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Standard Guide for Spiking Organics into Aqueous Samples [1]

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1. Scope

1.1 This guide covers the general technique of "spiking" aqueous samples with organic analytes or components. It is intended to be applicable to a broad range of organic materials in aqueous media. Although the specific details and handling procedures required for all types of compounds are not described, this general approach is given to serve as a guideline to the analyst in accurately preparing spiked samples for subsequent analysis or comparison. Guidance is also provided to aid the analyst in calculating recoveries and interpreting results. It is the responsibility of the analyst to determine whether the methods and materials cited here are compatible with the analytes of interest.

1.2 The procedures in this guide are focused on "matrix spike" preparation, analysis, results, and interpretation. The applicability of these procedures to the preparation of calibration standards, calibration check standards, laboratory control standards, reference materials, and other quality control materials by spiking is incidental. A sample (the matrix) is fortified (spiked) with the analyte of interest for a variety of analytical and quality control purposes. While the spiking of multiple sample test portions is discussed, the method of standard additions is not covered.

1.3 This guide is intended for use in conjunction with the individual analytical test method that provides procedures for analysis of the analyte or component of interest. The test method is used to determine an analyte or component's background level and, again after spiking, its now elevated level. Each test method typically provides procedures not only for samples, but also for calibration standards or analytical control solutions, or both. These procedures include preparation, handling, storage, preservation, and analysis techniques. These procedures are applicable by extension, using the analyst's judgement on a case-by-case basis, to spiking solutions, and are not reiterated in this guide. See also Practice E 200 for preparation and storage information.

1.4 These procedures apply only to analytes that are soluble in water at the concentration of the spike plus any background material, or to analytes soluble in a solvent that is itself water-soluble. The system used in the later case must result in a homogeneous solution of analyte and sample. Meaningful recovery data cannot be obtained if an aqueous solution or homogeneous suspension of the analyte of interest in the sample cannot be attained.

1.5 Matrix spiking may be performed in the field or in the laboratory, depending on which part of the analytical process is to be tested. Field spiking tests the recovery of the overall process, including preservation and shipping of the sample. Laboratory spiking tests the laboratory process only. Spiking of sample extracts, concentrates, or dilutions will test only that portion of the process subsequent to the addition of the spike.

1.6 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]

D 1193 Specification for Reagent Water[2]

D 3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents[3]

D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water[2]

D 4375 Practice for Basic Statistics in Committee D19 on Water[2]

E 200 Practice for Preparation, Standardization, and Storage of Standard and Reagent Solutions for Chemical Analysis[2]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *matrix spike, n*—the quantity (mass) of a component (analyte) of interest which is added to a sample (matrix) in

[1] This guide is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.02.

order to test bias as measured by recovery (of that component under specific analytical conditions) and reported as percent recovery (*P*).

3.2.2 *spike, v*—the addition of a known amount of an analyte of known identity to a measured volume of a sample (from a specific matrix) to determine the efficiency with which the added analyte can be ″recovered" from (measured in) that matrix by the analytical system after exposure to a specific portion of an analytical process. Matrix spiking is a process for accomplishing this. The precision and bias estimates from several trials under specific analytical conditions represent the measurement efficiency with which the analyte may be determined under these conditions.

3.2.3 *spiking solution*—the solution in which one or more spikes are dissolved (along with any necessary preservatives). This solution acts as a carrier to provide ease of measurement and more rapid and thorough mixing of the spike into the sample, as compared to adding the spike as a pure compound.

4. Summary of Guide

4.1 This guide describes a technique for the addition of a known amount of an organic analyte to an aqueous sample. Instructions are given to help prevent loss of volatile analytes in the sample headspace and to provide a homogeneous solution for subsequent analysis. Appropriate concentrations of the spike relative to the original concentration in the sample are discussed. Applications of the technique and aids in the interpretation of results obtained are described.

5. Significance and Use

5.1 Matrix spiking of samples is commonly used to determine the bias under specific analytical conditions, or the applicability of a test method to a particular sample matrix, by determining the extent to which the added spike is recovered from the sample matrix under these conditions. Reactions or interactions of the analyte or component of interest with the sample matrix may cause a significant positive or negative effect on recovery and may render the chosen analytical, or monitoring, process ineffectual for that sample matrix.

5.2 Matrix spiking of samples can also be used to monitor the performance of a laboratory, individual instrument, or analyst as part of a regular quality assurance program. Changes in spike recoveries from the same or similar matrices over time may indicate variations in the quality of analyses and analytical results.

5.3 Spiking of samples may be performed in the field or in the laboratory, depending on what part of the analytical process is to be tested. Field spiking tests the recovery of the overall process, including preservation and shipping of the sample and may be considered a measure of the stability of the analytes in the matrix. Laboratory spiking tests the laboratory process only. Spiking of sample extracts, concentrates, or dilutions will be reflective of only that portion of the process subsequent to the addition of the spike.

5.4 Special precautions shall be observed when nonlaboratory personnel perform spiking in the field. It is recommended that all spike preparation work be performed in a laboratory by experienced analysts so that the field operation consists solely of adding a prepared spiking solution to the sample matrix. Training of field personnel and validation of their spiking techniques are necessary to ensure that spikes are added accurately and reproducibly. Consistent and acceptable recoveries from duplicate field spikes can be used to document the reproducibility of sampling and the spiking technique. When environmentally labile compounds are used as spikes, the spiking solution shall be protected up to the time of use by appropriate means such as chilling, protection from sunlight and oxygen, or chemical preservation.

NOTE 1—Any field spiked sample, if known to the laboratory, should be labeled as a field spike in the final results report. Also, whenever possible, field spiking of volatile compounds should be avoided.

5.5 It is often tacitly assumed that the analyte component is recovered from the sample to approximately the same extent that a spike of the same analyte is recovered from a spiked sample. One reason that this assumption may be incorrect is that the spike may not be bound up in the sample (for example, with suspended matter) in the same way that the naturally occurring analyte is bound in the sample. The spike may therefore be recovered from the sample differently than the background level of the analyte. For this reason, as well as the fact that bias corrections can add variability, it is not good practice to correct analytical data using spike recoveries. Spike recovery information should, however, be reported along with the related sample analysis results.

5.6 This guide is also applicable to the preparation and use of spikes for quantification by the method of standard additions and to the addition of surrogates and internal standards.

6. Apparatus

6.1 *Stirring Apparatus*—Borosilicate glass beads, 4 to 6 mm in diameter, or small TFE-coated magnetic stirring bars. A small non-heating variable-speed magnetic stirrer is recommended for use with the stirring bar.

6.2 *Microsyringes*—Standard gas chromatographic microsyringes of borosilicate glass with stainless steel needles, suitable for injection of spiking solutions through a TFE-coated silicone septum. The TFE-tipped plungers may be contaminated by certain analytes. If this is determined to be likely, a syringe may be dedicated to a single process, or a plain-tipped stainless steel plunger may be used to avoid cross-contamination. Sizes from 10 to 500 µL are appropriate, depending on the concentration and sample volumes used.

6.3 *Micropipettors*—Stainless steel micropipettors with disposable glass tips are preferable to syringes for introduction of spiking solutions into open sample containers, since they deliver more reproducibly and are less prone to cross-contamination. Sizes from 5 to 200 µL are appropriate.

6.4 *Syringes*—Borosilicate glass syringes with demountable stainless steel needles may be used to measure volumes of samples (spiked or unspiked) to be injected into purge-and-trap sample introduction systems.

6.5 *Volumetric Transfer Pipets*—Class A, used to deliver known volumes of sample and to add larger volumes of spiking solutions.

6.6 *Volumetric Flasks*—Class A volumetric flasks may be used to measure known volumes of sample.

6.7 *Balance*—An analytical (0.1-mg), semimicro (0.01-mg), or micro (0.001-mg) balance.

7. Reagents

7.1 *Purity of Reagents*—At a minimum, reagent grade chemicals shall be used in all spike preparations. Spectrograde, high-pressure liquid chromatography (HPLC) grade, pesticide grade, or ultrapure grade solvents shall be used to prepare spiking solutions. Reagents of the highest available purity shall be used for spike analytes and demonstrated to be free of interfering substances for the subsequent test methods to be performed. If possible, a primary standard grade shall be used. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[4] Other grades may be used, provided (*1*) that reagent purity is unspecified and (*2*) that it is first ascertained that the reagent is of sufficiently high purity to permit its use without adversely affecting the bias and precision of subsequent determinations. Purchased spiking solutions shall be demonstrated to be free of substances that would interfere with subsequent analyses being performed, and the supplier's stated concentration shall be verified by analysis prior to use. Compensatory errors associated with self-referencing should be prevented by using spiking solutions of a standard originating from a source, when available, different from that of the routine method calibration standards.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by the individual test method to be used to analyze a sample after spiking. If more than one test method is to be utilized, the minimum criteria of each test method must be met. If test method reagent water specifications are not available, references to water shall be understood to mean reagent water conforming to Type I of Specification D 1193 and demonstrated to be free of interfering substances for the test(s) being performed.

7.3 *Methanol*—Spectrograde, HPLC grade, or ultrapure grade methanol is preferable for use as a solvent for water-insoluble analytes in most trace-level analyses. Other water-soluble solvents may be useful for certain analytes. Solvents shall be checked before use for interfering substances by analysis.

7.4 *Spiking Solutions*—Spiking solutions of each analyte of interest are prepared individually or in combination, either gravimetrically or volumetrically, correcting for density (for liquid or solution standards). The preservation and storage criteria found in the applicable analytical test method for its calibration or check standards apply likewise to spiking solutions. The stability of a stored spiking solution shall be verified routinely by the appropriate dilution of a portion of spiking solution to the laboratory's analyte concentration of interest. Stability is demonstrated whenever the analyzed concentration of a diluted spiking solution falls within the control limits for a routine laboratory control sample of the same concentration. Where solubilities permit, stock spiking solutions are customarily prepared 25 to 1000 times as concentrated as the working spiking solution, and are diluted volumetrically to produce the working spiking solution at the time of use. In some cases, concentrated solutions may be stable at 4°C for substantially longer periods than dilute solutions. Alternatively, prepare spike or spiking solution fresh for each batch of samples.

8. Sampling

8.1 Although sampling methodology is beyond the scope of this guide, a properly split or duplicate sample is of utmost importance to the successful measurement of spike recovery. This is especially critical in samples containing suspended sediment or volatile analytes.

8.2 Sample containers shall be selected and prepared, and samples shall be preserved in accordance with Practice D 3694.

9. Procedure

9.1 Use relevant good laboratory practices in accordance with Guide D 3856 and Practice E 200.

9.2 *Nonvolatile Compounds*—Except for volatile analytes, this category includes all analytes or components of interest. Semi-volatile compounds, for which volatility is not a concern for these spiking procedures, are included in this classification.

9.2.1 Analyze one portion of the sample for the analyte(s) of interest. Duplicate analyses are recommended to determine the overall precision of the sample splitting and analysis process. If this is not possible, estimate the concentrations of analytes of interest, based upon knowledge of the sample source.

9.2.2 Use the result of this analysis or estimation to determine the appropriate amount of spike to be added to the sample.

9.2.2.1 To be of maximum value for quantification of the analyte(s) or for the evaluation of method accuracy, the concentration in the spiked sample should be at least double, but ideally not over five times, the concentration of the analyte in the unspiked sample, as long as the total analyte concentration can be brought within the test method's dynamic range. Spike concentrations below this range lead to highly variable spike recoveries, as described in Section 11. Higher spike concentrations may mask the effect that real interferences, such as matrix effects, are having on the analyte at its background levels, leading to over-optimistic estimates of analyte recovery.

9.2.2.2 If the spiked analyte is not necessarily present in the sample, but is added only to validate the general recovery of an analytical method or technique, then adjust the concentration after spiking to two to five times the "action level," the analyte concentration of primary interest to the data user, for example, the detection limit or the regulatory limit for an environmental sample, or at a critical set point or process optimization point for a process sample. Otherwise, adjust the spike to two to five times the anticipated concentration of the samples, or to two to five times the detection level, if the analyte is not present.

9.2.3 Determine the volume of the sample test portion to be spiked, as prescribed by the analytical test method to be used or for convenience of preparation.

[4] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

9.2.4 Determine the volume of the aliquot of spiking solution that will be added to each sample test portion. Never let the volume of spiking solution exceed 2 % of the total volume of the sample. The maximum recommended spiking solution volume is much lower, in the range from 0.01 to 0.1 % of the sample volume, so that the sample matrix is not appreciably altered, for example, through matrix solubilizing by the spiking solution carrier solvent. An exact volume is based upon the volume marking on the available spiking pipet or on the micropipettor to be used to spike an aliquot into the sample test portion. Also, the carrier solvent must not interfere in the test method.

9.2.5 Determine the appropriate concentration of the spiking solution. Pertinent factors in determining the appropriate concentration of the spiking solution include the following:

9.2.5.1 The desired final concentration of the spike in the sample as determined in 9.2.2;

9.2.5.2 The sample test portion volume as determined in 9.2.3;

9.2.5.3 The volume of the spiking solution aliquot to be added to each sample test portion as determined in 9.2.4;

9.2.5.4 The working calibration range of the test method for the analyte of interest (the total of the analyte already present in the sample and the spiked amount shall fall within this range to obtain a useful result);

9.2.5.5 The solubility of the analyte of interest in the solvent (water or a water-soluble carrier) of the spiking solution; and

9.2.5.6 The volume markings on the available pipets or micropipettors to be used for preparation of the spiking solution.

9.2.6 Prepare a stock spiking solution of suitable concentration using the appropriate solvent as described in 7.4.

9.2.7 Using a micropipettor, add the desired volume of spiking solution to the sample in a volumetric flask. Cap the sample and mix well.

9.2.8 Examine the spiked sample for any increased turbidity. If turbidity persists after extensive mixing, it may be necessary to respike a new portion of sample using a lower concentration of analyte, a smaller volume of more concentrated spiking solution, or a new spiking solution prepared in a more miscible solvent.

9.3 *Volatile Analytes*:

9.3.1 Analyze one portion of the sample for the analyte(s) of interest. Duplicate analyses are recommended to determine the overall precision of the sample splitting (see Section 6) and analysis process. If this is not possible, estimate the concentration of the analytes of interest, based on knowledge of the sample source.

9.3.2 Use the result of this analysis or estimation to determine the appropriate amount of spike to be added to the sample.

9.3.2.1 To be of maximum value for quantification of the analyte(s) or for the evaluation of method accuracy, the concentration in the spiked sample should be at least double, but ideally not over five times, the concentration of the analyte in the unspiked sample, as long as the total analyte concentration can be brought within the test method's dynamic range. Spike concentrations below this range lead to highly variable spike recoveries, as described in Section 11. Higher spike concentrations may mask the effect that real interferences, such as matrix effects, are having on the analyte at its background levels, leading to over-optimistic estimates of analyte recovery.

9.3.2.2 If the spiked analyte is not necessarily present in the sample, but is added only to validate the general recovery of an analytical method or technique, then adjust the concentration after spiking to two to five times the "action level," the analyte concentration of primary interest to the data user, for example, the detection limit or the regulatory limit for an environmental sample, or at a critical set point or process optimization point for a process sample. Otherwise, adjust the spike to two to five times the anticipated concentration of the samples, or to two to five times the detection level, if the analyte is not present.

9.3.3 The volume of the sample is determined in one of two ways: in the septum-capped vial, or in the syringe used to inject the sample into a purge-and-trap sample-introduction system. Because of the need to maintain zero headspace, the volume of sample to be spiked shall be the difference between the volume of spiking solution required and the volume of the septum-capped vial or syringe to be used during the analysis.

9.3.3.1 If the spiked sample is to be prepared in the septum-capped vial, the exact volume of the vial must be determined before the spiking procedure is performed. Tare weigh the clean, dry septum-capped vial containing the glass beads or stirring magnet to be used for mixing. Fill the vial to overflowing with water. Place the septum carefully atop the vial so as to allow no headspace. Screw the cap down firmly and dry the outside of the vial. Weigh the vial and its contents with the septum-sealed cap in place, to an accuracy of 0.1 % of the contents of the vial; for example, a 40 mL vial is weighed to 0.04 g. Compute the volume of the vial. Dry the vial, cap, and glass beads or stirring magnet before filling with sample by rinsing with methanol and drying in an oven at 105°C for 1 h.

9.3.4 Determine the volume of the aliquot of spiking solution that will be added to each sample test portion. Never let the volume of spiking solution exceed 2 % of the total volume of the sample. The maximum recommended spiking solution volume is much lower, in the range of 0.01 to 0.1 % of the sample volume, so that the sample matrix is not appreciably altered, for example, through matrix solubilizing by the spiking solution carrier solvent. An exact volume is based upon the volume marking on the available spiking pipettor, micropipettor, or syringe to be used to spike an aliquot into the sample test portion. Also, the carrier solvent must not interfere in the test method. For example, 1 to 2 µL of methanol, a common spiking solution solvent for purge and trap volatile organic analytes, in the standard 5-mL sample test portion used will cause false negatives for some ion-trap systems in the area where methanol elutes. The spiking solution volume should be less than 0.02 % of the sample volume in this case.

9.3.5 Determine the appropriate concentration of the spiking solution. Pertinent factors in determining the appropriate concentration of the spiking solution include the following:

9.3.5.1 The desired final concentration of the spike in the sample as determined in 9.3.2;

9.3.5.2 The sample test portion volume as determined in 9.3.3;

9.3.5.3 The volume of the spiking solution aliquot to be added to each sample test portion as determined in 9.3.4;

9.3.5.4 The working calibration range of the test method for the analyte of interest (the total of the analyte already present in the sample and the spiked amount must fall within this range to obtain a useful result);

9.3.5.5 The solubility of the analyte of interest in the solvent (water or a water-soluble carrier) of the spiking solution; and

9.3.5.6 The volume markings on the available micropipettors or syringes to be used for preparation of the spiking solution.

9.3.6 Prepare a spiking solution of suitable concentration using the appropriate solvent as described in 7.4.

9.3.7 Add the desired volume of spiking solution to the sample using a syringe or micropipettor.

9.3.7.1 Remove the septum cap and inject the spiking solution beneath the liquid surface using a micropipettor. Replace the septum and cap immediately to avoid loss or contamination. Mix thoroughly.

9.3.7.2 In the case of small spike volumes (<1 % of sample volume), use a syringe to inject the spike through the septum without removing the cap. This method may be preferable for highly volatile analytes because it reduces the possibility of loss of spike analytes or contamination of the sample by analytes in the atmosphere. However, once the septum is pierced, the integrity of the seal may be compromised.

9.3.7.3 Alternatively, pour the desired volume of the sample into the large glass syringe used to inject the sample into a purge-and-trap apparatus, and the spiking solution added to it. This procedure avoids the necessity of measuring the volume of the septum-capped vial, but results in a somewhat less accurate measure of sample volume.

9.3.8 Examine the spiked sample for any increased turbidity. If turbidity persists after extensive mixing, it may be necessary to spike a new portion of sample using a lower concentration of analyte, a smaller volume of more concentrated spiking solution, or a new spiking solution prepared in a more miscible solvent.

NOTE 2—Although the preceding procedures are designed for use within a laboratory, adaptations to field spiking are readily apparent.

9.4 Using the chosen test method for analysis of the analyte of interest, analyze spiked and unspiked test portions of sample to obtain the desired number or degrees of freedom (see Sections 10 and 11).

10. Calculation

10.1 In the following discussion, units of measure are not given but shall be consistent. That is, the user shall determine the appropriate concentration units on a case-by-case basis, for example, percent (weight per volume), milligram per litre, or microgram per litre. Once chosen, the units of measure shall remain consistent throughout these calculations. For example, if microgram per litre is selected as the concentration units then microgram per litre shall be used wherever concentration is indicated, litre shall be used wherever volume is indicated, and microgram shall be used wherever mass is indicated.

10.2 An estimate of the volume of the spiking solution, V, to be added to the sample may be calculated as follows:

$$V = \frac{F \times B \times V_s}{C} \quad (1)$$

where:

F = desired ratio of the mass of the analyte added in the spike to the background mass of the analyte in the unspiked sample. The value of F should lie between one and four; see 9.3.2.1. If B is at or below the limit of detectability for subsequent testing, F should equal four and B set at the limit of detectability; see 9.3.2.2,

B = measured background concentration of analyte (or component of interest) in unspiked sample (in volume, V_s),

V_s = volume of sample test portion to which spike is added (with background concentration, B), and

C = concentration of analyte (or component of interest) in spiking solution (in volume, V), known by weights and measures from preparation.

It may be observed from Eq 1 that if V becomes large relative to $V + V_s$ then the effect (for example, matrix effect) of the spiking solution may overwhelm the experiment. In this case, either increase C (but not beyond the limits of solubility) or choose an alternative sample with a smaller background concentration, B, for testing.

10.3 The percent recovery, P, of the spike is always expressed as a percentage and, in general, is calculated from the ratio of the measured amount (mass), M, of the matrix spike found through analysis in the spiked sample to the theoretical amount (mass), T, of the matrix spike calculated by weights and measures during preparation of the spiking solution. This can be expressed as follows:

$$P = 100\,\frac{M}{T} \quad (2)$$

As a practical matter, an analyst may wish to use concentration determinations to calculate P. Readily determined concentrations and volumes (or masses) may be substituted as shown in the following paragraphs. Note that dilution of the sample by the spiking solution and compensation for background levels of the analyte in the sample are considered.

10.3.1 Assuming that V_s and V are additive (that the final volume of the spiked sample is $V_s + V$), then for each analyte, $A\ (V_s + V) - (B \times V_s)$ is substituted for M and $C \times V$ is substituted for T. The percentage recovery, P is then calculated as follows:

$$P = \frac{100\ [A(V_s + V) - (B \times V_s)]}{C \times V} \quad (3)$$

where A is the concentration determined by analysis of the analyte in the spiked sample.

10.3.2 Where V_s and V are not additive, for example, when the spiking solution solute is methanol, then instead of $A\ (V_s + V)$ use the mass, M_s, of the analyte determined by analysis of the spiked sample in the following equation:

$$P = \frac{100\ [M_s - (B \times V_s)]}{C \times V} \quad (4)$$

10.4 Since both *A* and *B* are determined experimentally, the acceptable recovery for any spike is a function of the combined error in determining both *A* and *B* and the relative standard deviation of the method at those concentrations. The combined error, *CE*, is determined using the following formula:

$$CE = \sqrt{(A \times RSD)^2 + (B \times RSD)^2} \quad (5)$$

where:

CE = combined error in the same concentration units as *A* and *B*, and

RSD = relative overall standard deviation of the test at the concentrations found, expressed as a ratio in accordance with Terminology D 4375.

10.5 *Initial Approach to Assessing P*—When the percent spike recovery, P_i, falls within the generic limits, as follows:

$$\left(\bar{P}_A - \frac{3 \times 100 \times CE \times (V_s + V)}{C \times V}\right) \leq P_i \leq \left(\bar{P}_A + \frac{3 \times 100 \times CE \times (V_s + V)}{C \times V}\right) \quad (6)$$

where $\bar{P}_A$ is the mean percent recovery expected at concentration *A*, then the recovery of the spike is "in control" and there is no evidence of a significant matrix effect. See *ASTM MNL 7*[5] for an explanation of "in control" and how the variability in values of *P* within these generic limits may be attributable to chance.

10.6 *Control Charting Approach to Assessing P*—An analyst or laboratory may accumulate percent recovery values, $P_{i,i = 1 \text{ to } n}$, and, after accumulating at least 8, but preferably 15 (or more) P_i values, calculate the mean of the percent recovery, *P* for the number of results available, *n*, and the standard deviation of the P_i, or s_P value, using $n-1$ df for the sample of percent recoveries included in these calculations. Based on Terminology D 4375, these calculations are as follows:

$$\bar{P} = \sum_{i=1}^{n} \frac{P_i}{n} \quad (7)$$

$$s_P = \sqrt{\sum_{i=1}^{n} \frac{(P_i - \bar{P})^2}{n-1}} \quad (8)$$

The recommended control limits are $\bar{P}$ plus and minus three times the $s_P$value, and this can be expressed as $\bar{P} \pm 3\, s_P$. These are control limits for individual future percent recovery values, *P*. For control charting of *P* and for alternative statistical procedures for calculating control limits, see *ASTM MNL 7*.[5]

11. Interpretation of Results

11.1 Spike recovery is dependent on the test method used for analysis, sample matrix, and concentration of the component of interest in the spiked and unspiked sample.[6] Table 1 illustrates the effect of various spike-to-background ratios on the 95 % tolerance interval when the mean recovery is 100 % and the relative standard deviation of each analytical measurement is 10 %. It is clear that the variability of the percent recovery is not identical to that of the analytical measurement, except when the spike-to-background mass ratio is very large.

TABLE 1 Effect of Spike-to-Background Ratio on Variability of Percent Recovery

Spike-to-Background Ratio [A]	95 % Tolerance Interval in Expected % Recovery [B]
100	80 to 120
50	80 to 120
10	78 to 122
5	76 to 124
1	55 to 145
0.5	28 to 170
0.1	−200 to 400
0.05	−480 to 680

[A] This is *F* as defined in 10.2.

[B] Based on a relative standard deviation of 10 % in each individual determination, *assuming 100 % recovery.* Statistical results, including negative recovery values are included for reference and are not intended to suggest that actual negative recovery values are expected.

11.2 Results outside of three standard deviations about the mean of historical percent recoveries should be investigated. First consider matrix effects. Bias will typically be in the same direction for a given matrix each time that matrix is encountered and will appear only for the problem matrix or matrices. To obtain a valid result for the sample and to confirm the matrix interference, the analyst should choose another test method or invoke other procedures for resolution, for example, the method of standard additions or the method of standard dilutions.

11.3 When compared to existing control limits (see 10.6), percent recoveries that show a trend, or that are often outside $\bar{P} \pm 3\, s_P$ for different matrices, may indicate a bias in the measurement system. Typically, the analytical instrument (if there is one involved) or the analyst is causative. Investigation should proceed accordingly. Other sources of out-of-control-limit results are possible, for example, a difficult matrix or an interference. If, as recommended in this guide, the spike originates from a source different from the calibration standards used for the test method, out-of-specification standards could explain the observed out-of-control-limit results and should be investigated further.

11.4 If possible, the standard deviation of the percent recovery, s_P, determined should be compared with other analysts, instrument systems, and laboratories running the same or similar matrices. If the s_P value is significantly larger than those of other analysts, instruments, or laboratories, the measurement system should be investigated for the cause. However, note that s_P can be quite variable when based on small numbers of measurements, *n*. To overcome this limitation, it is suggested that a history of results, $P_{i,i = 1 \text{ to } n}$, be accumulated (see 10.6) for both analysts, for both instruments, or in both laboratories, until *n* is large for each alternative.

[5] *ASTM Manual on Presentation of Data and Control Chart Analysis, ASTM MNL 7,* Sixth Edition, ASTM, 1990.

[6] Provost, L. P., and Elder, R. S., "Interpretation of Percent Recovery Data," *American Laboratory*, Vol 15, No. 12, December 1983, pp. 57–63.

Then the two sets of accumulated results are compared before conclusions are drawn.

11.5 If all or most s_P values exceed, for example, 7 % (chosen according to the data quality objectives, 7 % is a reasonable objective for most quantitative water test methods); that is, if the $\bar{P} \pm 3\ s_P$ is outside the range of 80 to 120 %, the test method variability is considered excessive for the analytes and matrices tested, and corrective action should be taken, for example, to retrain analysts, repair or replace the instrument, or find a better test method. See the applicable test method for exceptions and alternative guidance for individual analytes. Note that the suggested 80 to 120 % range may be too narrow if, for example, background analyte levels are high (see 11.1) or when working at the extreme lower levels of test method sensitivities, for example, below five times the lower limit of detection (see 9.2.2.1, 9.2.2.2, 9.3.2.1, and 9.3.2.2).

12. Keywords

12.1 bias; internal standards; matrix spike; organics; percent recovery; quality assurance; recovery; spike; spiking; standard additions; surrogates