

# Elemental Analysis Manual

## for Food and Related Products

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## 3.3 Uncertainty

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### GLOSSARY

Total combined standard uncertainty can vary widely and is influenced by analytical parameters. However, for EAM methods, the procedure and control specifications keep uncertainties within a range useful for most applications. For simplicity in this discussion, “uncertainty” is used in place of “standard uncertainty” and uncertainties are expressed at a confidence level of about 67% (commonly said to be at a “1-sigma level”). Uncertainties at 95% or 99% confidence levels are obtained using coverage factors. For example, the use of a coverage factor of 2 (multiplying uncertainty by 2; a “2-sigma” level) generates an expanded uncertainty at a confidence level of about 95%.

Statistical guidelines<sup>1-2</sup> were used to account for the various sources of uncertainty associated with results generated using the EAM methods. The guidelines provide for components of uncertainty to be based on a variety of information, such as data generated during analyses, approximations based on previous scientific data, experience, and scientific judgment. Depending on the data obtained and the number and type of assumptions used, the expression of uncertainty can vary even within a given method.

Unique uncertainties based on data from individual analyses are specific and unique to individual results. A calculation of a result's uncertainty is needed for every result to demonstrate low uncertainties of highly-accurate values. Alternatively, uncertainties based on general information apply universally to all results and indicate accuracies that are generally attainable using a method. These method uncertainties are generated using extreme, or worst-case, assumptions that are typically much worse than actual situations. By using worst-case uncertainty calculations, accuracy is never presented as being better than may actually be obtained if calculated for the individual result. Method uncertainties generated in this manner can be considered limits of uncertainty.

Although the generation of individual uncertainties is discussed below, the EAM is written with the expectation that the routine presentation of results will include method uncertainties only. For each result that is above detection, the only accuracy designation would be either quantified (uncertainty estimated to be  $\leq 10\%$ ) or qualitative (uncertainty  $> 10\%$ ). These are very broad accuracy regions defined by LOQ, which is set according to  $ASQL = 30 \times s$  where  $s$  is the standard deviation of the concentration measurements of the MBKs (or MBKs fortified).

### 3.3.1 UNCERTAINTY CALCULATIONS

Total combined uncertainty arises from many individual components, which are determined then combined using the root-sum-squares calculation. This process is sometimes referred to as performing an uncertainty budget.

Uncertainty for rectangular distributions, such as for long-term sensitivity stability and accuracy, are treated by dividing the distribution limits (check solution control limit and accuracy limit, respectively) by  $\sqrt{3}$ . Uncertainty for normal distributions, such as imprecisions for instrument alignment, plasma stability, sample introduction, autosampler deviations and emission integration variations, are estimated by using standard deviations. Note that when  $n$  measurements are taken for a solution, the uncertainty for the average of the  $n$  readings is equal to  $RSD/\sqrt{n}$ .

Below is the equation for ASDL, using  $s$  for  $\sigma_{MBK}$  and assuming  $n=5$  blanks ( $t = 2.132$ ). The last factor is commonly omitted because it is negligible when the standard deviation results from a large number of blanks. It is needed in this treatment, however, because it accounts for the use of only a relatively small number of blanks. This factor raises ASDL by 10% for the minimum 5 blanks case and decreases to 1% for  $n=50$  blanks.

$$ASDL = 2 \times t \times \sigma_{MBK} \times \sqrt{1 + \frac{1}{n}} = 4.67 \times s$$

#### 3.3.1.1 Uncertainty Components and Total Analytical Uncertainty

For practical reasons, a balance has been chosen for the EAM between treating a minutia of small uncertainty components for completeness and omitting some of the more significant ones for simplification. Below focuses on the main components, their magnitudes at control limits, and how they combine to give the total combined uncertainty of  $\leq 10\%$  which applies generically as an upper limit for analysis results generated using EAM methods.

(1) Signal measurement, blank variability, and net uncertainty

The signal measurement and method blank subtraction processes were examined independently but are inter-related and logically combined to form a net component. The control specifications for signal measurement are 7% maximum RSD and minimum of 3 repeated measurements (aspirations, etc.) for generation of the final (average) value. The graphite furnace atomic absorption technique is an exception and only requires a minimum of 2 repeated measurements, which result in a slightly increased total uncertainty. Therefore, at control limits, the uncertainty component of the signal measurement is equal to 7% divided by  $\sqrt{3}$ , which is 4.04%. Method blank uncertainty is estimated by the standard deviation of the method blanks (s).

Net uncertainty results from combining the signal measurement uncertainty and method blank uncertainty (root-sum-squares) and normalizing to the net analyte level.

$$unc_{net} = \frac{\sqrt{unc_{measurement}^2 + unc_{blank}^2}}{signal - blank} \times 100\%$$

For a specific analysis, absolute values for analyte signal uncertainty and method blank uncertainty would be used. For the purposes of this discussion, however, definitive values are assumed to be not known and the calculation is done using relative values at control limits and at ASQL. The signal measurement relative uncertainty is converted to an absolute value so it can be combined with the method blank standard deviation. The root-sum value is ratioed to the net level (signal minus blank), the factor s cancels, and the result is converted to percent. Assuming level scedascity (s constant for levels  $\leq$ ASQL),  $MBK_L = ASD_L = 4.67 \times s$  (for 5 blanks), analytical solution uncertainty = 4.04% (see above), and analyte level = ASQL =  $30 \times s$ , the net uncertainty component is 6.20%.

$$unc_{net} = \frac{\sqrt{\left(\frac{4.04}{100} \times 30 \times s\right)^2 + s^2}}{(30 - 4.67) \times s} \times 100\% = \frac{\sqrt{(1.21)^2 + 1} \times s}{25.33 \times s} \times 100\% = 6.20\%$$

(2) Standard signal measurement and sensitivity instability

Standardization uncertainty has a relatively small contribution. However, sensitivity stability has a loose control limit to facilitate high sample throughput, so this component is very significant. The control specifications for standard signal measurement are a 5% maximum RSD and minimum of 3 repeated measurements (aspirations, etc.) for generation of final (average) values. The uncertainty component is therefore equal to the RSD (5%) divided by the square root of the number of repetitions (3) to equal 2.89%. Standardization sensitivity has a control limit of 10% (check solution  $\pm 10\%$ ). Since this represents a square distribution, the uncertainty component is equal to 10% divided by the  $\sqrt{3}$ , or 5.77%.

$$UNC_{std} = \frac{5}{\sqrt{3}} \times 100\% = 2.89\%$$

$$UNC_{CS} = \frac{10}{\sqrt{3}} \times 100\% = 5.77\%$$

### (3) Matrix Effect Uncertainty

Uncertainty associated with matrix effects is treated as a separate component. Although procedures are incorporated into the EAM methods to correct for matrix effects, the corrections cannot be considered perfect. Also, small matrix effects may go unnoticed. Therefore, there may be a bias associated with matrix effects and/or the correction procedures. The limit for this bias is estimated to be 5%. Therefore, since this represents a square distribution, the uncertainty component is equal to 5% divided by  $\sqrt{3}$ , or 2.89%.

Note that very large matrix effects that cannot be properly treated within a method are not considered within the context of this discussion because in this case an analysis would be outside the scope of the method.

### (4) Miscellaneous Uncertainty

Several components of uncertainty have been combined since they have a relatively small effect on total uncertainty (see 3.3 Table 1). These individual components account for standard stock solution purity, reagent blank for working standard solutions, standard curve generation, standard solution dilutions, unknown mass measurement, solution dilutions, and nominal digestion losses. These components were based on manufacturer's specifications (*e.g.*, pipet and volumetric accuracy/imprecision) or assigned according to past laboratory experiences.

Up to 3 dilutions were assumed for standard solutions and up to 2 dilutions for unknown solutions. The digestion loss limit ( $<0.2\%$ ) was considered appropriate for materials posing no exceptional problems, such as routinely-used reference materials. Accuracies and limits were rectangular distributions and imprecisions were normal distributions.

**3.3 Table 1. Miscellaneous Uncertainty**

Dilutions	uncertainty (%)
pipettor accuracy $\leq 1\%$	0.58 <sup>a</sup>
pipettor imprecision 0.2%	0.2
volumetric flask accuracy $\leq 0.1\%$	0.06 <sup>a</sup>
combined (root-sum-sq)	0.61
2 dilutions	0.87
3 dilutions	1.06
Miscellaneous	uncertainty (%)
standard stock purity/accuracy 0.2%	0.12 <sup>a</sup>
dilutions (up to 3)	1.06
standard blank $\leq 0.1\%$	0.0058 <sup>a</sup>
curve generation $\leq 0.2\%$	0.2
mass measurement (RM 100 $\pm$ 0.1 mg)	0.058 <sup>a</sup>
digestion losses $\leq 0.2\%$	0.115 <sup>a</sup>
dilutions (up to 2)	0.87
combined (root-sum-sq)	1.40
<sup>a</sup> Rectangular distribution	

(5) Total combined uncertainty

Combining the above values (6.20%, 2.89%, 5.77%, 2.89%, and 1.40%) gives 9.5% total combined uncertainty.

$$UNC_{total} = \sqrt{UNC_{net}^2 + UNC_{std}^2 + UNC_{CS}^2 + UNC_{matrix}^2 + UNC_{misc}^2}$$
$$= \sqrt{6.20^2 + 2.89^2 + 5.77^2 + 2.89^2 + 1.40^2} = 9.5\%$$

Rounding up gives the  $\leq 10\%$  upper limit that applies generically to results generated using EAM methods.

### 3.3.2 DETERMINATION OF A SPECIFIC UNCERTAINTY

For a given analysis, specific components of uncertainty unique to that analysis may be used instead of assigning a generic method uncertainty of  $\leq 10\%$ . To do this, some of the uncertainty components must be determined. Although many components could be uniquely determined, the most useful components to determine are the net and standard signal measurement uncertainties.

The sensitivity instability component is a constant at 5.77% because it arises from method control limits. This would not be the case, however, if a correction were performed for sensitivity drift based on check solution measurements. This is a deviation from the EAM methods and not discussed here. Alternatively, should ongoing laboratory records show that sensitivity drift varies according to a measured standard deviation or with a maximum drift significantly less than 10%, a different value may be appropriate (see also the note at the end of the example below).

A different value may be appropriate for the matrix uncertainty component and the values giving rise to the miscellaneous component would probably vary. However, these factors are relatively small so adjusting them would have only a small effect.

Note that although a blank below ASDL could carry a large relative error, the uncertainty component would be low compared to ASQL.

The following parameters are used to assign an uncertainty for a specific analysis:

s ----- standard deviation for MBK (mg/L)  
MBK<sub>L</sub> ----- method laboratory blank (mg/L)  
AS ----- analytical solution analyte level (mg/L)  
STDEV<sub>AS</sub> ----- standard deviation for analytical solution (mg/L)  
n<sub>AS</sub> ----- number replicate measurements of analytical solution (unitless)  
RSD<sub>STD</sub> ----- relative standard deviation control limit for standard solution (%)  
n<sub>STD</sub> ----- number replicate measurements required for standards (unitless)

$$UNC_{net} = \frac{\sqrt{\left(\frac{STDEV_{AS}}{\sqrt{n_{AS}}}\right)^2 + s^2}}{AS - MBK_L} \times 100$$

$$UNC_{std} = \frac{RSD_{std}}{\sqrt{n_{std}}}$$

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*Example:*

*Zn concentration is slightly above ASQL, MBK is below detection, and there is a small measurement imprecision.*

$$s \text{-----} 0.00412 \text{ mg/L}$$

$$MBK_L \text{-----} 0.00113 \text{ mg/L (from 492 blank measurements)}$$

$$AS \text{-----} 0.50 \text{ mg/L}$$

$$STDEV_{AS} \text{--} 0.0075 \text{ mg/L}$$

$$n_{AS} \text{-----} 3$$

$$RSD_{STD} \text{----} 2 \%$$

$$n_{STD} \text{-----} 3$$

*Performance values (MBK n=492; t=1.645)*

$$ASDL = 2 \times 1.645 \times 0.00412 \times \sqrt{1 + 1/492} = 0.0136 \text{ mg/L}$$

$$ASQL = 30 \times 0.00412 = 0.13 \text{ mg/L}$$

*Generic statements on uncertainty:*

*“results above 0.13 mg/L, quantified with uncertainty  $\leq 10\%$ ”*

*“results between 0.014 and 0.13 mg/L, “trace” with uncertainty above 10%”*

*Specific uncertainty, using generic values for sensitivity instability, matrix effect, and miscellaneous components:*

$$UNC_{\text{net}} = \frac{\sqrt{\left(\frac{0.0075}{\sqrt{3}}\right)^2 + (0.00412)^2}}{0.50 - 0.00113} \times 100 = 1.20\%$$

$$UNC_{\text{std}} = 2/\sqrt{3} = 1.15\%$$

$$UNC_{\text{CS}} = 5.77\%$$

$$UNC_{\text{matrix}} = 2.89\%$$

$$UNC_{\text{misc}} = 1.40\%$$

*Therefore,*

$$UNC_{\text{total}} = \sqrt{1.20^2 + 1.15^2 + 5.77^2 + 2.89^2 + 1.40^2} = \sqrt{38.0} = 6.8\%$$

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*Note that most of the uncertainty came from the check solution component. If a long-standing history shows that the check solution results never deviate more than 5% or a 5% control limit is used instead of 10%, this value would be*

*reduced considerably. Use of a correction factor to account for sensitivity changes would eliminate this uncertainty component but another would need to be added to account for using this factor.*

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### 3.3.3 SPECIAL SITUATIONS AND NONHOMOGENEITY

The uncertainty estimates described apply to typical analyses where analytical behavior is simple and straightforward where no special circumstances occur. Uncertainty from digestion losses, interference (such as from spectral line overlap and background emission effects), and matrix effects (such as those associated with ionization effects, viscosity and surface tension differences) are not included in this basic treatment. These are considered unique and would need to be treated individually.

Sample nonhomogeneity is extremely important and must be accounted for when using analysis results. However, this is a characteristic of sample selection/preparation and must be considered when interpreting results. However, it is not considered in this discussion because it is not part of the analysis.

### 3.3.4 CONSISTENCY WITH THE FDA/ORA LABORATORY MANUAL

The EAM treatment of uncertainty is consistent with FDA/ORA Laboratory Manual, Vol. II, Section 2, *Estimation of Uncertainty of Measurement* (ORA-LAB.5.4.6, v1.2).

## REFERENCES

- (1) Taylor, B. N. & Kuyatt, C. E. (1994) National Institute of Standards and Technology Technical Note 1297, Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results, U.S. Government Printing Office, Washington, DC 20402.
- (2) ISO TAG4 (1993) Guide to the Expression of Uncertainty in Measurement, GUM, International Organization for Standardization, 1 Rue Varamb , Case Postale 56, CH-1211 Geneva 20, Switzerland.