Designation: D 4515 – 85 (Reapproved 1995)ε1

AMERICAN SOCIETY FOR TESTING AND MATERIALS
100 Barr Harbor Dr., West Conshohocken, PA 19428
Reprinted from the Annual Book of ASTM Standards. Copyright ASTM

# Standard Practice for Estimation of Holding Time for Water Samples Containing Organic Constituents[1]

This standard is issued under the fixed designation D 4515; 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.

ε[1] NOTE—Section 12 was added editorially in June 1995.

## 1. Scope

1.1 This practice describes the means of estimating the period of time during which a water sample can be stored after collection and preservation without significantly affecting the accuracy of analysis.

1.2 The maximum holding time is highly matrix-dependent and is also dependent on the specific analyte of interest. Therefore, water samples from a specific source must be tested to determine the period of time that sample integrity is maintained by standard preservation practices.

1.3 In those cases where it is not possible to analyze the sample immediately at the time of collection, this practice does not provide information regarding degradation of the constituent of interest or changes in matrix that may occur from the time of sample collection to the time of the initial analysis.

1.4 This practice does not provide information regarding holding time for concentration of analyte less than one order of magnitude above the criterion of detection.

1.5 *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 2777 Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water[2]

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

## 3. Terminology

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

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *acceptable holding time*—acceptable holding time is any period of time less than or equal to the maximum holding time.

3.2.2 *maximum holding time*—maximum holding time is the maximum period of time during which a properly preserved sample can be stored before such degradation of the constituent of interest occurs or change in sample matrix occurs that the systematic error exceeds the 99 % confidence interval (not to exceed 15 %) of the test about the mean concentration found at zero time.

## 4. Summary of Practices

4.1 Holding time is estimated by means of replicate analysis at discrete time intervals of a large volume of a water sample that has been properly collected and preserved. Concentration of the constituent of interest is plotted versus time. The maximum holding time is the period of time from sample collection to such time that degradation of the constituent of interest occurs or change in sample matrix occurs that the systematic error exceeds the 99 % confidence interval (not to exceed 15 %) of the test about the mean concentration at zero time. Prior to determination of holding time, each laboratory must generate its own precision data for use in the calculation. For those tests which are relatively imprecise, replicate determinations are performed at each time interval to maintain the 99 % confidence interval within 15 % of the concentration found at zero time.

NOTE 1—This practice generates only limited data that may not lead to consistent conclusions each time the test is applied. In cases where the concentration of the constituent of interest changes very gradually over an extended period of time, the inherent variability in test results may lead to somewhat different conclusions each time that the practice is applied.

## 5. Significance and Use

5.1 In order to obtain meaningful analytical data, sample preservation techniques must be effective from the time of sample collection to the time of analysis. This period of time must be defined in order that the analyst may know how long samples may be stored prior to analysis.

## 6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall beused in all tests. Unless otherwise indicated, it is intended

[1] This practice is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

Current edition approved Aug. 30, 1985. Published October 1985.

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

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

that all reagents shall conform to the specifications of the committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.1.1 Refer to the specific test method and to Practices D 3694 for information regarding necessary equipment and preparation of reagents.

6.2 *Purity of Water*—Reference to water shall be understood to mean reagent water conforming to Specification D 1193, Type II and demonstrated to be free of specific interference for the test being performed.

## 7. Determination of Holding Time

7.1 *Collection of Sample*:

NOTE 2—In some instances, it may be of interest to determine the holding time of standard solutions prepared in water. In such cases, a large volume of properly preserved standard solution should be prepared and carried through the steps of the practice in the same manner as a sample. The volume of solution required can be estimated using the equation in 7.1.1.

7.1.1 Based on the estimated precision of the test in the matrix to be tested, calculate the estimated total volume of sample required to perform the holding time determination plus a precision study. The following formula may be used to estimate this volume.

$$V = (A \times B \times C) + 2(A \times D)$$

where:

$V$ = estimated volume of sample required, mL,
$A$ = volume of sample required to perform each separate analysis, mL,
$B$ = estimated number of replicate analyses required at each interval in the holding time study (see Table 1),
$C$ = estimated number of time intervals required for the holding time study (excluding the initial time zero precision study), and
$D$ = number of replicate determinations performed in initial precision study (usually 10).

7.1.2 Based on the volume calculated in 7.1.1, collect a sufficient volume of the specific matrix to be tested to perform the holding time study and a precision study. The sample must be collected in a properly prepared sample container or series of containers. Refer to Practices D 3694 and the procedure for the constituent of interest for specific instructions on sample collection procedures.

NOTE 3—The total volume of sample calculated in 7.1.1 is only an estimate. Depending upon the degree of certainty with which the precision can be estimated, it is recommended that a volume somewhat in excess of that calculated in 7.1.1 be collected in order to make certain that sufficient sample will be available to complete the holding time study. The analyst may want to consider performing a preliminary precision study prior to sample collection in order to be certain that the estimate of precision made in 7.1.1 is reasonably accurate.

**TABLE 1 Estimated Number of Replicate Determinations Required at Each Interval in the Holding Time Study Based on the Estimated Relative Standard Deviation of the Test in the Matrix Under Study**

| Estimated RSD, % | Approximate Number of Replicates |
|---|---|
| 1 to 4 | 1 |
| 5 to 6 | 2 |
| 7 to 8 | 3 |
| 9 | 4 |
| 10 | 5 |
| 11 | 6 |
| 12 | 7 |
| 13 | 8 |
| 14 | 10 |
| 15 | 11 |

7.1.3 Add the appropriate preservation reagents to the sample. Immediately proceed to 7.2.

7.2 *Determination of Single Operator Precision*:

7.2.1 *General Organic Constituent Methods*:

7.2.1.1 Immediately after sample collection, analyze an appropriate number (usually 10) of measured volumes of sample as described in the appropriate procedure. If a sufficiently high concentration of the constituent of interest is found (concentration must be at least one order of magnitude higher than the criterion of detection) proceed to 7.2.1.2. If not, collect another sample and repeat the analysis until a sample containing a sufficiently high concentration is obtained.

NOTE 4—Since there is no way of positively identifying all of the compounds which may be contributing to the values found in the General Organic Constituent Methods, the sample cannot be fortified. In order to carry out the holding time determination, a sample must be obtained which contains a sufficiently high concentration to carry out the study.

7.2.1.2 Calculate the mean concentration, the standard deviation, and the relative standard deviation of these replicate determinations. (See Practice D 2777.) Proceed to 8.1.

7.2.2 *Specific Organic Constituent Methods* (Applicable to methods that do not require extraction of the sample container.):

7.2.2.1 Immediately after sample collection, analyze an appropriate number (usually 10) of measured volumes of sample as described in the appropriate procedure. If a sufficiently high concentration of the constituent of interest is found (mean concentration must be at least one order of magnitude higher than the criterion of detection), proceed to 7.2.2.4. If not, fortify the sample as described in 7.2.2.2 and reanalyze.

7.2.2.2 Accurately measure the volume of the remainder of the sample and fortify with a known concentration of the constituent of interest. The fortified sample must contain a concentration of the constituent of interest which is at least one order of magnitude higher than the criterion of detection of the method.

7.2.2.3 Immediately perform an appropriate number (usually 10) of replicate analyses of the fortified sample as described in the appropriate procedure.

7.2.2.4 Calculate the mean concentration, the standard deviation and relative standard deviation of these replicate determinations. (See Practice D 2777.) Proceed to 8.1.

[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. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

7.2.3 *Specific Organic Constituent Methods* (Applicable to methods that require extraction of the sample container.):

7.2.3.1 If the sample was collected in a container other than litre glass bottles, immediately transfer shaken 1-L portions of the sample to separate properly prepared (see Practices D 3694) litre glass bottles which have had the litre mark placed on the neck of the container.

7.2.3.2 Immediately perform an appropriate number (usually 10) replicate determinations of the constituent of interest by analyzing the sample in the containers. If a sufficiently high concentration of the constituent of interest is found (mean concentration must be at least one order of magnitude higher than the criterion of detection), proceed to 7.2.3.5. If not, fortify the sample as described in 7.2.3.3 and reanalyze.

7.2.3.3 Fortify the sample in all of the remaining glass bottles with a known concentration of the constituent of interest by adding an accurately measured small volume of a concentrated standard solution of the analyte. The fortified sample must contain a concentration of the constituent of interest which is at least one order of magnitude higher than the criterion of detection of the method.

7.2.3.4 Immediately perform an appropriate number (usually 10) of replicate analyses of the fortified sample as described in the appropriate procedure.

7.2.3.5 Calculate the mean concentration, the standard deviation, and the relative standard deviation of these replicate determinations. (See Practice D 2777.) Proceed to 8.1.

7.2.4 *Purgeable Organic Compounds*:

7.2.4.1 Immediately after collection, perform an appropriate number (usually 10) of replicate determinations of the constituent of interest by analyzing separate aliquots of the sample that have been collected in hermetically sealed containers. If the concentration is sufficiently high (concentration must be at least one order of magnitude higher than the criterion of detection), proceed to 7.2.4.5.

7.2.4.2 If the concentration found in 7.2.4.1 is not sufficiently high to accurately determine holding time (concentration must be at least one order of magnitude higher than the criterion of detection of the method), collect another sample and repeat the analysis or fortify the sample as described in 7.2.4.3.

7.2.4.3 If the sample requires fortification, open all of the remaining containers and transfer the contents to a graduated cylinder to measure the total volume and the remaining sample. Then transfer the sample to an aspirator bottle fitted with a stopcock at the bottom. Transfer, by means of a syringe, a measured volume of stock solution containing a known concentration of the constituent of interest into the sample. The syringe needle should be below the surface of the liquid during the transfer. Stopper the bottle and mix well. Carefully transfer (by draining through the stopcock) the sample to separate small glass sample vials. Great care must be exercised to carry out the sample transfer with a minimum of sample agitation and aeration. Each sample vial must be filled to overflowing so that a convex miniscus forms at the top. Seal each vial as described in Practices D 3694.

NOTE 5—It is recommended that the operator test his or her technique in transferring solutions of purgeable organic compounds by preparation and analysis of replicates prepared from a standard solution. This should be done to make certain that no loss of purgeable organic compounds is occurring during transfer. Such loss can seriously bias the results of this test.

7.2.4.4 Perform an appropriate number (usually ten) replicate analyses of the fortified sample as described in the appropriate procedure.

7.2.4.5 Calculate mean concentration, the standard deviation and relative standard deviation of the values found in either 7.2.4.1 or 7.2.4.4. (See Practice D 2777.) Proceed to 8.1.

## 8. Calculation of Replicates Required for Holding Time Study

8.1 Based on the relative standard deviation found in 7.2, calculate the number of replicate determinations that will be required at each time interval in the holding time study. The following formula is used for the calculations:

$$n = \left(\frac{ts_0}{D}\right)^2$$

where:

$n$ = number of replicates required in the holding time determination,
$t$ = student's $t$ (based on number of replicates used in precision study. See Table 2.),
$s_0$ = relative standard deviation expressed as percent (Determined in 7.2.), and
$D$ = 15 % (maximum variation from mean concentration to be tolerated).

NOTE 6—The number of replicate determinations calculated using this formula is rounded off to the next highest whole number. For example, a value of 1.09 would be rounded to 2.

## 9. Analyses at Specified Time Intervals

9.1 At appropriate intervals following the initial analysis, perform the appropriate number of replicate analyses as calculated in 8.1. The intervals at which the subsequent analyses are carried out are left to the judgment of the analyst and are somewhat dependent on whether a measure of maximum or acceptable holding time is desired. For example, days 1, 5, 10, and 14 would be appropriate for a two week study. In some cases, shorter or longer time intervals may be

**TABLE 2 Values of Student *t* at 99 % Confidence Interval[A]**

| Number of Replicates | *t* Value |
|---|---|
| 2 | 63.657 |
| 3 | 9.925 |
| 4 | 5.841 |
| 5 | 4.604 |
| 6 | 4.032 |
| 7 | 3.707 |
| 8 | 3.499 |
| 9 | 3.355 |
| 10 | 3.250 |
| 11 | 3.169 |
| 12 | 3.106 |
| 13 | 3.055 |
| 14 | 3.012 |
| 15 | 2.977 |

[A] University of Kentucky College of Engineering," Design of Experiments Course", Vol 7, p. 146.

appropriate. During this period, the sample must be stored under the conditions defined for sample preservation.

NOTE 7—In some cases, degradation of the analyte may occur more rapidly than anticipated and acceptable range of variation is exceeded after the first or second chosen interval. In such cases, the holding time study should be repeated using shorter time intervals if an accurate estimation of maximum holding time is required.

NOTE 8—If it is desired to know only whether a specific time interval is an acceptable holding time, a single time interval may suffice.

## 10. Calculation and Evaluation of Data

10.1 Calculate the average concentration found at each time interval in the holding time study.

10.2 Calculate the tolerable range of variation (99 % confidence interval) from the initial mean concentration that will be used as the criterion for the holding time evaluation. Use the following equation:

$$d = \pm(ts/\sqrt{n})$$

where:

$d$ = range of tolerable variation from the initial mean concentration (in concentration terms),
$t$ = student's $t$ (based on the number of replicates used in the precision study),
$s$ = standard deviation (in concentration terms) calculated in 7.2, and
$n$ = number of replicate determinations used at each time interval in the holding time determination (calculated in 8.1).

10.3 Plot the average concentration found at each time interval versus time on linear graph paper. Indicate on the plot the range of variation from the initial mean concentration that can be tolerated before the holding time is exceeded.

10.4 Draw the best graphical fit of the data points. Evaluate the changes in concentration as a function of time to determine whether the changes represent a significant systematic error in analysis due to increase or decrease in analyte concentration. The maximum holding time is the maximum period of time during which a properly preserved sample can be stored before the systematic error exceeds the tolerable range of variation calculated in 10.2. See Note 1.

## 11. Example of Holding Time Evaluation

11.1 Assume that a laboratory is planning on determining the holding time for a specific organic constituent in a specific water. Historically, the concentration of the constituent of interest has ranged from below the criterion of detection (<1 mg/L) to as high as 80 mg/L. Based on limited precision studies performed in the past and experience with the method, the single operator precision is estimated to be in the range of 3 to 8 % (RSD) over the concentration range of 10 to 50 mg/L. The laboratory is interested in determining whether the analyte is stable in the water for a period of up to 30 days. The time intervals chosen for the study are 0, 6, 12, 18, 24, and 30 days. The volume required to perform each individual test is 100 mL.

11.2 The total amount of sample required for the study is calculated using the equation in 7.1.1.

$$V = (100 \times 3 \times 5) + 2(100 \times 10) = 3500\ \text{m}L$$

The laboratory decides to collect a total of 5000 mL of sample in case the estimate of precision is somewhat low.

11.3 Immediately after sample collection and preservation, ten measured aliquots of sample are analyzed according to the prescribed procedure. The mean concentration found is 8.5 mg/L. This value is less than one order of magnitude above the criterion of detection. The remaining sample is fortified with 40 mg/L of the constituent of interest. Ten measured aliquots of the fortified sample are then immediately analyzed. These data are tabulated and the mean, standard deviation, and relative standard deviation of the fortified values are calculated.

| Replicate Number | Concentration, mg/L |
|---|---|
| 1 | 44.8 |
| 2 | 46.5 |
| 3 | 52.2 |
| 4 | 46.2 |
| 5 | 46.6 |
| 6 | 49.5 |
| 7 | 47.6 |
| 8 | 51.1 |
| 9 | 55.2 |
| 10 | 46.3 |

The mean of the above values is calculated by summing the concentrations and dividing by the number of replicate determinations.

$$\text{Sum of concentrations} = 486.0$$

$$\text{Mean concentration, } \bar{X} = \frac{486.0}{10} = 48.6\ \text{mg}/L$$

Calculate the standard deviation of the concentration values using the following equation:

$$s = \sqrt{\Sigma(Xi - \bar{X})^2/(\text{n} - 1)}$$

where:

$s$ = estimated standard deviation of the series of results,
$Xi$ = each individual concentration value,
$\bar{X}$ = the mean concentration (calculated above), and
$n$ = number of replicate determinations.

| Replicate Number | $(Xi - \bar{X})$ | $(Xi - \bar{X})^2$ |
|---|---|---|
| 1 | −3.8 | 14.44 |
| 2 | −2.1 | 4.41 |
| 3 | 3.6 | 12.96 |
| 4 | −2.4 | 5.76 |
| 5 | −2.0 | 4.00 |
| 6 | 0.9 | 0.81 |
| 7 | −1.0 | 1.00 |
| 8 | 2.5 | 6.25 |
| 9 | 6.6 | 43.56 |
| 10 | −2.3 | 5.29 |
| | | 98.48 |

$$\Sigma(Xi - \bar{X})^2 = 98.48$$

$$s = \sqrt{98.48/9} = 3.3079 = 3.31\ mg/L$$

Replicate No. 9 is tested to determine whether it is an outlier (See Practice D 2777) and found not to be an outlier.

Calculate the relative standard deviation (RSD):

$$\text{RSD, \%} = \frac{s}{\bar{X}} \times 100 = \frac{3.31}{48.6} \times 100 = 6.8\ \%$$

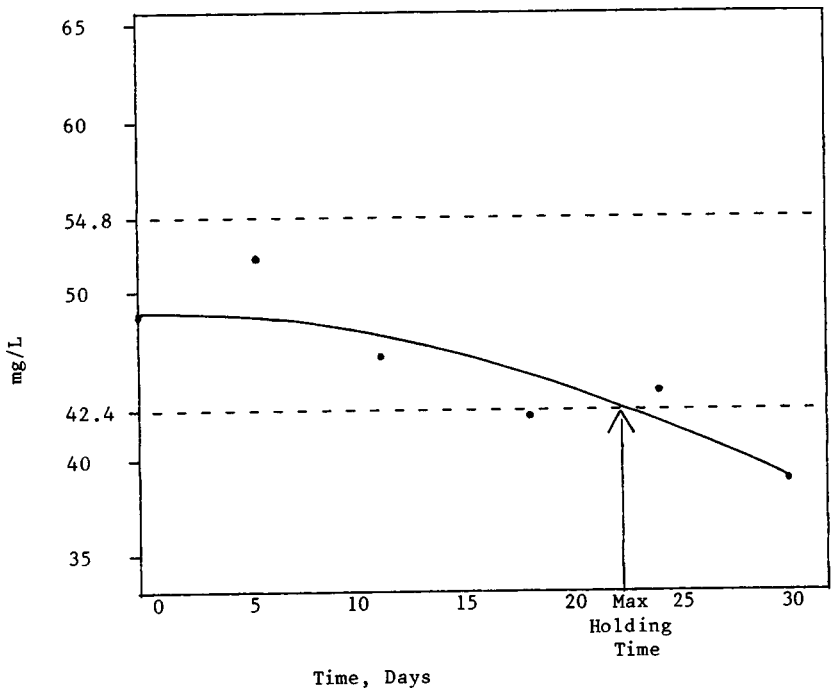


**FIG. 1 Plot of Data for Holding Time Estimation**

The final tabulation of the data is as follows:

| Number of Replicates | Mean, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|---|---|---|---|
| 10 | 48.6 | 3.31 | 6.8 |

11.4 Calculate the number of replicates required in the holding time study using the equation in 8.1.

$$n = \left(\frac{3.25 \times 6.8}{15}\right)^2 = 2.17$$

The calculated value of 2.17 is rounded to 3. Three replicate determinations will be required at each time interval in the holding time study.

11.5 All of the tests are carried out at the appropriate time intervals. The average concentration found at each time interval is calculated. The tolerable range of variation from the mean concentration (99 % confidence interval) is calculated using the equation in 10.2.

$$d = \pm \frac{3.25 \times 3.31}{\sqrt{3}} = \pm 6.2 \text{ mg/}L$$

The tolerable interval of variation is therefore, 48.6 ± 6.2 = 42.4 to 54.8 mg/L.

11.6 A plot of the data is prepared and the best graphical fit of the data is drawn (see Fig. 1). The point at which this line crosses the tolerable range of variation is the estimated maximum holding time.

Evaluation of Data for Holding Time Determination

| Day | Concentration Found, mg/L |
|---|---|
| 0 | 48.6 |
| 6 | 51.9 |
| 12 | 45.6 |
| 18 | 42.1 |
| 24 | 43.2 |
| 30 | 37.9 |

## 12. Keywords

12.1 acceptable holding time; maximum holding time; preserved samples; purgeable organic compounds; specific organic constituents