

Establishing Matrix Spike Control Limits from Laboratory Control Samples

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Abstract

A simple method for estimating matrix spike (MS) control and warning limits using laboratory control sample (LCS) recovery data is presented. When the native analyte concentration is large relative to the spiking concentration for the MS, the statistical control limits for the MS are approximately equal to the laboratory's in-house statistical control limits for the LCS (when the recoveries are normally distributed). However, when the native analyte concentration for the MS approaches the spike concentration, simple correction factor may be applied to the acceptance range for MS recoveries (when the relative uncertainty is essentially constant and the ratio of the native analyte to the MS spike concentration is no larger than 0.5). A matrix effect should be suspected when MS recovery falls outside of this range.

Matrix spike (MS) samples are routinely processed with environmental samples to evaluate the impact of matrix effects on overall method performance and the usability of the resulting analytical data. A matrix spike is a “representative” environmental sample that is spiked with target analytes of interest *prior* to being taken through the entire analytical process. The percent recoveries of the target analytes in a matrix spike sample provide a measure of method bias (and provide a measure of overall method precision when analyzed in duplicate).

To calculate an MS recovery, an environmental sample must be thoroughly homogenized and divided into two portions or aliquots (e.g., unless volatile compounds are being analyzed). A known amount of the analyte is “spiked” (i.e., added) to one of the sample aliquots and the aliquot is subsequently analyzed with the test method. The remaining or “unspiked” sample aliquot is directly analyzed. The percent recovery for the MS analysis, R , is defined by the following equation:

$$R = 100 (C_F - C_I) / C_S \quad (1)$$

where C_I is the measured amount of analyte for the unspiked sample aliquot, C_F is measured amount of analyte for the spiked sample aliquot and C_S is the amount of analyte that is spiked. For simplicity, it will be assumed that the variables on the right-hand side of Equation 1 represent analyte concentrations (e.g., for liquid samples, it will be assumed that the volumes of the unspiked and spiked samples are equal so that concentrations can be directly added or subtracted). Therefore, C_I will be referred to as the “initial concentration” and C_F as the “final concentration” for the MS analysis.

Ideally, the MS recovery is 100%. Matrix interferences may give rise to either high or low bias (e.g., R may be significantly greater or less than 100%, respectively). For

example, for chromatographic methods, high bias or “positive interferences” may arise from high concentrations of non-target analytes that coelute with the analytes of interest during the instrumental portion of the analytical procedure where measurements are taken. Substances such as peat and clay may bind the target of interest and prevent complete extraction during the sample preparatory portion of the analytical procedure (especially when the target analytes are present at low concentrations), giving rise to a low bias or “negative interference”. However, poor MS recoveries may also result from inadequate homogenization. For example, sludges, clayey soils, multiphase samples, and samples with macroscopic particles of analytes such as explosives and metals, may defy homogenization attempts (e.g., when the sample is divided for the MS analysis).

Documents such as laboratory SOPs (standard operating procedures) and QAPPs (Quality Assurance Project Plans) often state that acceptance limits for matrix spikes should be established from statistical control ranges generated from MS recovery data. For example, Method 8000B of SW-846 (Update III) states:

It is essential that laboratories calculate in-house performance criteria for matrix spike recoveries . . .

Calculate the average percent recovery (p) and the standard deviation (s) for each of the matrix spike compounds after analysis of 15-20 matrix spike samples of the same matrix . . .

After the analysis of 15-20 matrix spike samples of a particular matrix . . . calculate upper and lower control limit for each matrix spike . . .

$$\begin{aligned} \text{Upper control limit} &= p + 3s \\ \text{Lower control limit} &= p - 3s \end{aligned}$$

Calculate warning limits as

$$\begin{aligned} \text{Upper control limit} &= p + 2s \\ \text{Lower control limit} &= p - 2s \end{aligned}$$

For laboratories employing statistical software to determine these limits, the control limits approximate a 99% confidence interval around the mean recovery, while the warning limits approximate a 95% confidence interval . . .

Not only should the results all be from the same (or very similar) matrix, but the spiking levels should also be approximately the same (within a factor of 2).

Typically, once the MS control limits are established (e.g., as described above), a matrix effect is inferred for a batch of environmental samples when an associated MS recovery falls outside of the statistical (i.e., “three sigma”) control range. However, in order for this approach to be viable, the matrix used to establish the MS control range must be well

defined, similar in composition to the environmental matrix of interest, and known to lack significant interferences.

However, because of the variety and complexity of environmental matrices, establishing MS control limits for each matrix is usually impractical in an environmental production laboratory setting. Most environmental production laboratories that maintain statistical MS control limits establish the limits by analytical method rather than by matrix. For example, when calculating statistical MS control limits, groundwater, surface water, rain water and waste water samples processed using the same analytical method are often considered to be the same sample matrix--“water”. Furthermore, MS control limits are frequently calculated using MS recoveries that have been impacted by matrix effects, which tends to produce wide control ranges. Because MS control ranges are often calculated using spiked samples affected by significant matrix interferences, a MS recovery for a batch of environmental samples that falls within the MS control range does not demonstrate the absence of matrix interference. At best, the result may demonstrate that a matrix effect (if present) is no larger than typically observed for a variety of matrices analyzed by the same test method. These types of problems frequently result in very wide MS control limits that are difficult to interpret and frequently do not satisfy project objectives. When MS acceptance limits are established solely on the basis of a laboratory’s statistical control limits and these limits are developed using MS recoveries from a variety of dissimilar matrices impacted by interferences, the MS acceptance limits will probably be of very limited value.

For routine analyses, it is recommended that laboratory control sample (LCS) data be used to establish acceptance limits for MS recoveries. A LCS assesses the ability of a method to successfully recover the analytes of interest from a homogeneous matrix of known composition and typically consists of a “clean” matrix (e.g., reagent water, Ottawa sand, or some other purified material) that is spiked with known amounts of analytes. For environmental analyses, at least one LCS and one MS are typically processed with a batch of 20 or less samples using the same preparatory and measurement procedures for the environmental samples. The percent recovery, R_{LCS} , for each analyte in the LCS is calculated from the equation:

$$R_{LCS} = (C_m / C_s) 100 \quad (2)$$

where C_m is the measured amount of analyte and C_s is the “known” or “theoretical” analyte spike (e.g., determined from some certified reference standard). For simplicity, it will be assumed that the variables on the right-hand side of Equation 2 are concentrations. For an ideal test method, R_{LCS} is 100%. LCS recoveries are usually evaluated by establishing control and warning limits using “X-bar” or “mean” control charts. The procedure for establishing LCS control limits is similar to the SW-846 procedure for establishing MS control limits.

Note that Equations 1 and 2 indicate that two concentration measurements are required to calculate an MS recovery (C_I and C_F), but only one measurement is required to calculate a LCS recovery (C_m). (Since the LCS is essentially a spiked blank, the unspiked or initial

sample concentration is assumed to be zero and is not measured.) Therefore, in order to establish MS acceptance limits from statistical LCS warning or control limits, the random error associated with the “additional” MS measurement must be taken into account. If one assumes that the relative standard deviation is constant in the quantitative range of the method, a correction factor for the additional measurement uncertainty may be estimated and subsequently applied to the LCS warning or control limits to establish MS acceptance limits. The derivation of the correction factor is presented below.

Assume that MS recoveries are calculated using Equation 1 by “double spiking” a “clean” homogenous matrix (e.g., the same material used to prepare the laboratory control samples). For example, a known amount of analyte is added to a method blank to obtain the concentration, C_I , where C_I represents the native analyte concentration for an actual environmental sample. A spike, C_S , is then added to obtain the final concentration C_F . If the initial and final concentrations C_F and C_I are assumed to be statistically independent variables, then it follows from error propagation that the standard deviation for the MS percent recovery is^{1, 2}:

$$\sigma(R) = (100/C_S) \sigma(C_F - C_I) = (100/C_S) \sqrt{\sigma^2(C_F) + \sigma^2(C_I)}$$

where the symbol, $\sigma(Y)$, denotes the standard deviation of the variable Y . If it is assumed that the relative standard deviation for the sample concentration is constant (i.e., is a linear function of concentration) within the quantitative range of the method, then³

$$\sigma(C_F)/C_F = \sigma(C_I)/C_I = \sigma(C_m)/C_m$$

Using the above equations, the equation for $\sigma(R)$ may be written as:

$$\begin{aligned} \sigma(R) &= (100/C_S) \sqrt{C_F^2 [\sigma^2(C_F)/C_F^2] + C_I^2 [\sigma^2(C_I)/C_I^2]} \\ &= (100/C_S) [\sigma(C_m)/C_m] \sqrt{C_F^2 + C_I^2} \end{aligned} \quad (3)$$

It follows from Equation 2 that:

$$C_m = R_{LCS} (C_s / 100)$$

and

$$\sigma(C_m) = \sigma(R_{LCS}) (C_s / 100)$$

Substituting the two equations above into Equation (3) gives the result:

$$\sigma(R) = \sigma(R_{LCS}) (100/R_{LCS}) \left[\sqrt{C_F^2 + C_I^2} / C_S \right] \quad (4)$$

Note that $\sigma(R) \geq \sigma(R_{LCS})$ because the last two terms on the right-hand side of Equation 4 are greater than or equal to one. This occurs because one measurement is required to calculate a LCS recovery but two are required to calculate an MS recovery; the last two terms (i.e., factors) of Equation 4 represent the additional uncertainty associated with the second MS measurement.

The relative standard deviation $\sigma(R)/R_{LCS}$ in Equation 4 may be approximated by s_{LCS}/\bar{R}_{LCS} , where s_{LCS} and \bar{R}_{LCS} are standard deviation and the mean recovery, respectively, for the set of LCS recoveries used to generate the laboratory's LCS warning and control limits. Furthermore, because the LCS and MS recoveries are determined using the same matrix and test method, the mean LCS recovery can be used to estimate the mean MS recovery. Therefore, assuming that the LCS recoveries are approximately normally distributed and a sufficient number of recoveries are available (e.g., at least 20 recoveries), it follows from Equation 4 that control and warning limits for the MS recoveries can be estimated from the acceptance limits for the LCS recoveries as follows:

$$\bar{R}_{LCS} \pm t s_{LCS} (100/\bar{R}_{LCS}) \left[\sqrt{C_F^2 + C_I^2} / C_S \right] \quad (5)$$

The symbol t is the critical value for Student t : $t \approx 2$ for the warning limits and $t \approx 3$ for the control limits.

If method bias is small and the spiking concentration is large relative to the native analyte concentration, Equation 5 may be simplified. If method bias is not significant, that is, if

$$|100 - \bar{R}_{LCS}| / (s_{LCS} / \sqrt{n}) \leq t,$$

where n is the number of recoveries used to calculate the mean LCS recovery, then $(100/\bar{R}_{LCS}) \approx 1$. Furthermore, the initial and final spiking concentrations C_I and C_F may be written as:

$$C_I = k C_S \quad (6A)$$

$$C_F \approx k C_S + C_S \quad (6B)$$

where k is defined as the ratio of the initial concentration to the MS spiking concentration:

$$k = C_I / C_S$$

The substitution of Equations 6A and 6B into Equation 5 gives the following result:

$$\bar{R}_{LCS} \pm t s_{LCS} \sqrt{(1+k)^2 + k^2} \quad (7)$$

If the initial or native analyte concentration is small relative to the MS spike concentration, that is, if $k \ll 1$, then the last term on the right-hand side of the equation above is approximately equal to $1 + k$. This first-order approximation will be fairly accurate when the spiking concentration is at least twice as high as the native analyte concentration (when $k \leq 0.5$ the error is less than about 5%). Therefore, the confidence limits for the matrix spike recoveries are approximately the following:

$$\bar{R}_{LCS} \pm t s_{LCS} (1+k) \quad (8)$$

Note that, according to Equation 8, the MS acceptance limits approach the LCS acceptance limits as the MS spiking concentration increases (i.e., as k decreases). In other words, if the native analyte concentration for an environmental sample is very small, then the MS recovery is essentially the recovery for a spiked blank (i.e., a LCS). Equation 8 should not be used when the MS spiking concentration is close to the native analyte concentration (e.g., when $k \leq 0.5$) or when the statistical control or warning range for the LCS is wide (e.g., when the percent relative standard deviation of the LCS is greater than about 20%) as non-normal distributions often result in wide ranges (e.g., and can result in negative recoveries for the lower control limit when normality is erroneously assumed).

To illustrate the use of Equation 8, assume that the LCS control range is $100\% \pm 20\%$ (i.e., 80% - 120%) and that the MS spiking concentration is twice as great as the native analyte concentration. The control limits for the MS recoveries estimated from Equation 7 are as follows:

$$100\% \pm 20\% (1 + \frac{1}{2}) = 100\% \pm 30\% = 70\% - 130\%$$

Therefore, a matrix effect would be suspected if the LCS recovery for a batch of environmental samples were to fall within 80% - 120% but the recovery for the associated MS sample were to fall outside of 70% - 130%. The statistical acceptance range for MS recoveries may be set equal to the LCS warning or control range when the MS spiking concentration is much greater than the native analyte concentration (e.g., five or ten times greater).

However, it should be noted that MS samples can be analyzed because for two different objectives: (1) To determine whether or not matrix effects exist and (2) to determine whether or not project-specific objectives for bias (and, frequently, precision) were satisfied for the analytes in the matrices of interest. The distinction between the two objectives is somewhat subtle but is important to recognize when evaluating MS recoveries. To illustrate, assume that a laboratory's statistical control range for LCS recoveries for aqueous pesticide analyses is 70% - 130%, the project-required acceptance range for MS recoveries is 50% - 150%, and three separate sets of samples were analyzed with the associated matrix spike recoveries of 90%, 65%, and 40%. Assume that the spiking concentrations for all the samples are high relative to the native analyte concentration and quality control is otherwise acceptable. Since the 90% MS recovery

lies within the statistical LCS acceptance limits, this MS recovery suggests the absence of any matrix effects. The MS recovery of 65%, which falls well outside of the LCS statistical acceptance range, is indicative of a matrix effect that is within the project-required error tolerance for accuracy and bias (50% - 150%). Although the recovery is indicative of matrix interference, corrective actions (e.g., cleanup of sample extracts) would not normally be required. The recovery of 40% is indicative of a matrix effect that is greater than the project's error tolerance. At a minimum, data qualification would typically be required.

Conclusions:

A simple strategy for estimating statistical acceptance limits for matrix spikes using LCS warning and control limits is proposed. The estimate will be appropriate within the quantitative range of the analytical method when the LCS recoveries are normally distributed and the relative standard deviation is not strongly dependent upon analyte concentration (i.e., is approximately constant). For routine analyses performed by environmental production laboratories, it is believed that statistical confidence limits estimated for LCS recoveries will usually be more appropriate than those calculated from MS recoveries. The use of dissimilar matrices and matrices that have been affected by interferences to calculate statistical acceptance limits for matrix spikes tend to give rise to statistical limits that are not representative of method performance. The use of LCS recoveries to establish statistical acceptance limits for MS recoveries is a more viable strategy.

REFERENCES

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