Pure & Appl. Chem., Vol. 63, No. 9, pp. 1205–1211, 1991.
Printed in Great Britain.

ADONIS 206922209100114G

Statistical treatment of ETA-AAS (electrothermal atomisation – atomic absorption spectrometry) solid sampling data of heterogeneous samples

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Abstract - If pulverised samples contain rare particles of high analyte content ("nuggets"), skewed distributions of analytical results may be found. These distributions can be described by the Poisson probability function depending from the average number of nuggets in a subsample. For the solid sampling technique with electrothermal atomisation (graphite furnace) AAS skewed distributions must be accepted to get accurate results. However, if only a few replicates are performed, extreme values caused by several nuggets in a subsample must be rejected. The mean therefore often is somewhat too low. Compared to the errors of other methods the accuracy of the solid sampling method is sufficient even with samples which show micro-heterogeneity. The nugget effect is documentated and discussed for a bovine muscle sample (BCR CRM-184).

INTRODUCTION

The most common objection raised against the solid sampling with atomic absorption spectrometry and electrothermal atomisation (ETA-AAS) is that the subsampling error will be often too serious, when sample weights of one milligram or below are used. Background of this objection are the experiences with the classical approach for trace element analysis. These classical techniques consists of more of less elaborate digestion procedures of subsamples. From this approach and the results achieved, it has been concluded that even in well prepared solids of environmental or biological origin subsample amounts of 100 - 200 mg at least should be taken for digestion due to heterogeneity within the mostly powdered materials.

If this assessment would be correct, subsamples of considerably lower mass would lead to a drastic increase of the subsampling error, e. g. more than 100%. This would make solid sampling AAS due to the necessary low sample amounts only a semi-quantitative approach.

"However, from the applications of the method it appears that most materials have been analysed with acceptable precision, very few analyses have been reported to give such a poor precision that the data had to be rejected" Langmyhr and Wibetoe concluded in a review about the direct analysis of solid samples by atomic absorption spectrometry (1).

Because the error in the mg- and submg- range of pulverised samples is often dominated by the sample heterogeneity (subsampling error) a homogeneity factor can be determined by this method, which is a characteristic value for the material to be analysed (2). Mostly homogeneity factors of 5 - 30 $mg^{1/2}$ are reported for pulverised materials. These materials produce a relative standard deviations (RSD) of 5 - 30% with sample amounts of 1 mg (2, 3).

But, as been stated in ref. (1), "admittedly, a combination of unfavourable conditions may introduce a large sampling errror, this error may be so large that reliable analytical data cannot be obtained". They gave an example by a calculation for the expected precision in a mixture of cadmium sulfide in a matrix of iron sulfide. The obtained RSDs for subsamples of 1 mg ranged from 0.5% to 513%, depending on the particle size and the mean content of cadmium. They did not discuss the kind of distributions following from the average number of particles in a subsample, which infuence the most probable result.

At that time no analytical data were available which indicate that such strong heterogeneity can accure in real biological and environmental samples. In 1987 Mohl et al. presented some time frequency diagrams of determinations with up to one hundred replicates, achieved with the solid sampling technique, which demonstrated heterogeneity effects in biological reference materials (4). A proposal for statistical treatment of these data was not given.

This study gives an example of the statistical treatment of solid sampling data, which are not normally distributed caused by heterogeneous distribution of the analyte in the sample. The certified reference material BCR CRM-184 Bovine Muscle (16) is used as a "guinea pig". The theory is based on the fundamental papers of Ingamels et al. about the evaluation of geochemical and exploration data (5 ,6 ,7). Recently, Kurfürst et al. have applied and extended this theory for the characterisation of puverized laboratory and reference samples from biological and environmental origin (8).

Dedicated to Professor Tetsuo Hadeishi († 1990)

THEORY

Large sampling errors, skewed distributions and even "outliers" of analytical results from powdered materials can be explained by the existence of rare particles with very high content of the analyte ("nuggets") in the sample. If subsamples are taken so that only a very few of these nuggets are included, the distribution of the number of nuggets in subsamples can be described by the Poisson probability function (9)

$$P(x) = z^x e^{-z} / x! \qquad (1)$$

where
x is the number of nuggets in a subsample and
z is the average number of nuggets in subsamples of mass m (with respect to a unsegregated laboratory sample).

If z is smaller than 1, than the Poisson probability function is only rightsided, because a subsample which include no nugget is most probable. If z is larger, then the probability function is bothsided but skewed, with a tail to larger numbers. With increasing z the probability function becomes more symmetric. From z = 9 it is identical with the normal probability function.

If it is possible to separate the subsample results into fractions which contain different numbers of nuggets, then the average number z of nuggets can be determined by the Poisson distribution. Then the mean content c of the analyte in the laboratory sample can be calculated by

$$c = z\, c_n + c_b \qquad (2)$$

where
c_n is the contribution of a single nugget to the content with a subsample of mass m, and
c_b is the basic content of the matrix.

As the variance of the Poisson distribution is $s^2 = z$, the standard deviation can be calculated by

$$s = z^{1/2}\, c_n \qquad (3).$$

The average number of nuggets z for the subsample mass m can be transformed to the average number Z for the subsample mass M by

$$z = (m / M)\, Z \qquad (4).$$

With these relations the sample mass M, which gives a normal distribution (Z = 9), and the resulting standard deviation S can be calculated. With this values the homogeneity factor h_E (for the analyte E) is given by

$$h_E = S\, M^{1/2} \qquad (5)$$

and with the RSD the relative homogeneity factor H_E is

$$H_E = RSD\, M^{1/2} \qquad (6).$$

INSTRUMENTATION

The lead determinations have been carried out with the Zeeman Atomic Absorption Spectrometer SM 20 (GRÜN-Optik, Wetzlar, Germany). The background compensation is based on the direct application of the Zeemaneffect (10, 11), where a special spectral source (EDL, GRÜN-Optik) is placed in a strong permanent magnet (12).

The large number of replicates where carried out by the use of an automated sampler for powdered samples which was described recently (13).

The calibration was performed with the BCR CRM-150 Milk Powder. The methodology of solid sampling ETA-AAS analysis is described elsewhere in detail (14 ,15).

RESULTS AND DISCUSSION

Fig. 1 shows the histogram of lead determinations of the BCR CRM-184 Bovine Muscle with 360 replicates. The mean of the subsamples mass is 0.55 mg (upper and lower percentil: 0.3 - 0.85 mg).

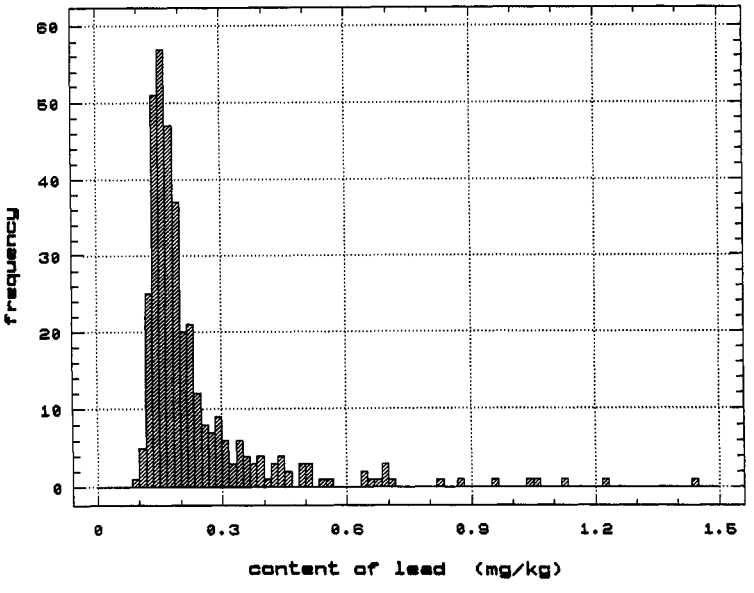

Fig. 1 Histogram of results for the lead determination of BCR CRM-184 Bovine Muscle (n = 360)

The distribution is similar to the data of this sample which were documentated in ref. (4). It is highly skewed and a multimodality is indicated.

To group the single results into fractions which include different numbers of nuggets with high lead content, the following considerations are made, which are discussed in more detail in ref. (8):
- The basic content is normally distributed.
- The fractions of subsamples with increasing number of nuggets are broadened successively by the distributions of subsample mass and nugget size.
- The distribution of the estimated nugget fractions must serve a good fit for a Poisson probability function.

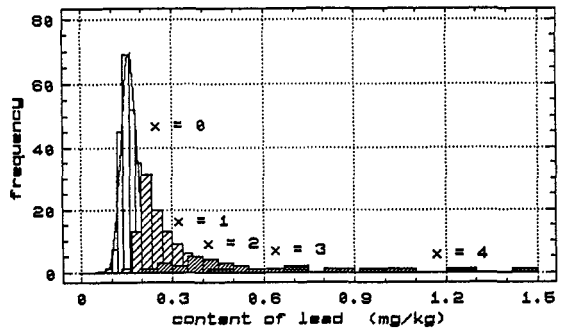

Fig. 2 Estimation of subsample fractions with 0 - 4 nuggets of high lead content from the determination in Fig. 1

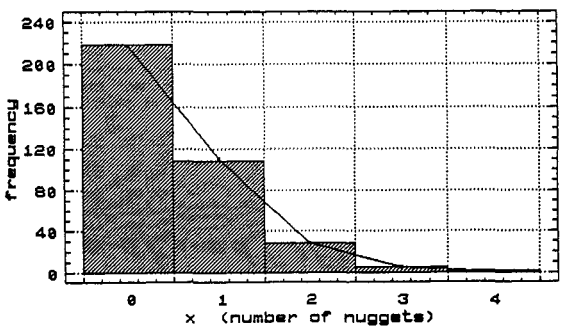

Fig. 3 Distribution of the subsample fractions from the estimation in Fig. 2 and the Poisson probability fit of z = 0.508

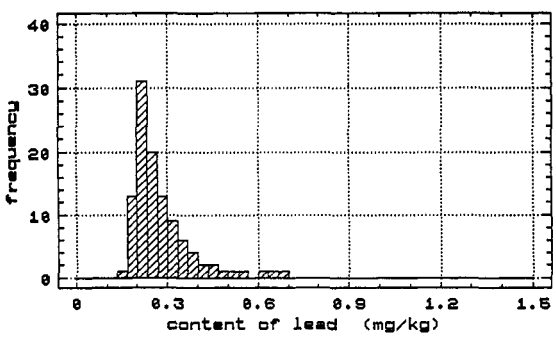

Fig. 4 Distribution of the content for the estimated fraction with one nugget (see Fig. 2, x = 1)

Table 1 Evaluation of the lead determination of BCR CRM-184 (Bovine Muscle)

Calculation from all 360 subsamples:	
mean value	0.236 mg/kg
standard deviation (RSD)	0.168 mg/kg (71 %)
Calculation from the nugget model:	
content	0.233 mg/kg
standard deviation (RSD)	0.130 mg/kg (56 %)
Expected values calculated from the nugget model:	
Subsample mass M for	
- norm. distr. results (Z = 9)	9.7 mg
- 5 % RSD	68 mg
- 1 % RSD	1700 mg
RSD for M = 200 mg	2.9 %
Homogeneity factor H_{Pb}	41 mg $^{1/2}$

In Fig. 2 the so designated fractions for x = 0 to x = 4 are shown. The basic content is c_b = 0.141 mg/kg and a single nugget gives a contribution to the content of c_n = 0.182 mg/kg, calculated from the averages of the fractions with no, one and two nuggets.

The distribution based on the nugget model for the different fractions of nuggets is shown in Fig. 3. The Poisson probability function for z = 0.508 (approximately an average of one nugget in every second subsample) shows a very good fit with the histogram columns.

Tab. 1 gives some evaluated values from these data, calculated from the relations (2, 3, 4, 5).

The application of the nugget model to the analytical results for this sample gives a good agreement for the mean content, while the standard deviation is significantly lower. However, the standard deviation calculated from the nugget model is determined mainly by the first and second fraction (thus by 80% of all measurements with this sample) while the direct calculated standard deviation depends strongly from a few extrem values. Therefore the values calculated from the nugget model are less influenced by extreme values with low probability.

Fig. 4 shows the fraction which contain one nugget . The shape of this fraction gives an impression of the nugget size distribution. The minimum contribution (and the mode) is c_n = 0.04 mg/kg and a right sided tail gives a contribution up to c_n = 0.4 mg/kg. Considering the difference in the sample mass, the nuggets should show a size difference of approximately a factor of 3. The long tail to larger nuggets is responsible for the very high contents within the fractions with more than one nugget ("outliers").

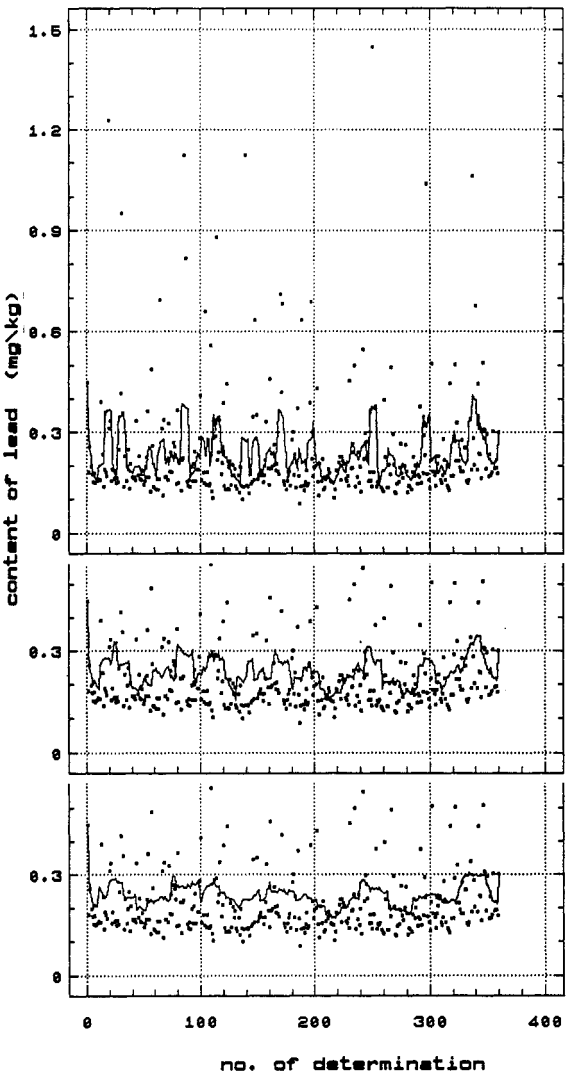

Fig. 5 Moving averages of the 360 single lead determinations: 7 terms (up), 13 terms (mid), 25 terms (low)

Tab. 2 Influence of rejected "outliers" over 0.6 mg/kg to the mean of 36 successively determined groups with n = 10

No. of group	mean overall (mg/kg)	mean after rej. (mg/kg)	n (values after rej.)
1	0.205		
2	0.316	0.214	9
3	0.213		
4	0.278	0.203	9
5	0.187		
6	0.222		
7	0.237	0.186	9
8	0.238		
9	0.333	0.173	8
10	0.216		
11	0.199	0.148	9
12	0.316	0.253	9
13	0.159		
14	0.241	0.143	9
15	0.215	0.198	9
16	0.222		
17	0.323	0.229	8
18	0.183		
19	0.219	0.173	9
20	0.277	0.231	9
21	0.163		
22	0.167		
23	0.207		
24	0.250		
25	0.327	0.203	9
26	0.198		
27	0.203		
28	0.187		
29	0.190		
30	0.308	0.227	9
31	0.188		
32	0.240		
33	0.280		
34	0.348	0.217	8
35	0.288		
36	0.212		

In routine analysis only 5 - 30 replicates for one sample are possible (even with an automated solid sampler). However, the nugget model evaluated by the Poisson distribution can only be applied when the number of replicates is large, e. g. n > 100.

Fig. 5 a - c gives an impression about the range of results which appear, when smaller number of replicates are performed by plotting the moving averages of 7, 13 and 25 single results of the performed determinations.

Fig. 6 shows 36 patterns which occure, when the 360 replicates are grouped to n = 10 single determinations.

Because the number of replicates is too low, it cannot be decided if the "god of chance" gave the extreme values representatively to the laboratory sample. So a few single results must be rejected! From Tab. 2 the influence to the mean can be read, when all results over 0.6 mg/kg are rejected.

From Fig. 6 and Tab. 2 some typical cases can be recognized:

Some patterns represent more or less the real distribution with an accurate mean without rejecting (no. 32) or after rejecting (no. 30) "outliers" of >0.6 mg/kg. The mean values over 0.3 mg/kg no longer occur after rejecting the extreme values (no. 17), but on the other hand also "accurate means" are reduced (no. 7), sometimes down to the basic content (no. 14).

When no "outlier" occur, the distribution seems to be normal and the mean is near the basic content (no. 21). This case however is very rare. The probability p of getting - in a series of n determinations - a number x of nuggets, which have the probability P is

$$p = 1 - (1 - P)^n$$

In the case of the performed analysis for one nugget p is 97%, for two nuggets p is 55% and for three nuggets p is still 12% in a series of 10 determinations. So the skewness will be (more or less) indicated (see Fig. 6).

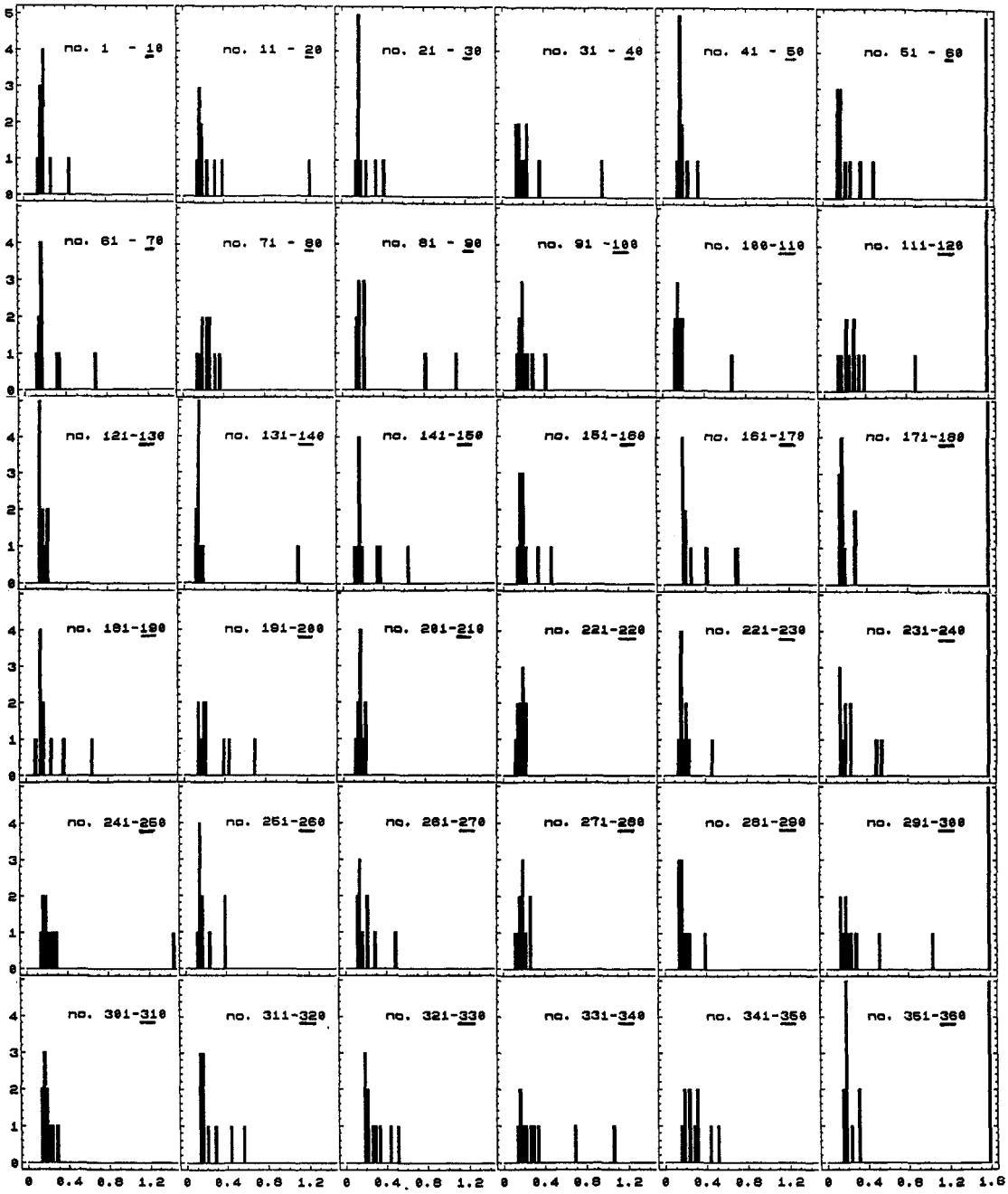

Fig 6 Histograms of 36 groups of 10 replicates each from the performed analysis. The numbers indicate the number of determination (see Fig. 5) and the underlining the number of the group (see Tab. 2)

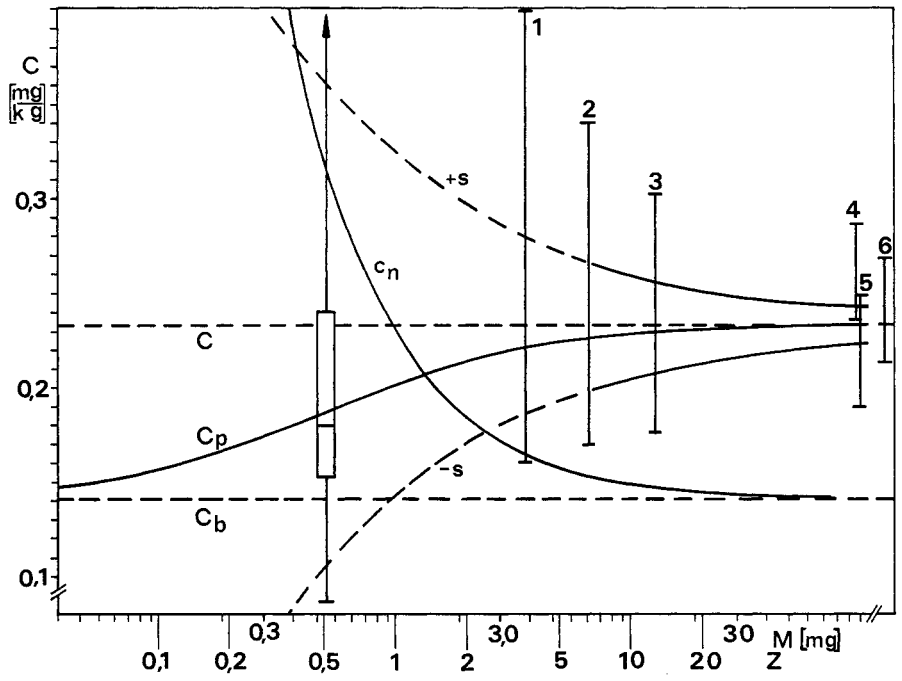

Fig. 7 Sampling diagram for lead in the certified reference material BCR CRM-184 Bovine Muscle.
The most probable result c_p was calculated by relation (7) and is plotted between the basic content c_b and the total content c.
From the homogeneity factor h_{Pb} the standard deviation s was calculated by rel. (3, 4, 5) and plotted symmetrically to the total content. Below 10 mg mass of subsamples (Z = 9) the standard deviation cannot be interpretated by the normal statistics (68 % of results between), because of the skewed distribution.
From the model of equal nuggets the contribution c_n of one nugget is calculated by the relations (2, 4).
The vertical bars (error bars) are marking the range of results. The box-and-whisker• plot at m = 0.55 mg for the performed measurement is favourable, indicating the skewness. The bars no. 1, 2, 3 at larger subsample masses correspond to the moving averages in Fig. 5.
The bars no. 4, 5, 6 are representing results from the certification campaign of the BCR (16). The ranges are based of 5 single results of determinations with ETA-AAS (no. 4) and Anodic Stripping Voltammetry (no. 5), using subsamples of 100 mg after pressure digestion. Error bar no. 6 shows the range of 5 subsamples of 2000 mg after dry ashing and 5 determinations with ETA-AAS.
• 50% of results are "in the box", each whisker represents 25% of results, in the box the median is indicated.

Ingamells has described the method of establishing sampling diagrams of mixtures for visualizing and controlling the sampling error dependig from the subsample mass (6). Fig. 7 shows the sampling diagram for the bovine muscle sample. It shows the basic (matrix) content, the total content, the resulting standard deviation and the ranges of results against the subsample mass. In addition the contribution of one nugget (of average size) to the total content for a sample of the mass M is calculated from relation (2).

For a real mixture, where the nuggets are not of equal size, a semiempirical relationship of the most probable result for a single determination is given in ref. (5) by:

$$c_p = c - (c - c_b) / (2Z + 1) \qquad (7).$$

This relation meet the requirement that with very small subsamples c_p approches to the basic content c_b and with large subsamples to the overall content c.

The calculated relations plotted in Fig. 7, which are based on the nugget model and the Poisson distribution, show a good agreement with the determined values (error bars). It must be pointed out, that the results of determination of subsamples >10 mg are symmetric (normal) to the total content and that the most probable result for a single determination is very close to the real content.

From the subsampling diagram it can be read, that with the solid sampling method sufficient accurate and precise results can achieve also with very heterogenious samples, when the analysed total sample mass is large enough, to get the chance of fetching the nuggets representatively.

By the central limit theorem the interpretation of several subsamples as a single subsample of the total mass is possible. So, if for this bovine muscle sample 10 mg total sample mass is necessary, an accurate determination can be performed by n = 10 of 1 mg subsamples or n = 18 of m = 0.55 mg subsamples. But it is necessary to accept skewed distributions for the single results, and no outlier test should be performed which is based on a normal distribution.

CONCLUSION

The test material examined by this study shows, that also biological materials can contain particles with extreme high analyte content. For bovine muscle samples Lücker has shown the origin of the nuggets (17). In cystic regions of the muscle calcanious parts are built, where lead was accumulated up to a 300 times higher content compared to the tissue. For the BCR CRM-186 Bovine Kidney was reported that very small crystals of equine kidney stones with a content of lead up to 0.1 % exist in the sample powder, which cause a strong sampling error also with subsamples of 100 mg (16). In (8) the nugget effet was described for cadmium in a codfish filet material, which have the origin in rests of bones.

These examples gave a hint that especially biological materials with a very low analyte content are endangered for containing nuggets.

For the solid sampling method it can be concluded, that in routine analysis there is a danger of getting results, which are systematically low, when the sample include rare analyte nuggets.

This danger can be reduced if the subsamples were chosen as large as possible. So the actual lead determination was performed near the limit of determination for the coming out of the nugget effect. It would be possible to increase the sample mass up to 2 mg. Than z becomes approximately 2, the resulting distribution still would be skewed, but no longer onesided and without extreme values. Of the same meaning is the increase of the number of replicates, when the sample mass cannot be enlarged by methodological reasons.

If the sample mass is too small, the most probable result and the mean value will be the basic content. This is true for the ETA-slurry technique, where the effective subsample mass is less than 0.05 mg, when the analyte was not dissolved from the particles.

The lack of accuracy with the solid sampling method by decreased precision and too small total subsample mass is in the same range as it is with classical methods using larger amounts of samples when only one to three replicates are performed. The methodical error is dominating with these approaches (see ref. 16 and Fig. 7, error bar no. 6). In particular the blind value of the used reagents and vessels can produce a large error with this low lead content when the subsample mass is below 100 mg (18). So the grade of confidence for the result of all of these methods is similar.

Reference samples which show this kind of microheterogeneity are not suited for the use of calibration in solid sampling AAS, because no accurate fit for the calibration curve is possible (with or without "outlier"-rejcection). In the light of the results of this study it is explicable, why the best accuracy with the solid sampling technique is achieved when ideal homogenious reference samples like milk powder are used for calibration (19).
Moreover, this investigation is suited for encouraging to accept "outliers" and skewed distributions of analytical results. Like Ingamells pointed out with respect to the classical analytical methods, "it appears, ... that an observed low variance in the result may be a symptom of gross error" (5).

Acknowledgement

I want to thank Dr. J. Pauwels and the members of the sample preparation group from the Joint Research Centre (CBNM) in Geel/Belgium for the many helpful discussions and their assistence with the experiments during my presence as a scientific visitor.

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