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Solid sampling Zeeman atomic absorption spectrometry in production and use of certified reference materials

J. Pauwels, L. De Angelis and K.H. Grobecker

Commission of the European Communities, Joint Research Centre, Central Bureau for Nuclear Measurements, B-2440 GEEL, Belgium

Abstract - Solid sampling Zeeman atomic absorption spectrometry (SS-ZAAS) was developed and commercialised some 10 years ago, and since that time a large number of applications have been published. However, in the process of production and certification of certified reference materials (CRMs), the method has never been fully accepted and, thus, was used only marginally. SS-ZAAS is ,nevertheless, ideally suited for CRM production control purposes, and, moreover, constitutes an interesting source of information on the micro-constitution of candidate CRMs. In the process of certification, the major problem is the independent and absolute calibration of the method with calibrants matching the matrix of the analysis samples. For routine analyses most of the presently existing CRMs can be used for calibration purposes.

INTRODUCTION

Solid sampling Zeeman atomic absorption spectrometry (SS-ZAAS) made its first steps to become a routine analysis method for the determination of trace elements, especially in biological and environmental samples some 10 years ago when the SM-1 spectrometer was developed and commercialised by Grün Optik (Wetzlar, Germany). As major advantages of the new method were especially mentioned :

1. Its fastness as a consequence of the fact that sample processing (dissolution, chemical matrix separation or modification, etc.) was no longer required;
2. Its accuracy as a consequence of operational simplicity and of decreased risks of contamination and/or losses;
3. Its low cost of operation.

Today, the absence of production of liquid waste is a significant advantage too.

During the first decade of existence of the method, a large number of applications have been developed and published in various fields (environmental, biological, medical, industrial, ...) (ref. 1-3). However, in the process of certification of candidate reference materials the method has never been appreciated nor accepted by the certification agencies, and thus was only used marginally.

The major reasons for this are :

1. the small sample size, which is often considered as being too small to be representative for the batch of material;
2. the difficulty of independent and absolute calibration of the method with calibrants matching the matrix of the analysis samples.

It is felt that these reasons are not fully objective, and that especially the first one is more an advantage rather than to be a drawback, as it constitutes a supplemental and not negligible source of information on the micro-constitution of the material.

THE USE OF SS-ZAAS IN CRM PRODUCTION CONTROL

In the production of certified reference materials (CRM) for trace elements in biological, environmental or medical matrices, SS-ZAAS is an ideally suited control method. Here accuracy is not of the highest importance : the main concern is to compare materials of almost identical composition before and after a given production step. Due to the simplicity and fastness of the method, an appropriate analysis planning not only allows to detect eventual contaminations or losses of analyte elements, but also to evaluate changes in microdistribution. Therefore, it is important that possible artefacts are ruled out or identified, such as accidental outliers (not to be confused with microheterogeneities), drifts in the response of the equipment, etc.

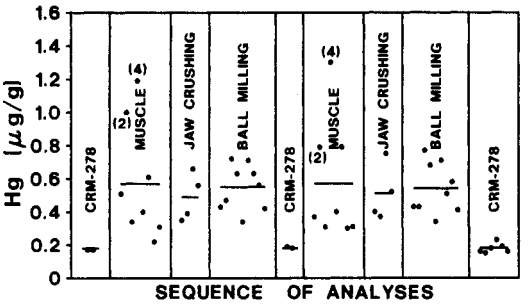

Fig. 1. Homogenising effect of the cryogrinding process on the Hg content of BCR Candidate CRM-422

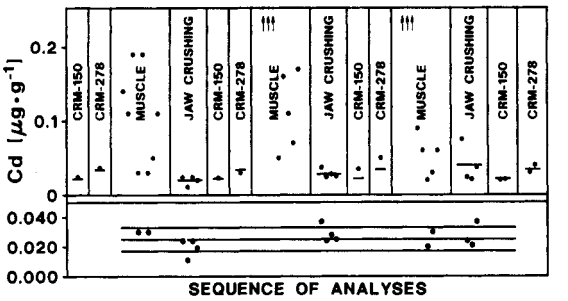

Fig. 2 Loss of Cd during jaw crushing of BCR Candidate CRM-422

It is, therefore, suggested that in CRM production control :

- Samples of each of the production steps to be studied are repeated once or possibly twice at regular intervals (see Figs. 1 and 2). Even with rather limited series of analyses the same type of distribution has to be found back. If this is not the case artefacts cannot be excluded and analyses should be repeated.
- Quality control samples of good homogeneity (possibly a CRM, eventually an aqueous solution) are analysed intermittantly. This should guarantee the stability of the system and demonstrate that its reliability (no drift, no abnormal scattering of the results) is under control. If not, also here analyses should be repeated.
- Masses are chosen properly in order that the measured signals are located in the "sensitive" part of the calibration line. Near to saturation "artificial" homogeneities (at only slightly varying intakes) or heterogeneities (at highly varying intakes) can be "generated" by the analyst : therefore, in the latter case the establishment of a sample mass vs concentration plot may be useful as well.

Figure 1 illustrates the homogenising effect of the cryo-grinding process on the Hg content of BCR candidate CRM-422 (ref. 4) :

- The stability of the response was confirmed using BCR CRM-278 (mussel tissue);
- The same type of distribution is found back on repetition of subsamples;
- Repetition of high values in muscle samples (No. 2 and No. 4) proves that "outliers" do not correspond to microheterogeneities or to micro-contamination, but to differences between specimens;
- Smaller and less skewed distributions after jaw crushing and ball milling compared to fresh muscle samples demonstrate the homogenizing effect of cryo-grinding. This is still accentuated in the next processing steps (Fig. 3).

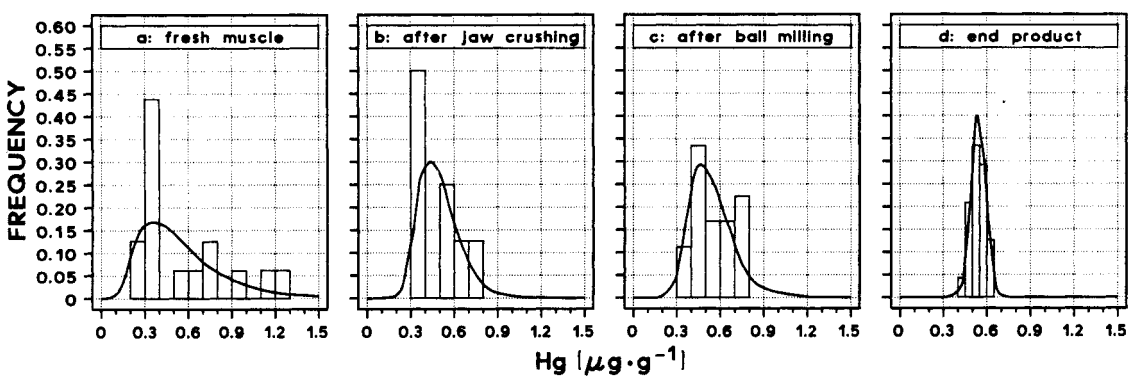

Fig. 3 Analysis results of Hg in BCR Candidate CRM-422 at various production steps

Figure 2 illustrates the loss of Cd during jaw crushing of the same candidate CRM (ref. 4) :

- The stability of the response was confirmed using BCR CRM-150 (milk powder) and CRM-278 (mussel tissue);
- The same type of distributions are found back on repetition of subsamples i.e. that high values are not artefacts, but correspond to high microlevels, possibly local spot contaminations eliminated during dipping of the fillets in liquid nitrogen;
- A "blank level" of 20-30 ng·g-1 is found for both fresh muscle and jaw crushed material (n=15). This level still seems to decrease in the further production process as illustrated in Fig. 4.

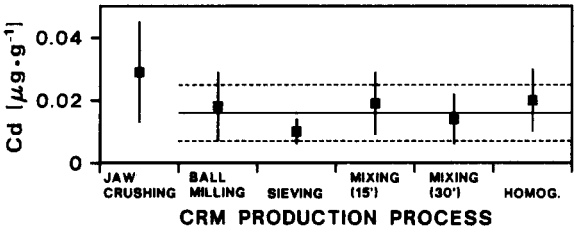

Fig. 4 Evolution of the Cd content of BCR candidate CRM-422 during the production process

METHOD

All determinations have been carried out with a Zeeman Atomic Absorption Spectrometer SM-20 (Grün Optik, Wetzlar, Germany). The background compensation is based on the direct application of the Zeemaneffect, where the lamp is placed in a strong permanent magnet. Calibrations were performed using either BCR certified reference materials, aqueous solutions or quantitatively doped samples.

THE USE OF SS-ZAAS IN CRM HOMOGENEITY CONTROL

The small sample size of the analysis samples used in SS-ZAAS constitutes an important source of information on the microconstitution of material, and several papers have already emphasized the power of the technique in this field (ref. 5-8). Despite this, almost no use was made of SS-ZAAS in the homogeneity control of CRMs.

The only examples found are :

- an "additional homogeneity control of Hg, Pb, Cu, Zn, Cr, Ni, Cd, As and Tl at lower levels of intake" of BCR-176, city waste incineration ash (ref. 9). However, this additional homogeneity control was restriced to 5 replicates per element, so that no conclusions were drawn from it;
- the homogeneity control of Ag in candidate reference material EC-NRM 522, copper for reactor neutron dosimetry (ref. 10);
- the preliminary micro-homogeneity control of Pb, Cd, Hg, Zn and Fe of BCR candidate reference material CRM-422, codfish (ref. 4).

According to the ISO Guide 35 (ref. 11) a principal test for homogeneity must be performed after the candidate RM has been packaged into final form to confirm that the between-units variation is not statistically significant. In general, this is carried out using a fast, preferably multi-element, method with good repeatability on samples of a size similar to that used by the users of the CRM. As a result of it, it can then be concluded that the mean value and confidence interval found for the batch can be applied for each specimen, or if differences between units are significant that a statistical concept called "statistical tolerance interval" must be used. This is valid for sample sizes comparable to those used in the homogeneity study itself.

It allows, however, not to determine a realistic minimum representative sample intake, nor to calculate which uncertainty should be allocated to the CRM if very small samples are used.

Based on the fact that for normal distributions

$$\sigma_t^2 - \sigma_a^2 = \sigma_h^2$$

$$(\sigma_h)_m^2 : (M/m) = (\sigma_h)_M^2$$

with σ_t, σ_a, σ_h = total, analytical method and heterogeneity standard deviations and m,M = the mass of a small and a large sample (expressed in mg), one can say that :

- <u>if very small samples are analysed</u> (e.g. using SS-ZAAS)
  - generally $\sigma_t \gg \sigma_a$, so that $\sigma_t \approx \sigma_h$;
  - even if not, the overestimation of $(\sigma_h)_M^2$ is relatively small, as σ_a^2 will also be divided by (M/m);
  - extrapolation to larger samples always yields a realistic value of $(\sigma_h)_M$, provided the original distribution is normal;

- extrapolation to a given uncertainty (e.g. the one on the certificate) allows the determination of a corresponding mass M, equal to the minimum representative sample intake.

- if larger samples are analysed an extrapolation to smaller samples is not possible because :
  - generally $\sigma_t > \sigma_h$ and an accurate estimation of σ_h is difficult;
  - the approximation $\sigma_h = \sigma_t$ cannot be justified, as by going from M to m the error is amplified by M/m instead of decreased;
  - no proof exists that when going from M to m the population will remain normal.

The consequence of this is that almost no attempts were made to determine realistic minimum sample sizes of CRMs, and that conservative statements (generally 0.1 to 1 g) are made on the certificates, which theoretically prohibit the use of these CRMs in microtechniques such as SS-ZAAS.

Therefore, we state that trace element CRMs should systematically be analysed for their micro-constitution as well, possibly using
- a sufficiently large number of intakes (e.g. 100) to demonstrate that the distribution of the results is normal;
- samples of the smallest possible size so that $\sigma_t \approx \sigma_h$ and relative homogeneity factors H_E can be calculated (ref. 5).

The result of this micro-heterogeneity determination should then be reflected in a "minimum sample weight to be used in an individual analysis" statement, whereby the homogeneity factors as described by Kurfürst et al. (ref. 5) can be used if the distribution is normal.

To calculate the minimal sample size to be used it is suggested to use the 95%/95% statistical tolerance interval

$$\Delta = k_2' \cdot s$$

whereby : k_2' = factor for two-sided tolerance limits for a proportion p = 0.95 and a probability level 1 - α = 0.95;
s = is an estimate of the measure of dispersion σ.

For 1 mg, this interval is

$$\Delta_{1mg} = k_2' \cdot H_E$$

For a large mass M it corresponds to :

$$\Delta_M = k_2' \cdot H_E / M^{1/2}$$

or if the microheterogeneity was determined on intakes of mass m ;

$$\Delta_M = k_2' \cdot s_m \cdot (m/M)^{1/2}$$

This is illustrated here for Ag in candidate reference material EC-NRM 522 (ref. 10) :

- micro-heterogeneity determination : Fig. 5
- results normally distributed : Fig. 6
- Δ_M = 4.2 % (uncertainty on certificate)
- k_2'(p = 0.95; 1 - α = 0.95; n = 54) = 2.358
- s_m = 9.5 %
- m = 0.450 mg

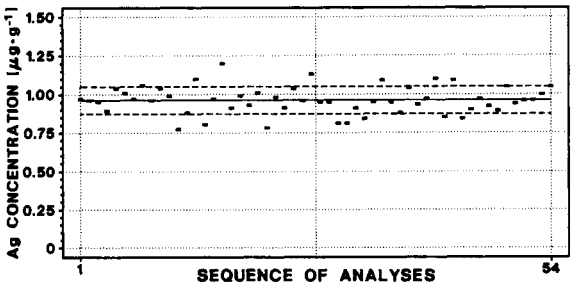

Fig. 5 Results of the homogeneity study of neutron dosimetry reference material EC-NRM 522 for Ag in Cu

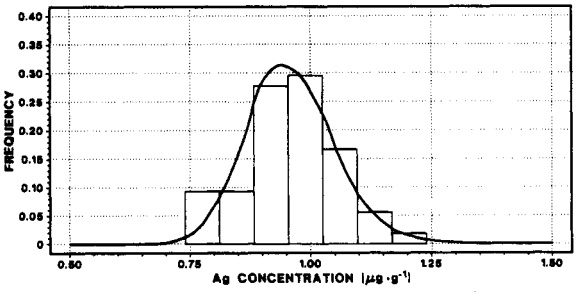

Fig. 6 Distribution of the homogeneity results of neutron dosimetry reference material EC-NRM 522 for Ag in Cu

The certified confidence interval is valid for samples of mass ≥ 13 mg. If 1 mg samples are used, the CRM uncertainty should be increased from 4.2 % to 15.0 % (0.95 ± 0.14 $\mu g \cdot g^{-1}$ instead of the certified 0.95 ± 0.04 $\mu g \cdot g^{-1}$).

As these uncertainties are mainly statistical ones, it is recommended that calibration using solid CRMs should be carried out on a relatively large number of subsamples, e.g. 20, as this reduces the statistical uncertainty coming from the CRM. The systematic uncertainty generated by the CRM can be reduced by using several CRMs per calibration whenever possible. A more sophisticated treatment is required in case the results do not follow a normal distribution (lognormal or Poisson) (see also ref. 8).

THE USE OF SS-ZAAS IN THE CERTIFICATION OF CRMs

In the process of certification, the major problem is the independent and absolute calibration of SS-ZAAS with calibrants matching the matrix of the analysis samples.

In certification analysis, neither the use of aqueous solutions, although still frequently used in routine operations, nor the use of other CRMs is acceptable, so that only the preparation of synthetic solid calibrants and the use of the standard addition method remain. In the latter it is, however, not acceptable to add a second (liquid) phase to the original (solid) one. All these reasons led to the situation that the method was only marginally used and accepted for certification purposes, the only examples being :

- BCR CRM-74 and CRM-75, copper metal (ref. 12) : analysis of Ag using synthetic solid calibrants prepared by quantitative alloying (ref. 13)
- BCR CRM-278, mussel tissue (ref. 14) : analysis of Zn, accepted notwithstanding aqueous calibration and higher standard deviation
- EC NRM-522, copper metal for neutron dosimetry (ref. 10): analysis of Ag using standard addition by quantitative alloying (ref. 13).

In all cases the results were excellent (Table 1).

TABLE 1. SS-ZAAS results used for certification purposes (all results in $\mu g \cdot g^{-1}$)

CRM	Analyte	Certified Value	SS-ZAAS
BCR-74	Ag	13.0 ± 0.4*	12.90 ± 0.36 (n = 6)
BCR-75	Ag	12.3 ± 0.4	12.42 ± 0.24 (n = 6)
EC-NRM 522	Ag	0.95 ± 0.04	0.96 ± 0.05 (n = 9)
BCR-278	Zn	76 ± 2	75.86 ± 4.50 (n = 5)

* Indicative value

However, to make the method of more general acceptance, the development of solid synthetic standards or of solid standard addition methods remains necessary. For metal samples quantitative alloying by high frequency levitation melting is a proven possibility. For organic materials a scheme :

"quantitative absorption → freeze drying → homogenisation"

as discussed by Hofmann et al. (ref. 15) may constitute an alternative. The problem is, however, that in both cases :

- a very pure base material must be available;
- the useful calibration range of SS-ZAAS is generally short.

THE USE OF EXISTING CRMs IN SS-ZAAS

Although it is generally accepted that in the proper use of CRMs :
- samples and CRMs should have matrices as similar as possible;
- CRMs should never be used as primary standards;
- the minimum sample intake as mentioned on the certificate should always be used;

in routine operation it must clearly be advised, as long as no specific SS-ZAAS RMs are produced, to use existing BCR, NBS, NIES, ...CRMs for calibration. Experience shows that generally matrices "of the same kind" are acceptable, provided :
- the concentration of CRMs and samples are comparable;
- two or three different CRMs yield a single calibration line;
- peak form of sample and CRM are similar;
- a relatively large number of shots (10 to 20) are distributed over the complete range used subsequently for analysis.

Only in marginal cases, e.g.
- when CRMs are known to have a micro-heterogeneity which is large;
- when the element is present in different chemical forms having quite different volatilities;
- when very different furnace programmes have to be used;

really significant errors must be feared.

REFERENCES

1. W. Fresenius and I. Lüderwald Fresenius Z. Anal. Chem. 322 (1985)
2. W. Fresenius and I. Lüderwald Fresenius Z. Anal. Chem. 328 (1987)
3. W. Fresenius and I. Lüderwald Fresenius J. Anal. Chem. 337 (1990)
4. J. Pauwels, G.N. Kramer, L. De Angelis, K.-H. Grobecker Fresenius J. Anal. Chem. 338, 515-519 (1990)
5. U. Kurfürst, K.-H. Grobecker, M. Stoeppler in : P. Brätter, P. Schramel (Eds.) Trace elements, No. 3., W de Gruyter, Berlin pp. 591-601 (1984)
6. M. Stoeppler, U. Kurfürst, K.-H. Grobecker Fresenius Z. Anal. Chem. 322, 687-691 (1985)
7. C. Mohl, K.-H. Grobecker, M. Stoeppler Fresenius Z. Anal. Chem. 328, 413-418 (1987)
8. U. Kurfürst 4th International Colloquium on Solid Sampling with Atomic Spectroscopy, Jülich FRG, 8-10 October 1990, Book of Abstracts
9. B. Griepink and H. Muntau EUR-9664 EN (1984)
10. F. Lievens, C. Ingelbrecht and J. Pauwels, EUR Report (to be published)
11. ISO Guide 35 Certification of reference materials - General and statistical principles, Second Edition (1989)
12. S. Vandendriessche and B. Griepink EUR Report (to be published)
13. J. Pauwels, L. De Angelis, F. Peetermans and C. Ingelbrecht(1990) Fresenius J. Anal. Chem. 337, 290-293 (1990)
14. B. Griepink and H. Muntau EUR 11838 EN (1988)
15. C. Hofmann, C. Vandecasteele and J. Pauwels, 4th International Colloquium on Solid Sampling with Atomic Spectroscopy, Jülich FRG, 8-10 October 1990, Book of Abstracts