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| **GUIDELINE for quantitative elemental analysis of glass samples using LA-ICP-MS** |
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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.

Beside this best practice manual also the standard method ASTM E2927-16E1 should be referred to.

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1. **AIMS**

This guideline describes quantitative elemental analysis
of glass samples using LA-ICP-MS within the field of forensic examination of glass.

The guideline is aimed towards experts in the field of forensic examination of glass.

1. **SCOPE**

This guideline provides recommendations of sample preparation, technical methods, calibration routines and analysis of glass samples by use of LA-ICP-MS. The guideline does also outline aspects of data analysis and interpretation, but relies on the assumption that each laboratory will have their own procedures on this topic, depending on national legal requirements.

LIMITATION: This guideline reflects the European Forensic Glass Community´s recommendation at the time of writing. This guideline does not serve as a textbook in the field of comparison of glass by use of LA-ICP-MS.

By application of the described procedure, elemental concentrations of main, minor and trace elements in glass samples can be determined. The described procedure can be used for comparative glass analysis case work and, in some instances, to classify glass samples.

1. **DEFINITIONS AND TERMS**

See document Best Practice Manual for forensic comparison of glass.

1. **INTRODUCTION**

Glass is a type of trace evidence commonly encountered in crime. Elemental analysis can be applied to discriminate between glass samples from different sources, especially when combined with refractive index measurements. When compared to (micro) X-ray fluorescence (XRF or µXRF) and scanning electron microscopy in combination with energy dispersive X-Ray spectrometry (SEM/EDX), LA-ICP-MS provides significantly lower limits of detection and limits of quantification. This results in a higher number of elements that can be quantified in glass samples and therefore a greater level of discrimination between glass samples from different sources. LA-ICP-MS can be applied to irregularly shaped glass fragments.

This guideline is based on research conducted, amongst others, by the NITECRIME network (“Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensics”) and the subsequent working groups EAWG (“Elemental Analysis Working Group”) and GIWG (“Glass Interpretation Working Group”) (see references in chapter 6).

Laser Ablation ICP-MS can detect the elemental composition of solid samples without prior digestion of the sample. Limits of quantification below 1 mg/kg for many elements and a linear dynamic range of 9 or more orders of magnitude allow for the determination of major, minor and trace elements within one analysis. Surface roughness and orientation have a negligible influence on the analytical results.

A pulsed laser is focussed on the sample within an air-tight ablation chamber. The laser ablates minute amounts of the sample, creating an aerosol. The laser generated aerosol is transported to the inductively coupled plasma using a helium gas flow.

The inductively coupled plasma is an Argon plasma with temperatures of about 7000-8000 K. These temperatures lead to vaporisation, atomisation and ionisation of the particles of the aerosol. The resulting ions are subsequently transferred to the mass analyser through an interface.

The mass analyser separates the ions by their mass/charge (m/z) ratio. The detector records signals for the various m/z ratios. Based on the signal heights for the ions of interest, concentrations of a wide range of elements can be calculated utilising an external calibration standard and Si as an internal or normalisation standard.

The method removes material from the sample (producing craters with diameters of 40 to 100 µm and depths of approximately 50 to 120 µm per replicate). The rest of the sample is not consumed and can be preserved for subsequent analyses.

The method can analyse irregularly shaped glass fragments with dimensions of 0.4 mm x 0.2 mm x 0.1 mm or larger. Analyses with only two replicate measurements may be possible on fragments as small as 0.2 mm x 0.1 mm x 0.1 mm.

1. **TECHNOLOGY**

**Laser Ablation**

Pulsed lasers with pulse durations in the range of several nanoseconds are commonly used for the forensic analysis of glass samples. The most common types are Nd:YAG lasers operated at a wavelength of 213 nm and ArF excimer lasers at 193 nm. Nd:YAG lasers operated at 266 nm are not recommended due to increased elemental fractionation in comparison to shorter wavelengths.

Lasers with pulse durations in the femtosecond range may further improve the quality of analyses and may allow the analysis of even smaller glass fragments. The use of these types of lasers is not described in this guideline.

**ICP-MS**

Most commonly, quadrupole ICP-MS instruments are applied in forensic glass analysis.

The application of double focussing sector field ICP-MS instruments can increase the sensitivity of the method.

Time of flight (ToF) ICP-MS instruments can provide quasi-simultaneous measurements of all isotopes instead of sequential measurements. This can significantly reduce the necessary ablation time and therefore the required size of the glass samples [Becker P. et al (2020)]. The use of this type of ICP-MS instruments is not described in this guideline.

Although most quadrupole ICP-MS instruments of the newer generations are equipped with some type of collision or reaction cell technology, in general this technique is not currently applied in forensic glass analysis Therefore it is not discussed in this guideline. However, the use of Helium or Hydrogen as collision/reaction gas, possibly in combination with a triple quadrupole configuration, may help in reducing the negative influence of some spectral interferences, especially for the determination of Iron and Potassium.

1. **METHODOLOGY**

See Chapter 4 Introduction.

1. **SAMPLE PREPARATION**
	1. Sample cleaning

Generally, it is not necessary to clean the glass fragments prior to LA-ICP-MS analysis, because the signals from the first pulses at the sample surface are not used for the evaluation. In addition, a pre-ablation with a few laser pulses and a larger spot diameter may be applied prior to the actual analysis. Heavily contaminated samples may be cleaned with water, organic solvents and/or diluted nitric acid.

* 1. Sample preparation techniques

Fixation of the glass fragments with adhesives such as double-sided tape or putty (such as Blu-Tack™) is recommended. The samples should be positioned in order to avoid analysis of original surfaces of the glass samples. Original surfaces can differ from the bulk material in the elemental composition, especially in the Sn concentrations and other elements used in coatings.

In order to carry out refractive index measurements in addition to the LA-ICP-MS measurements, it is recommended to cut or break glasses into at least two fragments, since changes in refractive index of the glass may occur after laser ablation. Therefore, one or more of these fragments should be selected for the quantitative analysis by LA-ICP-MS, whereas the other fragment(s) should be used for the determination of the refractive index. Alternatively, the refractive index can be determined first and the fragments recovered from the immersion oil and be used for LA-ICP-MS measurements.

* 1. Tuning of the ICP-MS

In tuning the system, a compromise between maximum sensitivity in the mass range between m/z 7 and m/z 208, stability of analyte signal, minimum oxide ratios, and minimum rates of doubly charged ions should be found. A tuning standard such as NIST SRM 612, can be used during tuning.

If the NIST SRM 612 standard is used during the tuning procedure, it is recommended to use the ThO+/Th+ oxide ratio on m/z 248 and 232 and the Ca2+/Ca+ doubly charged ions ratio on m/z 21 and 42.

If both detector modes of a discrete dynode detector are used during the measurements, it is recommended to perform a cross calibration between the detector modes during the instrument setup on each measurement day.

* 1. Experimental Parameters

**Recommended isotopes to be measured**

Internal or normalization standard:

29Si

Major and minor elements:

24 or 25Mg, 27Al, 39K, 42Ca, (23Na)

Trace elements:

7Li, 49Ti, 55Mn, 57Fe, 85Rb, 88Sr, 90Zr, 137Ba, 139La, 140Ce, 146Nd, 208Pb, (178 or 180Hf), (118Sn)

This set of isotopes can easily be quantified by using the FGS 2 standard.

However, not all listed isotopes necessarily need to be included to the measurements or data evaluation. Additional elements/isotopes may be included in the element menu.

The presence of less common elements such as B, Co, Zn or Cu can be detected by performing a short mass scan while ablating the glass in question (typically the control sample(s)).

It is recommended to use the FGS 2 as the calibration standard. In case it is necessary to quantify additional elements, other glasses such as CFGS 1, CFGS 2, CFGS 3, NIST SRM 614, NIST SRM 612 or NIST 610 may be used as the calibration standard or as an additional calibration standard.

178 or 180Hf is highly correlated with 90Zr and does not provide additional discrimination.

118Sn determinations may be problematic depending on the laboratory environment (elevated results caused by contaminations), but original float glass surfaces can easily be recognized using the time resolved Sn signals.

23Na does not provide much discrimination between soda lime glass samples, but it helps to identify specialty glass samples (such as borate glass).

25Mg and 42Ca do not provide much discrimination between float glass samples, but can help to discriminate float glass samples from container glass samples.

It is recommended to include at least 10 elemental concentrations in the data evaluation.

**Dwell time**

For quadrupole instruments operated in a country with 50 Hz power supply AC frequency, a dwell time of 10 µs per isotope is generally recommended.
Lower or higher dwell times than 10 ms may be used for lower signal intensities (such as Li, Hf etc.) or higher signal intensities (such as Na, Al etc.), respectively.

**Ablation mode**

All analyses should be carried out as single spot analysis. Besides the better analytical performance for quantitative glass analysis, a time resolved depth profile of the sample can be achieved.

This may assist in the additional characterisation of glass samples and preventing misinterpretation of the analytical data.

Inhomogeneities of the samples may be recognized in the transient signal.

Elements present in a functional coating on the sample surface may be recognized.

The tin-coated side of the float glass can be recognized.

Chemically toughened glass, e.g., from cell phones, may be identified by the time resolved signals of Potassium and Sodium.

**Repetition rate**

A repetition rate of 10 Hz is recommended. Alternative settings may be applied, e.g., for the analysis of very small fragments.

**Crater diameter**

The crater diameter should be in the range between 40 µm – 100 µm.

**Carrier gas for laser ablation**

It is recommended to use Helium (He) as a carrier gas for laser ablation in order to improve analytical performance. In order to maintain stable plasma conditions, most ICP-MS instruments require an additional argon (Ar) gas stream to be added to the helium carrier gas.

* 1. Measurements

**Duration of data acquisition**

A gas blank (typically 15 - 40 seconds) should be acquired followed by the ablation of the corresponding sample (~ 25 – 60 seconds). From the counts during the gas blank, the background for each isotope/element is calculated. From the counts during the ablation of the sample the elemental concentrations are calculated using an external calibration standard. In case the glass fragment is so thin that the laser beam has interleafed it, a smaller integration interval must be chosen. Typically, the crater depth for a replicate is the range of 50 µm to 120 µm.

In order to minimise carryover from previous measurements, a sufficiently long washout delay should be applied after every ablation and before data acquisition for the next sample.

## Time resolved analysis

The samples should be analysed in transient mode and reported as counts per second (cps) versus time for each isotope. It is recommended that a time resolved analysis (TRA) software such as GLITTER™, SILLS™, AMS™, IOLITE™ or LAMTRACE™ should be used for data evaluation. Some ICP-MS software products include time resolved data evaluation modules and may also be used for data evaluation.

**Procedure/sample sequence**

In order to minimise the influence of the instrumental drift on the quantitative results, the sample sequence should be subdivided in several short “calibration blocks” of less than 40 measurements. A symmetrical arrangement of the measurements within these blocks, starting and ending with measurements (three replicates are recommended) of the calibration standard, is recommended (“standard-sample bracketing”). A second calibration standard may be added in order to quantify additional elements or to better represent the concentration range of specific elements in the samples.

If the sample size allows, the ablation spots should be spaced sufficiently apart from each other to avoid re-ablating debris from earlier ablations. In order to get enough information on sample homogeneity it is recommended to carry out four to six measurements of each unknown sample, when possible. If the sample size does not suffice, less replicate measurements may be applied.

At least one quality control standard should be included in each calibration block.

If possible, at least 12 replicate measurements of four different fragments of the control sample should be analysed in order to include some of the heterogeneity that might be present within the original glass source. In addition, the ablation spots should, when possible, be distributed over the diameter of the glass fragment (i.e., in the middle of the glass and close to, but not on, the original surfaces).

Example for a Sample Sequence



1. **Data calculation**

For data calculation, it may be necessary to import the intensity versus time measurement files from the ICP-MS software to a specialized time resolved analysis (TRA) software. 29Si should be applied as the internal or normalization standard. Quantification bases upon the assumption that the average silicon oxide concentration in float glass is 0.72 kg/kg SiO2 (= 336600 mg/kg silicon). If other glass types than float glass are analysed, a reasonable value for SiO2 should be used, either based on the known glass type, based on analyses with other methods, or based on the assumption that the sum of all oxides in the glass must be equal or close to 100 %.

The TRA software calculates the results using a set of integration intervals chosen by the operator. Large spikes in the signal can be eliminated. Instrumental drifts are corrected by the software.

Because particle size effects may lead to undesired elemental fractionation, it is recommended that the first few seconds (typically ~ 5 - 10 s) of data collected during ablation should not be integrated.

1. **Comparison of samples**

Several methods for the comparison of multivariate data from LA-ICP-MS analyses of glass samples have been successfully applied in casework.

A modified 4s criterion with minimum relative standard deviations has been described [Dorn H. et al (2015)] and is recommended in the standard method ASTM E2927-16E1. The authors report 0.27 % false exclusions for 48 fragments from a single float glass pane and a random match probability of 0.11 % for a set of 82 different float glass samples from casework.

A modified 4s criterion with fixed relative standard deviations has been described [Weis et al (2011)]. The authors report 1.1 % false exclusions for 44 measurements on 33 different fragments from a single float glass pane and 0.11 % random matches for a set of 62 different float glass samples.

The authors of [Van Es A. et al (2017)] report the implementation and assessment of a calibrated likelihood ratio approach. The glass database from casework with 979 glass samples was split into a training data set of 659 samples and a test set of 320 samples. A rate of 0.2 % of misleading evidence was determined for same-source comparisons, while 0.3 % of the different-source comparisons led to misleading results. Resulting LRs were limited to a range of 3.1 x 10-3 to 3.4 x 104.

The authors of [Corzo R. et al (2018)] applied a calibrated likelihood ratio approach using a Pool Adjacent Violators (PAV) algorithm to a data set of 385 casework samples and compared the results to the ASTM E2927-16E1 criterion. The LR approach led to misleading results for 0.52 % of the same source comparisons (ASTM criterion: 3.25 % false exclusions), while 0.21 % of the different source comparisons resulted in misleading evidence (ASTM criterion: 0.022 % random matches; Weis et al criterion (unpublished data): 0,034 %). Resulting LRs were limited to the range between 3.0 × 10-3 to 6.1 × 103.

Unmodified n sigma criteria, unmodified t-test criteria and uncalibrated likelihood ratio approaches result in high rates of false exclusions and are not recommended in this context.

1. **Validation**

The method has been extensively validated in the previously mentioned interlaboratory studies and in a dedicated validation study [Montero-Berends S. (2006)].

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1. **AMENDMENTS AGAINST PREVIOUS VERSION**

This Guideline is a complete rewrite of the Appendix G to the Best Practice Manual for Forensic Glass Examinations, approved by the Expert Working Group Paint & Glass in 2009.