U.S. Department of Health & Human Services

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Elemental Analysis Manual

for Food and Related Products

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3.2 Terminology

Version 3.0 (December 2021)

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GLOSSARY and ACRONYMS

3.2.1 FIGURES OF MERIT

Figures of merit are parameters used to judge performance characteristics of chemical analysis and ensure data quality and reliability. They can be general, such as the selectivity of a method, or specific, such as the resolution of a detector. Confusion is often associated with figures of merit

because definitions vary; and different figures of merit are sometimes so closely related that they are used interchangeably.

Figures of merit can be established (e.g., during method development or when preparing for analysis), verified periodically (e.g., annually or after changes in hardware, operating conditions, reagents, personnel, etc.), and/or monitored during routine analyses.

Selected figures of merit - associated with performance, detection, quantitation, and sensitivity - are discussed below.

3.2.1.1 Performance

Selected measurement performance terms, which are inter-related and cover over-lapping concepts, are described.

- *True Value* (τ) and *Reference Value* (R) - *True value* represents the actual value (the objective sought when analyzing a sample). *Reference value* is a certified, consensus, or otherwise accepted value. Since τ is an unknown (due to measurement uncertainty), R is used as a surrogate for τ in calculations.
- *Measurement mean* ($\bar{x}$) – The sample mean of n measurements. It is a practical estimation of the *limiting mean* (μ). In multi-laboratory studies, the *grand mean* ($\bar{\bar{x}}$) is used as a better estimation of the *limiting mean* (μ).
- *Limiting mean* (μ) [also sometimes called the *expectation* of the measurement, $E(\hat{x})$] - The asymptotic value or population mean of the distribution that characterizes the measured quantity; the value that is approached as the number of observations approaches infinity (*i.e.*, as random error approaches zero).
- *Measurement error* (ε) [also called *total error*] – The difference (positive or negative) between the measurement result (x_i), mean ($\bar{x}$), or grand mean ($\bar{\bar{x}}$) and the *reference value* (shown in Equation 1 for $\bar{x}$). *Measurement error* encompasses (shown in Equation 2) both systematic and random effects (Δ and e, respectively).

$$\varepsilon = \bar{x} - R \qquad \text{3.2 Equation 1}$$

$$\varepsilon = \Delta + e \qquad \text{3.2 Equation 2}$$

> *Note that Measurement Error not Measurement Uncertainty (EAM 3.3) and the two should not be confused or spoken of as equivalent.*

- *Random error* (e) - Component of *measurement error* that in replicate measurements varies in an unpredictable manner. Random error is the net result of the random variation of one or more effects (influence factors) and is most often estimated by the sample standard deviation, s (as a surrogate for the population standard deviation, σ). In practice, the estimate of e for a laboratory mean is minimized by analyzing replicates. As the number of replicates increases, the estimate of e decreases.
- *Bias, Systematic Error*, and *Trueness* (Δ, δ) - Three terms that describe the same basic quantity but from different perspectives. *Bias* is the difference between the limiting mean and the true value (Equation 3). This makes it a component of total measurement error - specifically, the *systematic error* (that which in replicate measurements remains constant or varies in a predictable manner, Equation 4). *Bias* and *systematic error* are therefore the same

mathematical quantity, but their usage depends on the context of the discussion. *Trueness* is a closeness concept that is essentially the qualitative inverse of *bias* (Equation 5).

$$\Delta = \mu - \tau \cong \mu - R \qquad \text{3.2 Equation 3}$$

$$\Delta = \varepsilon - e \cong \varepsilon - \sigma \cong \varepsilon - s \qquad \text{3.2 Equation 4}$$

$$Trueness = |\mu - \tau| = |\Delta| \cong |R - \bar{x}| \qquad \text{3.2 Equation 5}$$

Whereas bias is a signed quantity, trueness is the unsigned quality.

Individual specific biases are sometimes designated with subscripts (e.g., within laboratory bias, Δ_w; between laboratory bias, Δ_L). It is also not uncommon in these cases for the lower case delta (δ) to be used (e.g., method bias, δ_m).

Bias is an unknown (because the true value is an unknown). Therefore, while some types of systematic effects can be well characterized and corrected (in whole or in part), the bias term would be considered to remain part of the equations.

Bias can only be estimated in the context of replicate measurements, and is most often calculated as the residual portion of measurement error when random error is subtracted (Equation 4).

Since bias, systematic error, and trueness are so closely related, it is common for their usage to be interchanged in discussions and documents.

The association between bias and trueness is explained in ISO 5725. "The term bias has been in use for statistical matters for a very long time, but because it caused certain philosophical objections among members of some professions (such as medical and legal practitioners), the positive aspect has been emphasized by the invention of the term trueness."

- *Accuracy/Inaccuracy* – Accuracy and inaccuracy are qualitative 'closeness concepts' that represent the closeness of agreement a measurement procedure (or the results it produces) is/are to the accepted reference value.

Accuracy is the qualitative inverse of inaccuracy (or, measurement error).

Accuracy has two aspects: Trueness and Precision. A method, or the test results it produces, can be considered accurate when both trueness and precision are satisfactory for the intended purpose.

- *Precision/imprecision* - The qualitative 'closeness concept' associated with random measurement variability, which describes how close measurement results are to each other under stipulated conditions. The quantitative equivalent of imprecision is variance or population standard deviation (σ), and is most often estimated by the sample standard deviation (s) of replicate measurements.

- *Repeatability standard deviation* (σ_r, s_r) - The precision under relatively narrow variation in conditions (*e.g.*, results within a single analytical batch).
- *Intermediate precision standard deviation* (σ_w, s_w) - The precision under more varied conditions (*e.g.*, results from multiple analytical batches, instruments and/or analysts but all within a single laboratory).
- *Reproducibility standard deviation* (σ_R, s_R) - Precision under broad conditions, ideally representing the full range of method parameters expected during routine use of a method. (*e.g.*, laboratory averages in a multi-laboratory trial). Reproducibility is calculated from two precision estimates (the between laboratory standard deviation, s_L, and the average within laboratory standard deviation, $\bar{s}_w$) according to Equation 6.

$$\sigma_R \approx s_R = \sqrt{s_L^2 + \bar{s}_W^2} \qquad \text{3.2 Equation 6}$$

Imprecision should always be reported with enough context to make it clear what it is describing. For example:

"Pipettor imprecision and injector flow imprecision were ____ and ____, respectively."

"Repeatability was better for laboratory X than for laboratory Y."

"Reproducibility was excellent for Method Z in the MLV study"

3.2.1.2 Detection

Detection, in simple terms, means discernment (that something was "seen" - e.g., an analyte above blank, an instrument signal above baseline, etc.). Detection limits are defined because they are based on arbitrarily-chosen criteria and procedures for determining them vary. In the EAM, detection limits are relatively simplistic 'nominal' estimates, based on blanks and without a rigorous metrological treatment. They are calculated according to the statistics of hypothesis testing, such that the probabilities of false positives (α) and false negatives (β) are both 0.05 (*i.e.*, 95% confidence).

Detection limits are based on blanks and are specific to given methods. Ideally, blanks would be identical to the sample matrix and present the same matrix effects but without the analyte. Such blanks are seldom available, however, and standard blank solutions (which have no matrix or other method-relevant effects) are used.

- Instrument detection level (IDL) - Estimates the lowest level which can be reliably detected in the measurement phase by a measuring system (i.e., an analytical instrument). IDLs only provide information about the impact of the measuring system (the analytical instrument and the dilution solvents). IDLs are generally calculated based on replicate measurements of a standard blank and thus represent an ideal case (e.g., without matrix effect).
- Analytical solution detection limit (ASDL) Estimates the lowest level which can be reliably detected in the analytical solutions. ASDLs are generally calculated based on at least five independently prepared method blanks (MBKs) having (or spiked to have) analyte mass fractions between ASDL and ASQL. ASDLs provide information about the impact of the entire method. For analytical techniques where measurements are made on solid test portions (such as energy dispersive x-ray fluorescence), rather than on analytical solutions, a

corresponding analytical portion detection limit (APDL) can be based on analyte-free materials (when available) or empty sample containers.

- Limit of detection (LOD) - Estimates the lowest level which can be reliably detected in the analytical sample. LODs are related to the ASDL (or APDL) by the analytical dilution factor (and applicable mass correction factor).

Detection limits calculated from a single analytical batch only provide information pertaining the specific analytical batch (corresponding to repeatability conditions). For regulatory and results comparison purposes, it is generally preferable to estimate detection limits from results accumulated over time and from more than the minimum number of measurements (corresponding to intermediate precision or reproducibility conditions), representing the laboratory's normal operations.

IDL or ASDL may be calculated using Equation 7 (typically rounded up to the next greatest two significant-digit number; units can vary, e.g., µg/kg, ng/kg):

$$IDL\ (or)\ ASDL = 2 \times t_{95} \times s \times \sqrt{1 + \frac{1}{n}} \qquad \text{3.2 Equation 7}$$

where: n = number of blank measurements
(standard blanks for IDL and method blanks for ASDL)

t_{95} = one-sided Student's t at 95% confidence level
(value depends on n; selected values are provided in 3.2 Table 1)

s = standard deviation of blank measurements (3 significant digits)

MBK fortification level between ASDL and ASQL. Gravimetric fortification recommended.

3.2 Table 1. Student's one-sided t-distribution values at 95% Confidence Level

n	Degrees of freedom	$t_{0.95}$
2	1	6.314
3	2	2.920
4	3	2.353
5	4	2.132
6	5	2.015
7	6	1.943
8	7	1.895
9	8	1.860
10	9	1.833
11	10	1.812
12	11	1.796
13	12	1.782
14	13	1.771
15	14	1.761
∞	∞	1.645

LOD is related to ASDL by Equation 8 (typically rounded to two significant figures, expressed in the same units as the reported analytical result).

$$LOD(mg/kg) = ASDL \times \frac{mass_{solution}}{mass_{portion} \times MCF} \times DF \qquad \text{3.2 Equation 8}$$

where: $mass_{solution}$ = mass of analytical solution

$mass_{portion}$ = mass of analytical portion

MCF = mass correction factor
(=1 if no water or other solvent added to aid homogenization)

DF = dilution factor (1 if analytical solution not diluted)

3.2.1.3 Quantitation.

In a general sense, any analytical result expressed as a number is considered quantified but in the context of the EAM, it applies when the associated uncertainty is acceptably low. That is, the inaccuracy (or, bias plus random error) is acceptably small.

All methods in the EAM are considered "quantitative methods" and all analyses are "quantitative analyses". But, only results above quantitation limits would be considered "quantified results". The criteria for quantitation must be defined by the laboratory's customers and can be expressed in different ways. For example, a customer may specify a maximum value for total combined uncertainty or give a minimum reporting threshold. Generally, the quantitation requirement is to use a validated method, which is practical because the validation process accounts for real-world settings with various matrix-related challenges.

- Quantitation limit (LOQ, ASQL) - level at which total combined uncertainty is equal to the defined quantitation specification given in a method or as required by a laboratory's "customer".

Food analysis presents an array of matrix challenges for which total combined uncertainty or method specification may underestimate the precision at low concentrations. The EAM applies a conservative approximation of the quantitation limit based on the same standard deviation used to estimate the ASDL. This approximation is especially useful for routine or high-volume sample analyses such as for the Total Diet Study compliance program.

It is common to report "not detected" when an analyte is below detection, "trace" if between LOD and LOQ, and a numerical value when above LOQ. Numerical results are usually expressed with ± uncertainties at ~95% confidence level (sometimes called "two-sigma" because they are double the value at a 67% confidence level).

An analytical solution quantitation level (ASQL) is typically calculated from the same standard deviation used to estimate the ASDL using Equation 9 then converted to LOQ using Equation 10.

$$ASQL = 30s \qquad \text{3.2 Equation 9}$$

$$LOQ(mg/kg) = ASQL \times \frac{V}{m \times MCF} \times DF \qquad \text{3.2 Equation 10}$$

where: s = standard deviation of method blanks (MBKs)

IUPAC [1], Eurachem [2], and NIST [3] are good sources of information when discussing LOD and LOQ terminology, calculations, and conventions.

3.2 Equation 4 provides a generic, or nominal, estimate. It was initially determined for method 4.4 (Section 3.3.5.1) but is considered applicable to other spectroscopic methods, as well.

A common mistake is to base ASQL solely on signal-to-noise ratio (S/N) and set it equal to ten times the standard deviation of the standard (or reagent) blank measurements (i.e., ASQL=10s). This calculation accounts for only signal measurement and does not capture variance for the entire analysis. While S/N may account for the majority of uncertainty for some methods (e.g., chromatography), this is not usually the case for elemental analysis.

3.2.1.4 Sensitivity.

- Sensitivity - calibration slope. In the EAM, this is referred to as a quantitative perspective. An example statement with a quantitative perspective might be, "The spectrometer's sensitivity will be greatest when it is properly aligned". Sensitivity from ISO 17025 and VIM standpoints are that response slope is meaningful primarily at the solution level (i.e., not so relevant for method sensitivity).

- In contrast, some analysis techniques equate sensitivity with detection limit. In the EAM, this is considered a qualitative perspective. An example statement with a quantitative perspective might be, "Technique X is not sensitive enough to detect lead in food"

- Linear dynamic range (LDR) - linear portion of response curve used for quantification. The LDR lower limit is ASDL. For standard addition, the signal (native level plus additions) must be within the LDR.

3.2.2 SAMPLES AND SAMPLE SOLUTIONS

- "Sample" can be ambiguous in common usage. The EAM uses the following:

- Sample - portion of material selected from a larger quantity of material [4].

- Laboratory sample - sample or subsample sent to or received by the laboratory [4].

- Analytical (or test) sample - sample, prepared from the laboratory sample (by homogenization, grinding, blending, etc.), from which analytical portions are removed for analysis [4].

- Analytical (or test) portion - quantity of material removed from the analytical sample for analysis [4].

- Batch - group of analytical portions and quality control materials processed in a continuous sequence under relatively stable conditions. Specifically:
  1. Method is constant
  2. Instrument and its conditions (i.e., pertinent operating parameters) are constant
  3. Standardization is constant (except for when performing standard addition).

- Analytical (or test) solution - solution that is measured spectrometrically (e.g., digested analytical portion diluted to desired mass).

- Leach solution - solution obtained by leaching a foodware test vessel.

3.2.3 STANDARD SOLUTIONS

- Stock standard solution - solution containing a high-level mass fraction(s) of one (or more) analyte(s) and used to prepare other lower-level standard solutions and other analyte solutions. It may be prepared in the laboratory using assayed high purity materials or purchased from a reputable commercial source.

- Intermediate standard solution - solution containing one or more analytes prepared by diluting an aliquot of stock standard solution. The intermediate standard solution is used for further dilutions to prepare standard solutions and possibly for fortifications of FMBs, FAPs or FASs.

- Standard solution - solution prepared by diluting stock standard or intermediate standard solutions and used for standardization and/or standard additions (sometimes called “working standard solution”).

- Standard blank - zero analyte mass fraction standard solution and used for instrument standardization and to verify absence of analyte carry-over between measurements.

3.2.4 QC/QA MATERIALS AND SOLUTIONS

- Check solution (CS) - solution with analytes at known mass fractions that is analyzed periodically during and at the end of an analytical run. The CS is used to verify the stability of standardization during the analytical run (i.e., to verify instrument drift is in control) and that carry-over did not occur. A standard solution at the mid-level range is typically used for this purpose. Initial calibration verification (ICV) and continuing calibration verification (CCV) solutions are common examples of CSs.

- Fortified analytical portion (FAP) - analytical portion that was fortified (spiked) with analyte before digestion. The FAP is used to determine if the preparation procedure or sample matrix contribute bias to the analytical result.

- Fortified method blank (FMB) - MBK that was fortified (spiked) with analyte(s) before digestion. The FMB is used to determine if the fortification and analysis methodology is in control.

- Fortified analytical solution (FAS) - analytical solution that is fortified (spiked) with analyte(s) before instrumental determination of analyte mass fraction and used to determine the need for further dilution of the analytical solution (to account for matrix effects).

- Independent check solution (ICS) - solution with analytes at known mass fractions prepared in-house or obtained from a source external to the laboratory and different from the source used for instrument standardization. The ICS is used to ensure a valid standardization and to check instrument performance. Use of a commercial source material with a different lot number is acceptable, but a source material from a different manufacturer is preferred.

- Method blank (MBK) - solution obtained by processing an aliquot of water (distilled, deionized, etc., as specified in a method) through all of the method's sample preparation steps (using all reagents, exposing to all laboratory ware, apparatus, equipment, and carrying through the entire analytical procedures) in the same manner as with an analytical portion or test vessel. The MBK is analyzed to ensure analytes have not significantly been added to the analytical solution during the analytical procedures and in the laboratory environment.

- Reference material (RM) - material or substance one or more of whose property values are sufficiently homogeneous, stable, and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to material [5].

- Certified reference material (CRM) - reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. [5]

3.2.5 HISTORY

EAM 3.2 Table 2. History

Version	Revisions Made	Effective Date
1.0	*Analytical Figures of Merit*	June 2008
2.0	3.2 renamed to *Terminology* with a major re-organization, re-write, and expansion; *Figures of Merit* became subsection 3.2.1; *Samples and Solutions* (former 4.0.1.1) brought in and became 3.2.2; *Standard Solutions* (former 4.0.1.2) brought in and became 3.2.4; *Method Performance* (former 4.0.1.3) brought in, became 3.2.4, and was renamed to *QC/QA Materials and Solutions*; converted to PDF for web posting.	September 2014
3.0	Updated; added *History* section.	December 2021

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