat surfaces improve the accuracy and precision of XRF analyses. To enable grinding and polishing the surfaces of glass fragments, these should be embedded in a polymer matrix, such as a methacrylate resin. Commercially available light curing or chemically curing resins may also be used.

6.3 Sample Fixation and Mounting

The sample is fixed on a sample holder, using an inert material (without interfering elements), e.g. a polymer foil with an adhesive surface or playdough which do not interfere with the XRF analysis. Other methods may be used [1]. If the thickness of a glass particle is less than about 1 mm the incident X-ray beam can penetrate the particle and cause an enhanced background due to scattering originating from the composition of the sample holder. To reduce background effects, the particle can be supported by a thin polymer foil elevated above the stage. The distance from the stage should be large enough to minimise the scattered X-ray intensity.

7. ELEMENTAL ANALYSIS

7.1 Functionality and Calibration

7.1.1 Functionality Check

The functionality or quality of the detection system should be checked on a regular basis or when there are concerns about the performance of the system. This should be measured and monitored using a well characterized sample. The spectra obtained should be inspected critically, by examining peak shapes and peak energy levels to reveal potential problems.

7.1.2 Energy Calibration

The detection system must be calibrated for the correct relationship between channels and absolute energy in keV, on a regular basis or when there are concerns about the performance of the system. Modern spectrometer software allows for an automatic two-point energy calibration using a standard composite material of known composition (e. g. Cu/Al), preferably providing high and low energy characteristic energies ($E(\text{Al-K}\alpha) = 1.468 \text{ keV}$ and $E(\text{Cu-K}\alpha) = 8.040 \text{ keV}$). For calibration methods it is necessary to follow your XRF software's application

notes. The detector and counting electronics are sensitive to temperature changes, therefore any fluctuation in the room temperature of the laboratory should be kept to a minimum.

The standardized FWHM (usually of the Mn-K α peak) is usually an output by the system and should be documented. It is strongly recommended to keep records of all calibration spectra as a quality assurance measure and to detect and backtrack any malfunctions.

7.1.3 Spatial Calibration

To achieve the theoretical spatial resolution determined by the spot size (usually for a monocapillary system >100 μm , polycapillary system >20 μm) it is important to check the coincidence point of the X-ray beam with the marked measurement spot of the video system. Especially when the capillary is tilted from the vertical line, a small defocussing will give a different point of impact of the X-ray beam in the horizontal plane. This can be checked by a special sample with a thin fluorescent layer or any other suitable sample for spatial calibration. For calibration follow your XRF software's application notes. Any uncertainties or anomalies noted at this point must be considered and steps made to rectify any issues.

7.2 Qualitative Analysis

7.2.1 Settings

The following instrumental settings are recommended for a standard glass analysis by XRF:

- 1) All measurements should be performed under vacuum or in helium.
- 2) The use of primary X-ray filters is not necessary.
- 3) The excitation voltage should be set to maximum (usually 40-50 kV for a Rh X-ray tube. This type of anode materials shows a minimum of interferences with common glass elements.)
- 4) A channel resolution ≤ 10 eV/channel is recommended.
- 5) Process time¹: the process time should be chosen according a suitable peak resolution (preferably ≤ 140 eV for the FWHM of the Mn K α peak) with regard to the points (6) and (7). Shorter process times allow higher beam currents and thus maximize the throughput while longer process times improve the resolution (FWHM).
- 6) Dead time/ tube current: the tube current should be chosen to achieve a dead time ≤ 30 % for selected process time, preferably < 20% to minimize coincidence peaks with respect to the acquisition time.
- 7) Acquisition time: The combination of selected parameters (1) to (6) and acquisition time should be set so a minimum of 500,000 (preferably 2,500,000) counts are stored in the spectral range in a reasonable measurement time.

Example: An input count rate (ICR) of 3,250 cps with a dead time of 30 % results in an output count rate (OCR) of 2,500 cps. That yields a total live time of 200 s for a statistically accurate analysis with a total of 500,000 counts.

With modern SD detectors usually much higher output count rates are possible compared to Si(Li) technology and measurement times can be shortened significantly.

7.2.2 Spectrum Acquisition

For every (sub)sample at least three measurements (at different locations on the sample if possible), under the same conditions determined in 7.2.1, should be performed. For a float

¹ May also be called: *time constant, amplification time, peaking time.*

glass it must be ensured that matching sides (float or anti-float) are acquired for unknown and reference sample.

After identification by the procedure described in 7.2.3 the three elemental composition spectra must 'match' (i.e. be indistinguishable from one another). If this is not the case the sample has to be checked for inhomogeneity and surface contamination. The full analysis including preparation/cleaning should be repeated with these factors in mind.

7.2.3 Identification Procedure

Modern XRF analysis software allows an automatic peak identification. If the automatic identification is used, a critical check must be performed, as there is the possibility of misidentification due to severe peak-overlap from the energy similarity of elemental peaks (e. g. Ba/Ti, Mo/S, Pb/S besides others) [2]. These algorithms are far from perfect and as such the spectrum must be inspected critically performing the following procedure:

- (1) The acquired spectrum has to be checked for statistically significant peaks. A peak is considered significant if its *netto* intensity I_{netto} is greater the 3 times the standard deviation of the background intensity in the framing background regions [3].
- (2) The Rayleigh peaks i. e. K-/L-lines corresponding to the anode material are assigned first.
- (3) Afterwards the peaks are assigned from highest energy descending to the next unidentified peak. If two line sets of the same element are visible the low energy lines are assigned in the same step.
- (4) To positively identify an element all members of a line family (e.g. $K\alpha$ and $K\beta$) these should be present in the correct intensity ratios. Depending on the absolute peak intensity some line family members may not be distinguishable from the background signal. In the low energy regime sublines may not be fully resolved into separated peaks. For strongly overlapping peaks of different elements, it may not be possible to determine the intensity or even the presence of a peak directly and the presence of an additional element may only be hinted by another peak's deviation from a symmetric gaussian shape. This can be approached by a mathematical peak deconvolution offered by most modern software.
- (5) Potential *escape and coincidence peaks* of the element identified are determined and assigned (cf. 7.2.4).
- (6) Step (3) to (5) are repeated with the next peak until all peaks in the spectrum are identified as an elemental peak, *sum/escape peak*, or *Compton peak* (slightly lower in energy than *Rayleigh peaks* due to inelastic scattering events).

If the analysis program offers a peak deconvolution option, the total convoluted spectrum should match the measured spectrum without severe differences. If this is not the case, check for coincidence peaks of two identified elements or mis- and unidentified elements (cf. 7.2.4).

7.2.4 Escape and Sum/Coincidence Peaks

X-ray photon interaction with the detector material (mostly silicon) leads to *escape peaks* with an energy difference of 1.740 keV from its parent peak. This can lead to a potential element misidentification when the *coincidence peak* has the same energy as another element line.

If two X-ray photons arrive simultaneously at the detector, which cannot be resolved by the detection electronics, an artificial peak is created using the summed energy of the two parent photons (*sum peak* or *coincidence peak*). Like escape peaks, this may lead to wrong element identification. For example, the presence of high Si ($E = 1.740$ keV) and Ca ($E = 3.690$ keV) amounts can produce a sum peak, that can be misidentified as Cr ($E = 5.412$ keV).

The intensity of *coincidence* (and to a smaller extent *escape*) *peaks* is dependent on the deadtime respectively the total input count rate. Therefore, it is possible to suppress (but not avoid) *coincidence peaks* by measuring at lower count rate and deadtimes. To discriminate element peaks from *coincidence peaks* measurements at different dead times can be considered.

Modern XRF software packages are able to label and even correct measured spectra for *escape and coincidence peaks*. Please consult your instruments handbook for these processes. These algorithms are however far from perfect, so the existence of *escape and coincidence peaks* should be expected and considered in every measurement.

7.3 Comparative glass analysis

Comparative analysis is the most reliable when analysis is conducted on smooth and horizontally oriented surfaces of particles larger than 1 mm. It can be performed without any further preparation on original anti-float surfaces when there are no coatings present. In each case the accuracy must be determined by repeated measurements at different points on the sample surface. If there are no flat and horizontally oriented surfaces present on the sample, it should be rotated between the measurements to allow for different inclinations. Questioned and known samples must be prepared in the same manner. For example, if the sample size, thickness and/or shape of the questioned particle are not ideal and do not allow embedding, the known sample should be prepared to mimic the state of the questioned glass by preparing several small, thin and/or irregularly shaped particles which can be measured for comparison.

7.3.1 Qualitative glass comparison

The following procedure for a comparative qualitative measurement is recommended:

- Collect replicate spectra from known and questioned samples under the conditions mentioned in section 7.2.1.
- If possible, at least six measurements should be made on the known sample and on each questioned glass sample.
- Identify the elements present and check the result by the spectrum deconvolution (cf. section 7.2.3).
- Compare known and questioned samples by the lists of elements which are identified.

7.3.2 Semi-quantitative glass comparison

If the questioned and known samples have the same qualitative elemental composition the following additional steps can be performed based on ASTM E2926-17 [4]:

- Determine the background corrected net peak intensities for a set of elements, which are chosen for quantitative comparison. The chosen peaks should be free from overlapping and artefacts (cf. section 7.2) and have a signal-to-noise ratio of greater 10:1. This set may include: Ca, Mg, K, Ti, Fe, Sr, Zr especially K and Fe provide good discrimination power.
- Calculate intensity ratios of these elements (e.g. Ca/Mg, Ca/Ti, Ca/Fe, Sr/Zr, Fe/Zr or Ca/K for the chosen set).
- Calculate the mean (\bar{x}) and the standard deviation σ_{n-1} of the intensity ratios of the known sample. Calculate match intervals $\bar{x} \pm 3 \sigma_{n-1}$ for each ratio. Decide whether the mean of the questioned sample (\bar{y}) falls within the match interval. If one or more intensity ratios yield a mismatch, the samples can be discriminated (i.e. different).

In addition to the mean and standard deviation the following two additional statistical approaches can be followed for comparative glass analysis (cf. [5]):

- Perform a *Bonferroni* corrected Student's t-test between known and questioned sample for each intensity ratio.
- Perform a Hotelling's T2-test on the intensity ratios (for details see [6]).

For the comparative glass analysis and any of these methods, it is essential for each individual laboratory to perform a validation procedure on different standard glass samples (e.g. SRM NIST 1831, Schott FGS 1 / FGS 2 or similar) which are of known composition used for the specific equipment, in use under conditions determined by this guideline.

8. REFERENCES

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9. AMENDMENTS TO PREVIOUS VERSIONS

Not applicable.