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

This standard is issued under the fixed designation D 4841; 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 practice covers 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 dependent upon the matrix used and 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 the event that 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 the matrix that may occur from the time of sample collection to the time of the initial analysis.

1.4 *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 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits[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]
D 4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data[2]
D 4375 Practice for Basic Statistics in Committee D-19 on Water[2]

E 178 Practice for Dealing with Outlying Observations[4]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this practice, refer to Terminology D 1129.

3.1.2 *criterion of detection*—the minimum quantity that must be observed before it can be stated that a substance has been discerned with an acceptable probability that the statement is true (see Practice D 4210).

3.2 *Definitions of Terms Specific to This Standard:*

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

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

4. Summary of Practice

4.1 Holding time is estimated by means of replicate analyses at discrete time intervals using a large volume of a water sample that has been properly collected and preserved. A sufficient number of replicate analyses are performed to maintain the 99 % confidence interval within 15 % of the concentration found at zero time. 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 or change in sample matrix occurs and the systematic error exceeds the 99 % confidence interval (not to exceed 15 %) of the test calculated around the mean concentration at zero time. Prior to the determination of holding time, each laboratory must generate its own precision data in matrix water. These data are compared to the pooled single-operator precision data on reagent water reported in the test method and, the less precise of the two sets of data are used in the calculation.

NOTE 1—This practice generates only limited data which may not lead to consistent conclusions each time that the test is applied. In cases where the concentration of the constituent of interest changes gradually over an

extended period of time, the inherent variability in test results may lead to somewhat different conclusions each time that this 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. A laboratory must confirm that sample integrity is maintained throughout maximum time periods between sample collection and analysis. In many cases, it is useful to know the maximum holding time. An evaluation of holding time is useful also in judging the efficacy of various preservation techniques.

6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[5] Other grades may be used provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering 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 (determined from past experience or from precision data reported in the test method), calculate the estimated total volume of sample required to perform the holding time determination plus a precision study. Estimate this volume as follows:

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

(1)

where:
V = estimated volume of sample required, mL,
A = volume of sample required to perform each separate analysis, mL,
B = estimated number of replicate determinations 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 a precision study and the holding time study. Collect the sample in a properly prepared sample container or series of containers. Refer to 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 used in 7.1.1 is reasonably accurate.

7.1.3 Add the appropriate preservation reagents to the sample immediately after collection. Immediately proceed to 7.2 or 7.3 depending upon whether inorganic or organic compounds are being determined.

7.2 *Determination of Single Operator Precision—Inorganic Methods*:

7.2.1 Immediately after sample collection, analyze an appropriate number (usually 10) of measured volumes of sample as described in the appropriate procedure. If a measurable concentration of the constituent of interest is found, proceed to 7.2.4. If the concentration of the constituent of interest is below the criterion of detection at a P level of ≤ 0.05, fortify the sample as described in 7.2.2 and reanalyze or collect another sample.

NOTE 4—If the concentration of the constituent of interest is very low such that it approaches the criterion of detection at a P level of ≤ 0.05, the precision will be very poor. At such very low concentrations, a fairly large number of replicate determinations will be required to bring the 99 % confidence interval to within 15 % of the concentration found. Under these circumstances, it may be desirable to fortify the sample with the constituent of interest to increase the concentration to a point where the precision will be improved and fewer replicates will be required for the holding time determination. However, the holding time may be different at the higher concentration than it would be at the lower concentration. This decision is left to the judgement of the analyst.

7.2.2 Accurately measure the volume of the remainder of the sample and fortify with a known concentration of the

TABLE 1 Approximate 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
1–4	1
5–6	2
7–8	3
9	4
10	5
11	6
12	7
13	8
14	10
15	11

[5] "Reagent Chemicals, American Chemical Society Specifications,'' Am. Chemical Soc., 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."

constituent of interest.

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

7.2.4 Calculate the mean concentration, the standard deviation, and relative standard deviation of these replicate determinations (see Practice D 4375). Proceed to 8.1.

7.3 *Determination of Single-Operator Precision—Organic Methods*:

7.3.1 *General Organic Constituent Methods*—Immediately after sample collection, analyze an appropriate number (usually 10) of measured volumes of sample as described in the appropriate procedure. If a measurable concentration of organics is found, proceed to 7.3.1.1. If the concentration of the organic compounds is below the criterion of detection at a *P* level of ≤ 0.05, collect another sample and repeat the analysis until a sample containing a measurable concentration is obtained (see Note 4).

NOTE 5—Since there is no way of positively identifying all of the compounds that may be contributing to the values found in the general organic constituent methods, the sample cannot be fortified. To carry out the holding time determination, a sample must be obtained that contains a measurable concentration of organics in order to carry out the study.

7.3.1.1 Calculate the mean concentration, the standard deviation, and the relative standard deviation of these replicate determinations (see Practice D 4375). Proceed to 8.1.

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

7.3.2.1 Immediately after sample collection, analyze an appropriate number (usually 10) of measured volumes of sample as determined in the appropriate procedure. If a measurable concentration of the constituent of interest is found, proceed to 7.3.2.4. If not, either collect another sample or fortify the sample as described in 7.3.2.2 and reanalyze (see Note 4).

7.3.2.2 Accurately measure the volume of the remainder of the sample and fortify it with a known concentration of the constituent of interest.

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

7.3.2.4 Calculate the mean concentration, the standard deviation, and the relative standard deviation of these replicate determinations (see Practice D 4375). Proceed to 8.1.

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

7.3.3.1 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.

7.3.3.2 Immediately perform an appropriate number (usually 10) of replicate determinations of the constituent of interest by analyzing the sample in the containers. If a measurable concentration of the constituent of interest is found, proceed to 7.3.3.5. If not, fortify the sample as described in 7.3.3.3 and reanalyze (see Note 4).

7.3.3.3 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.

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

7.3.3.5 Calculate the mean concentration, the standard deviation, and the relative standard deviation of these replicate determinations (see Practice D 4375). Proceed to 8.1.

7.3.4 *Purgeable Organic Compounds*:

7.3.4.1 Immediately after collection, perform an appropriate number (usually 10) of replicate determinations of the constituent of interest by analyzing separate aliquots of sample that have been collected in hermetically sealed containers. If a measurable concentration is found, proceed to 7.3.4.3. If the concentration is below the criterion of detection at a *P* level of ≤ 0.05, either fortify the sample as described in 7.3.4.2 or collect another sample and repeat the analysis (see Note 4).

7.3.4.2 If the sample requires fortification, open all of the remaining containers and transfer the contents to a graduated cylinder to measure the total volume of 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 vials. Take care to carry out the sample transfer with a minimum of sample agitation and aeration. Fill each sample vial to overflowing so that a convex meniscus forms at the top. Seal each vial as described in Practices D 3694.

NOTE 6—It is recommended that the operator's technique used in transferring solutions of purgeable organic compounds be tested 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.

7.3.4.3 Perform an appropriate number (usually 10) of replicate analyses of the fortified sample as described in the appropriate procedure.

7.3.4.4 Calculate the mean concentration, standard deviation, and relative standard deviation of the values found in either 7.3.4.1 or 7.3.4.3 (see Practice D 4374). Proceed to 8.1.

8. Calculation of Replicates Required for Holding Time Study

NOTE 7—Since some analytical methods are very precise (especially those used in determination of inorganic constituents), it is possible that the single operator precision as generated by the laboratory on a single day may be significantly better than the day-to-day variation caused by random errors. If so, this would significantly bias the results of the test. Consequently, the pooled single-operator precision on reagent water generated in the round-robin testing of the method should be used as the basis for calculation of the 99 % confidence interval if these data show poorer precision than the data generated in 7.2 or 7.3. It is recognized that such data do not include the variability caused by the matrix. However, it is assumed that if the single-operator precision as determined in matrix water is better than the pooled single-operator precision found in reagent water, the contribution of the matrix to the variability is negligible.

8.1 Based on the relative standard deviation found in 7.2 or 7.3 or the pooled single-operator precision in reagent water (see Note 7), calculate the number of replicate determinations that will be required at each time interval in the holding time study (See Table 2). Calculate the number of replicate determinations as follows :

$$n = \left(\frac{t\, RSD_o}{D} \right)^2 \qquad (2)$$

where:

n = number of replicates required in the holding time determination,

t = Student's t (Based on the number of replicates used in the precision study. See Table 2 and Note 8),

RSD_o = relative standard deviation, %, (Determined in 7.2 or 7.3 or use pooled single operator precision in reagent water), and

D = 15 % (maximum variation from mean concentration to be tolerated).

NOTE 8—If the pooled single-operator precision in reagent water reported in the test method is used in this calculation, information on the number of replicates used in the precision study may not be available. Under these circumstances, use $t = 3.00$ to obtain a reasonably accurate estimate of the 99 % confidence interval.

NOTE 9—The number of replicate determinations calculated using this formula is rounded to the next highest whole number. For example, a value of 1.09 would be rounded to 2.

NOTE 10—The value of 15 % was chosen as the maximum variation from the mean concentration to avoid the need to run an unrealistic number of replicates on tests that are very imprecise. Note that only one determination will be required on tests with a relative standard deviation (RSD) of about 4.5 % or less.

9. Analysis 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 2-week study. In some cases, shorter or longer time intervals may be appropriate. During this period, the sample must be stored under the conditions defined for sample preservation.

TABLE 2 Values of Student's t for a Two-Tailed 99 % Confidence Interval

No. 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

NOTE 11—In some cases, degradation of the analyte may occur more rapidly than anticipated and the 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 12—If it is desired to know only whether a specific time interval is an acceptable holding time, a single time interval may suffice.

10. Calculations 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 as follows:

$$d = \pm \frac{t\, s}{\sqrt{n}} \qquad (3)$$

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 or use 3.00 if the pooled single-operator precision in reagent water is used),

s = standard deviation (in concentration terms) calculated in 7.2 or 7.3 or based on pooled single-operator precision in reagent water, 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 If the loss of analyte versus time appears to be a linear relationship, calculate and plot the best straight line through the points using the method of least squares. Otherwise, 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 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 from 3 to 8 % RSD over the concentration range from 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.

$$V = (100 \times 3 \times 5) + 2(100 \times 10) = 3500 \text{ mL} \qquad (4)$$

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. To improve the precision of the measurement, 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 (see Table 3) and the mean, standard deviation, and relative standard deviation of the fortified values are calculated.

11.3.1 The mean of the values in Table 3 are calculated by summing the concentrations and dividing by the number of replicate determinations as follows:

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

$$\text{Mean Concentration } (\bar{X}) = \frac{486.0}{10} = 48.6 \text{ mg/L}$$

11.3.2 The standard deviation of the concentration values (see Table 4) is then calculated as follows:

$$s = \sqrt{\Sigma(X_i - \bar{X})^2/(n-1)} \qquad (5)$$

where:
s = estimated standard deviation of the series of results,
X_i = each individual concentration value,
$\bar{X}$ = the mean concentration (calculated in Eq 5), and
n = number of replicate determinations.

$$\Sigma(X_i - \bar{X})^2 = 98.48 \qquad (6)$$

$$s = \sqrt{98.48/9} = 3.3079 = 3.31 \text{ mg/L} \qquad (7)$$

11.3.3 Replicate No. 9 is tested to determine whether it is an outlier (see Recommended Practice E 178 and Practice D 2777) and found not to be an outlier.

11.3.4 The RSD is then calculated as follows:

$$RSD\,(\%) = \frac{s}{\bar{X}}(100) = \frac{3.31}{48.6}(100) = 6.8\,\% \qquad (8)$$

TABLE 3 Example Data

Replicate No.	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

TABLE 4 Standard Deviation of Concentration Values

Replicate No.	$(X_i - \bar{X})$	$(X_i - \bar{X})^2$
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
Total		98.48

11.3.5 The final tabulation of the statistics is shown in Table 5.

11.4 Calculate the number of replicates required in the holding time study using Eq 2 in 8.1.

$$n = \left(\frac{3.25\,(6.8)}{15}\right)^2 = 2.17 \qquad (9)$$

11.4.1 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 then 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 then calculated using Eq 3 in 10.2.

$$d = \frac{\pm 3.25\,(3.31)}{\sqrt{3}} = \pm 6.2 \text{ mg/L} \qquad (10)$$

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 then prepared as shown in Table 6 and Fig. 1. Since the loss of analyte does not appear to be linear with time, the best graphical fit of the data is drawn. The point at which this line crosses the tolerable range of variation is the estimated maximum holding time.

12. Keywords

12.1 degradation; estimation; holding time; storage limit; water

TABLE 5 Tabulation of Statistics

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

TABLE 6 Evaluation of Data for Holding Time Determination

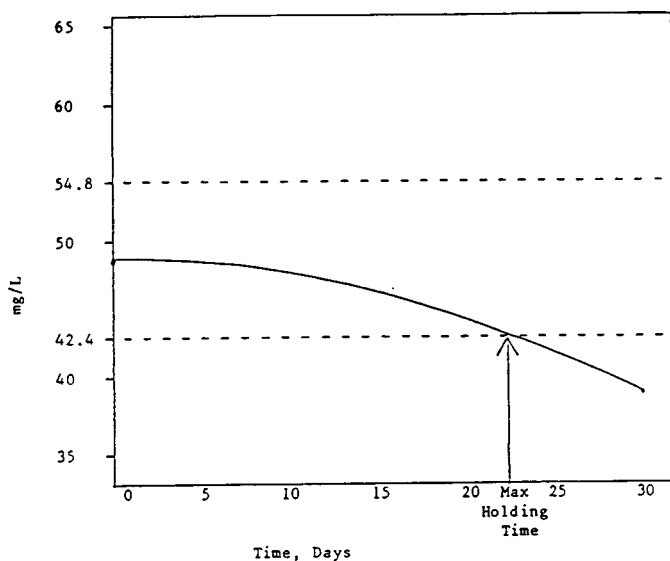| Day	Concentration Found, mg/L
0	48.6
6	51.9
12	45.6
18	42.1
24	43.2
30	37.9

FIG. 1 Plot of Data for Holding Time Determination

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