Elemental Analysis Manual

for Food and Related Products

The following is a section of the Elemental Analysis Manual for Food and Related Products.

For additional information and to view other sections of the manual, visit the Elemental Analysis Manual for Food and Related Products web page at http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006954.htm.

U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

Elemental Analysis Manual

for Food and Related Products

3.2 Terminology

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Table of Contents

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 if changes in hardware, operating conditions, reagents, personnel, etc.), and/or monitored during routine analyses.

Selected figures of merit are discussed below. Not all of these apply to all methods and this listing is not comprehensive.

Accuracy and trueness - how close results are to true values.

Accuracy applies to individual results whereas trueness applies to groups of results. One would speak of the accuracy of a result as opposed to trueness of a method.

Precision, repeatability and reproducibility - how close results are to each other; obtained via replicate, or repeat measurements.

Precision is a general term but specific in application and requires a descriptor for clarity. For example, pipettor precision and injector flow precision are attributes of analytical equipment whereas method precision characterizes an entire analytical method.

Repeatability and reproducibility are different types of method precision. Repeatability applies to replicate analyses by one person or one method whereas reproducibility applies to replicate analyses but by different analysts, different methods, etc. For example, “Repeatability is better for John Smith than for Mike Jones.” Or, “Reproducibility is excellent for method X even when using five different mass spec models.”

Detection and detection limit (LOD, IDL, ASDL) - lowest level that can be detected

In simple terms, “detection” means discernment. This is a general concept that is widely understood and would not, necessarily, require defining. In analytical chemistry, however, detection limits must be defined because they are based on arbitrarily-chosen criteria and procedures for determining them vary. In the EAM, detection limits are relatively simplistic estimates, based on blanks and without a rigorous metrological treatment. They are calculated according to the statistics of hypothesis testing, with a 95% confidence such that the probabilities of false positives (α) and false negatives (β) are both 0.05.

Three types of detection limits are discussed - method limit of detection (LOD), instrument detection limit (IDL), and analytical solution detection limit (ASDL). The terms LOD and IDL are relevant to all methods, although the exact procedure for calculating them differs. In contrast, ASDL is relevant for only some methods.

LOD is the lowest level that can be detected in a test sample and it accounts for the entire chemical measurement process (i.e., all processing effects such as blank uncertainty, dilutions, separations, chemical yields, etc.). IDL is the lowest level that an instrument's detector can measure and ASDL is the lowest level that can be detected in a test solution obtained after a test portion is digested. Whereas IDL represents an ideal case (e.g., without matrix effect), LOD and ASDL apply to a real-world sample.

Detection limits are based on blanks and relevant to a given method. For example, the LOD for ICP-MS might be derived from method blank (MBK) solutions whereas an XRFS LOD may depend on scattering from a sample substrate and an INAA LOD on polyethylene sample packaging material. Ideally, the blank would be a sample matrix having none of the analyte. Since IDL is a characteristic of a measurement instrument (i.e., not the entire method), it would be based on a blank having no matrix or other method-relevant effects.

Some common detection limit equations are given below. 3.2 Equation 1 is generic and equation 2 relates LOD and ASDL for methods such as ICP-AES where signals are obtained from

digested sample solutions. Standard deviations are assumed to be from at least 5 independently prepared MBKs having (or spiked to have) analyte concentrations between ASDL and ASQL. The blank standard deviation represents a laboratory's normal operation so use of long MBK results accumulated over time and from more than the minimum number of measurements is optimal.

LOD, IDL or ASDL may be calculated as follows and rounded up to the next greatest two significant-digit number (units can vary, e.g., µg/kg, ng/kg):

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

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; see 3.2 Table 1)

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

Note: MBK may be fortified to a level between ASDL and ASQL. This fortification should be accomplished gravimetrically because pipetting imprecision could be great enough to affect the MBK standard deviation.

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

When a test portion is digested and an ASDL is calculated, LOD is related to ASDL.

$$LOD(mg/kg) = ASDL \cdot \frac{V}{m \cdot MCF} \cdot DF$$ 3.2 Equation 2

where: V = volume of analytical solution (L)

m = mass of analytical portion (kg)

MCF = mass correction factor
(1 if water or other solvent not added to aid homogenization)

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

Quantitation and limit of quantitation (LOQ, ASQL) - level at which total combined uncertainty (relative) is equal to the defined quantitation specification. In the EAM, unless noted differently, LOQ is defined using the IUPAC default criteria - the level at which the relative standard deviation is 10% about the true value.

Generally, any analytical result expressed as a number is considered quantified. For chemical measurements, however, criteria for quantitation are set because accuracy can be critically important. High accuracy (low uncertainty) is of low importance if a contaminant is either far above or far below a regulatory action level but very important when a contaminant is close to an action level. All methods in the EAM are therefore "quantitative methods" and all analyses are "quantitative analyses". However, only results above LOQ are considered "quantified results".

The protocol for some analytical programs calls for numerical results to be reported when above LOQ and "trace" to be reported if a result is between LOD and LOQ. As noted above, if numerical results are reported without uncertainties, they are generically accepted to have relative uncertainties ±10% or less (at 67% confidence level). The uncertainty would double to ±20% if expressed at about 95% confidence level. These uncertainty ranges are sometimes called "one-sigma" and "two-sigma", respectively.

For practical reasons, approximations are often used to assign nominal LOQs that apply generically to general classes of measurements. This is especially useful for routine, high-volume sample analyses such as for the Total Diet Study compliance program. For example, Method 4.4 (ICP-AES) involves sample digestion followed by production of an analytical solution which is measured spectrometrically. An analytical solution quantitation level (ASQL) is typically calculated (Equation 3) then converted to LOQ (Equation 4).

$$ASQL = 30 \cdot s$$ 3.2 Equation 3

$$LOQ(mg/kg) = ASQL \cdot \frac{V}{m \cdot MCF} \cdot DF$$ 3.2 Equation 4

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

Notes:

IUPAC1[1], Eurachem[2], and NIST[3] are good sources of information when discussing LOD and LOQ terminology, calculations, and conventions.

Equation 3 provides a generic, or nominal, estimate. It was determined for method 4.4 (Section 3.3.5.1) but may be applicable to other methods, as well. The assumptions were that method blank is below detection and not subtracted. The assumption that the actual blank level is unknown contributed significantly to the overall method uncertainty.

A common mistake is to base ASQL solely on signal-to-noise ratio (S/N) whereby ASQL is set equal to ten times the standard deviation of the blanks (i.e., ASQL=10*s*). This calculation accounts for only signal measurement and does not capture uncertainty for the entire analysis. Most notably, it does not account for blank subtraction and a host of other components. While S/N may account for the majority of uncertainty for some methods, this is not usually the case.

Characteristic mass (m_o) - mass of analyte that produces an integrated absorbance signal of 0.0044 A-sec (or 0.0044 absorbance if peak signal).

Characteristic mass is associated with graphite furnace atomic absorption spectrometry and is a function of instrument design, operating conditions and analyte-matrix-graphite interactions. It is related to the volume and concentration of solution injected into the graphite furnace.

The concentration of analyte in the solution used to calculate m_o should provide an instrument response in the middle of the working range (*i.e.*, approximately 0.050 A-sec). A mean m_o is calculated over time from daily values for a given set of operating conditions to represent an instrument-method combination. Several weeks of data should be used for calculating the m_o. If operating conditions are new and thus no historical mean value exists, then use the m_o given by the instrument manufacturer. Units of m_o are usually pg.

Linear dynamic range (LDR) - linear portion of response curve

For methods having an ASDL, this is the lower limit of the LDR. Knowing the upper limit is especially important when using the method of standard additions for quantification. The combined signal from the native level plus additions must be within the LDR.

Sensitivity - Ratio of instrument response to analyte level (units vary with instrument)

Sensitivity is needed to convert an instrumental reading to analyte level. The term "sensitivity" is often incorrectly used when talking about LOD.

3.2.2 SAMPLES AND SAMPLE SOLUTIONS

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 processed in a continuous sequence under relatively stable conditions. Generally, a batch includes the maximum number of samples and associated quality control materials that can be analyzed efficiently, maintained for sample integrity, and evaluated effectively for quality assurance. Specifically:

1. Method is constant
2. Instrument and its conditions (i.e., pertinent operating parameters) are constant
3. Standardization is constant, except for methods in which standardization is performed for each analytical solution (i.e., method of standard additions).

Analytical (or test) solution - solution prepared by digestion of an analytical portion and diluting to a fixed volume or mass or a solution obtained by leaching a test vessel.

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

3.2.3 STANDARD SOLUTIONS

Stock standard solution - solution containing a high concentration of one or more analytes prepared in the laboratory using assayed high purity materials or purchased from a reputable commercial source. Stock standard solutions are used to prepare standard solutions and other needed analyte solutions.

Intermediate standard solution - solution containing one or more analytes prepared in the laboratory by diluting an aliquot of stock standard solution or purchased from a reputable commercial source. 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 from the dilution of stock standard or intermediate standard solutions. Standard solutions are used to standardize instrument response with respect to analyte concentration.

Standard blank - zero concentration standard solution prepared with the same matrix as standard solutions but without the addition of analyte. Standard blank is used for instrument standardization and may be used to verify absence of analyte carry-over during instrumental measurements.

3.2.4 QC/QA MATERIALS AND SOLUTIONS

Check solution (CS) - solution with analytes at known concentrations 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-concentration range is typically used for this purpose.

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 concentration. The FAS is 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 concentrations 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 made from all method reagents, and exposed to all laboratory ware, apparatus, equipment, and carried through the entire analytical procedure in the same manner as 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 procedure and laboratory environment.

Reference material (RM) - materials closely related to the sample matrix that have a reference value concentration for the analyte of interest.

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