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Standard Guide for Spiking into Aqueous Samples [1]

This standard is issued under the fixed designation D5810; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers the general technique of "spiking" a broad range of materials into aqueous media. This guide will serve the analyst in preparing spiked samples for quality control purposes. 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 procedures and materials described here are appropriate to the task at hand.

1.2 The procedures in this guide are focused on "matrix spike" preparation, analysis, and interpretation of results. 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 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 E200 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 homogenous suspension of the analyte of interest in the sample cannot be attained. These procedures may be applicable to microbiological preparations if the homogeneity of the suspension can be adequately maintained throughout the course of the analysis, for example, by mechanical agitation or stirring.

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 addition of the spike.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D4375 Practice for Basic Statistics in Committee D19 on

[1] This guide is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.02 on Quality Systems, Specification, and Statistics.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

Water (Withdrawn 2018)[3]
E200 Practice for Preparation, Standardization, and Storage of Standard and Reagent Solutions for Chemical Analysis

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *matrix spike, n*—the quantity (mass) of a component (analyte) of interest that is added to a sample (matrix) in order to test the 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, n*—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 analyte to an aqueous sample. 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 is commonly used to determine the bias under specific analytical conditions, or the applicability of a test method to a particular sample matrix in that context, by determining the extent to which the spiked analyte or component 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 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 or recovery limits from the same or similar matrices over time may indicate variations in the quality of analytical results.

5.3 Spiking can be used to compare the recoveries of like spikes from reagent water samples and natural matrix samples (measured with and without spike) to distinguish between (*1*) unusual interference and (*2*) inherent method recovery and instability effects. This guide does not attempt to deal with the statistical significance of differences in spike recoveries from different matrices.

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. Duplicate field spikes can be used to document the reproducibility of the technique. When environmentally labile compounds are used as spikes, the spiking solution shall be protected up to the point 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 an analyte component is recovered from samples 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. It is not good practice to correct analytical data using spike recoveries for this reason, as well as the fact that bias corrections can add variability. However, spike recovery information should be reported along with related sample analysis results.

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

6. Apparatus

6.1 *Pipetters*—Plunger-actuated pipetters, to dispense small volumes of spike solutions. These must be calibrated and tested carefully for repeatability before use.

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

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

6.4 *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. Reagents of the highest available purity shall be used for spike analytes and

[3] The last approved version of this historical standard is referenced on www.astm.org.

demonstrated to be free of interfering substances for the subsequent tests 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 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 used, 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 as defined by Type I of Specification D1193 and demonstrated to be free of interfering substances for the test(s) being performed.

7.3 *Solvents*—Spectroscopic, high-pressure liquid chromatography (HPLC), or ultrapure grade methanol is preferable for use as a solvent for relatively water-insoluble components in most trace-organic analyses. Other water-soluble solvents may be useful as solvents for certain analytes. Most inorganic spiking solutions are prepared in water or dilute aqueous acid solution. Solvents shall be checked before use by analysis for interfering substances.

7.4 *Spiking Solutions*—Spiking solutions of each analyte of interest are prepared individually or in combination, either gravimetrically or volumetrically. 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 should 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 may be prepared 25 to 100 times as concentrated as the working spike solution and diluted volumetrically to produce the working spike solution at the time of use. In some cases, concentrated solutions may be stable 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 components.

8.2 Sample containers shall be selected and prepared, and samples shall be preserved in accordance with Practices D3694.

9. Procedure

9.1 Use relevant good laboratory practices in accordance with Guide D3856 and Practice E200.

9.2 Perform an analysis on at least one portion of the sample to estimate the concentration of the component(s) of interest.

9.3 Use the result of this analysis to determine the appropriate amount of spike and spiking solution to be added to the sample. If this is not possible (such as when spiking in the field), estimate the concentrations of the components of interest based on prior knowledge of the sample source.

9.3.1 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 If the spiked component is not present in the sample, but is added only to validate the recovery of an analytical method, the concentration after spiking should be at least five times the detection limit of the method or a concentration of interest to the data user, whichever is greater.

9.4 Determine the volume of the portion of sample to be spiked, depending on such factors as the sample volume required by the analytical method to be used, convenience of dilution factors, and amount of sample available.

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

9.5.1 Pertinent factors in determining the appropriate concentration of the spiking solution are as follows:

9.5.1.1 The desired final concentration of the spike in the sample;

9.5.1.2 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);

9.5.1.3 The solubility of the solute (the spiked analyte or component) in the solvent (water or a water-soluble carrier) of the spiking solution;

9.5.1.4 The volume of the sample; and

[4] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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.

9.5.1.5 The volume markings on the available pipets or pipettors.

9.5.2 The spiking solution will generally constitute less than, preferably much less than, 2 % of the total volume of the sample, so the matrix is not altered appreciably, for example, through matrix solubilizing by the spiking solution carrier solvent. 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 in which methanol elutes. Less than 0.02 % of the total volume of the sample should be used in this case. The calculation and correction of the volume of a spiking solution has no negative effect and may be beneficial in any case; see 10.2 and 10.3.

9.5.3 The spiking solution volume must be sufficient for the spiked analyte(s) to remain solubilized and for accurate volumetric dispensing. Solubility and handling considerations may require that the spike be added to the sample as a pure compound. Extra care is needed to ensure thorough mixing of pure compounds when used as spikes.

9.6 Add the desired volume or mass of spiking solution or spike to the sample. Cap the sample and mix well.

9.7 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 component, a smaller volume of more concentrated spiking solution, or a new spiking solution prepared in a more miscible solvent.

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/volume), milligram per litre, or microgram per litre. The units of measure must remain consistent throughout these calculations once chosen. For example, if microgram per litre is selected as the concentration units, 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 1 and 4; see 9.3.1. If B is at or below the limit of detectability for subsequent testing, F should equal 4 and B set at the limit of detectability; see 9.3.2,

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 $V + V_s$ as explained in 9.5.2, then 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 is generally 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 two 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 $A(V_s + V) - (B \times V_s)$ is substituted for M for each analyte and $C \times V$ is substituted for T. The percent recovery, P, is then calculated as follows:

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

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:

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

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 (RSD) of the method at those concentrations. The combined error (CE) is determined using the following formula:

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