Designation: D6259 – 23

An American National Standard

Standard Practice for
Determination of a Pooled Limit of Quantitation for a Test Method[1]

1. Scope*

1.1 This practice covers the use of standard regression techniques and data from an interlaboratory study to determine a lower quantitative limit for a test method. This determined lower limit represents the numerical limit at or above which the test results are considered to be quantitatively meaningful for commerce or regulatory activities by this practice. It is defined by this standard as the *pooled limit of quantitation (PLOQ) for the test method*.

1.2 This practice is applicable to test methods that are capable of producing numerical test results close to zero. Examples are those test methods that determine quantitatively the concentration of analyte(s) near zero.

1.3 *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]

D6300 Practice for Determination of Precision and Bias Data for Use in Test Methods for Petroleum Products, Liquid Fuels, and Lubricants

E456 Terminology Relating to Quality and Statistics

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions:*

3.1.1 *repeatability conditions, n*—conditions under which test results are obtained with the same test method in the same laboratory by the same operator with the same equipment in the shortest practical period of time using test units or test specimens taken at random from a single quantity of material that is as nearly homogeneous as possible.

Note 1—By *in the shortest practical period of time* is meant that the test results, at least for one material, are obtained in a time period not less than in normal testing and not so long as to permit significant change in test material, equipment, or environment. See Terminology E456.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *pooled limit of quantitation (PLOQ), n*—level of property or concentration for a test method at which the ratio: [10× pooled repeatability standard deviation of results for a concentration level/concentration level] = 1.

3.2.1.1 *Discussion*—Quantitative test results obtained using a test method at or below its PLOQ is expected to have an uncertainty of ±30 % or greater at the 95 % confidence level.

3.2.2 *pooled repeatability standard deviation, n*—the statistically pooled standard deviation for results obtained under repeatability conditions over multiple operators/laboratories for a sample that is obtained in accordance with Practice D6300 or other equivalent Interlaboratory Study (ILS) analysis (for example, Practice E691, ISO 4259).

3.2.3 *laboratory limit of quantitation (LLOQ), n*—level of property or concentration for a test method at which the ratio: [10× repeatability standard deviation of results for a concentration level determined in a single laboratory/concentration level] = 1.

3.2.3.1 *Discussion*—Quantitative test results obtained by this single laboratory at or below its LLOQ is expected to have an uncertainty of ±30 % or greater at the 95 % confidence level.

3.3 *Acronyms:*

3.3.1 *ILS, n*—interlaboratory study.

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*A Summary of Changes section appears at the end of this standard

3.3.2 *LLOQ, n*—laboratory limit of quanitation.

3.3.3 *PLOQ, n*—pooled limit of quantitation.

4. Summary of Practice

4.1 Statistically pooled repeatability standard deviations from multiple laboratories for at least seven samples meeting the requirements in Section 6 are obtained.

4.2 An empirical power function is fitted to the plot *Y* versus *X* where:

Y = [10× pooled repeatability standard deviation/mean] for each sample, and
X = [mean] of each sample.

4.3 The value of *X* at which the evaluated *Y* = 1 is determined using the function determined in 4.2. This value of *X* is defined as the PLOQ for the method.

5. Significance and Use

5.1 *Background*—In a single laboratory, the limit of quantitation, LOQ, equal to ten times the standard deviation obtained under repeatability conditions extrapolated to zero concentration (s_0) based on samples with close to zero concentrations has been recommended.[3] A test result at this LOQ has an uncertainty of ±30 % at the 99 % confidence level.

5.1.1 This practice uses a regression technique to determine a similar limit for a test method (PLOQ) using statistically pooled repeatability standard deviations over multiple operators/laboratories/samples from ILS data. This PLOQ can be used by industry to assess the reliability of a test method, or, compare reliability of different test methods, for quantitative measurement at concentrations near zero. Similarly, quantitative test results obtained using the test method for levels at or below the PLOQ can be expected by industry to have an uncertainty of ±30 % or greater at the 95 % confidence level.

5.1.2 The regression technique described in this practice can also be used to determine a limit of quantitation specific for a single laboratory (LLOQ). The limit thus quantified for one laboratory is defined by this standard as the laboratory limit of quantitation (LLOQ).

5.1.3 It should be noted that since differences in repeatability testing capabilities between different laboratories can exist, therefore, LLOQ determined at a single laboratory can be different than the PLOQ determined for the test method.

5.2 Values below the PLOQ are deemed by this practice to be too uncertain for meaningful use in commerce, or in regulatory activities.

6. Procedure

6.1 Make the preparations outlined in 6.2, then carry out the procedures described in Section 7.

6.2 *Preparations:*

6.2.1 *Select Test Levels*—Decide the objective of the test method, the range of typical samples it is expected to cover. Determine a set of at least seven test levels intended to be used

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[3] Taylor, J. K., *Quality Assurance of Chemical Measurements,* Lewis Publishers, Michigan, 1987.

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to estimate the PLOQ (see Note 2). The ILS can include more than seven test levels. However, for use of regression for PLOQ only, use results from samples meeting the following requirements:

6.2.1.1 At least four shall have *Y* values (see Section 4, Summary of Practice) greater than 0.5,

6.2.1.2 At least one shall have a *Y* value less than 0.5,

6.2.1.3 At least one shall have a *Y* value between 0.5 and 1,

6.2.1.4 At least two of the seven samples shall have a *Y* value greater than 1.2, and

6.2.1.5 No sample should exceed 4 times the estimated PLOQ.

6.2.1.6 See Table 1 for an example.

NOTE 2—Mean and standard deviation data from experienced laboratories, archived research reports, and known limitations of the test method or equipment can give a preliminary notion of the PLOQ.

6.2.2 *Select Sample Materials*—Use sample materials that are typical of those to which the test method is applied. In special cases, the method of standard additions (spiking) can be necessary to achieve the selected test levels. Synthetic blends may be required because of cost or other practical considerations.

6.2.2.1 For a multi-analyte test method, an analyte-specific plan is required to determine the PLOQ of each analyte of interest. PLOQs determined for one analyte cannot be extrapolated to another.

6.2.3 *Select Number of Laboratories*—Practice D6300 requires at least six laboratories be used in the ILS study.

6.2.4 *Determine Number of Runs Per Sample*—For this practice, the required minimum degrees of freedom for the repeatability standard deviation for each sample is six. Therefore, for determination of PLOQ using an ILS involving six laboratories, the minimum number of runs per sample is two.

6.2.4.1 For determination of LLOQ at a single laboratory, the minimum number of runs per sample is seven.

6.2.4.2 If a blank measurement is required to calculate the measured level of analyte, the analyst must obtain a separate blank measurement for each sample aliquot analyzed.

7. Calculation

7.1 Arrange the averages and corresponding pooled repeatability standard deviations in ascending order of the averages for each sample.

7.2 Calculate the ratio: *Y* = [10× pooled repeatability standard deviation/average] for each sample.

TABLE 1 Example Data from an Interlaboratory Study

| Sample | Mean = X | Within Laboratories | | Y = 10 × Standard Deviation/Mean |
		Standard Deviation	Degrees of Freedom	
S8	110	44.72	10	4.065
S1	640	100	10	1.563
S3	870	89.44	10	1.028
S6	1180	94.87	10	0.804
S2	1272	78.17	9	0.615
S7	2505	136	10	0.543
S4	3165	125	8	0.395
S5	3338	112.4	10	0.337

7.3 Plot *Y* versus *X* where *X* = average of each sample.

7.4 Fit a power function for *Y* versus *X* using standard regression software.

NOTE 3—Step 7.4 can be easily achieved by utilizing the "format trendline" option in Excel and select the "power" function option.

7.5 Solve for the value of *X* for which the value of *Y* = 1 using the fitted function in 7.4. The value of *X* for which *Y* = 1 is the PLOQ.

7.6 For LLOQ, replace pooled repeatability standard deviations in this section by repeatability standard deviations determined at a single laboratory meeting the requirement of 6.2.4.

8. Reporting Low-level Data

8.1 Should a test result be reported that is below the PLOQ of the test method, it is recommended that the PLOQ for the test method be reported in parentheses after the data, for example, chlorine content = 110 mg/kg (PLOQ = 1000 mg/kg).

8.2 A statement about the PLOQ should appear in the test method in the Scope section of the test method.

9. Example of PLOQ Calculation

9.1 Example of data from an interlaboratory study used to execute this practice are provided in Table 1.

9.1.1 The fitted power function and PLOQ solved using the fitted function is provided in Fig. 1.

10. Keywords

10.1 limit of quantitation; pooled limit of quantitation

[10 × Standard Deviation / Mean] versus Mean

function: $Y = 127.28 (X)^{-0.7164}$

set Y = 1
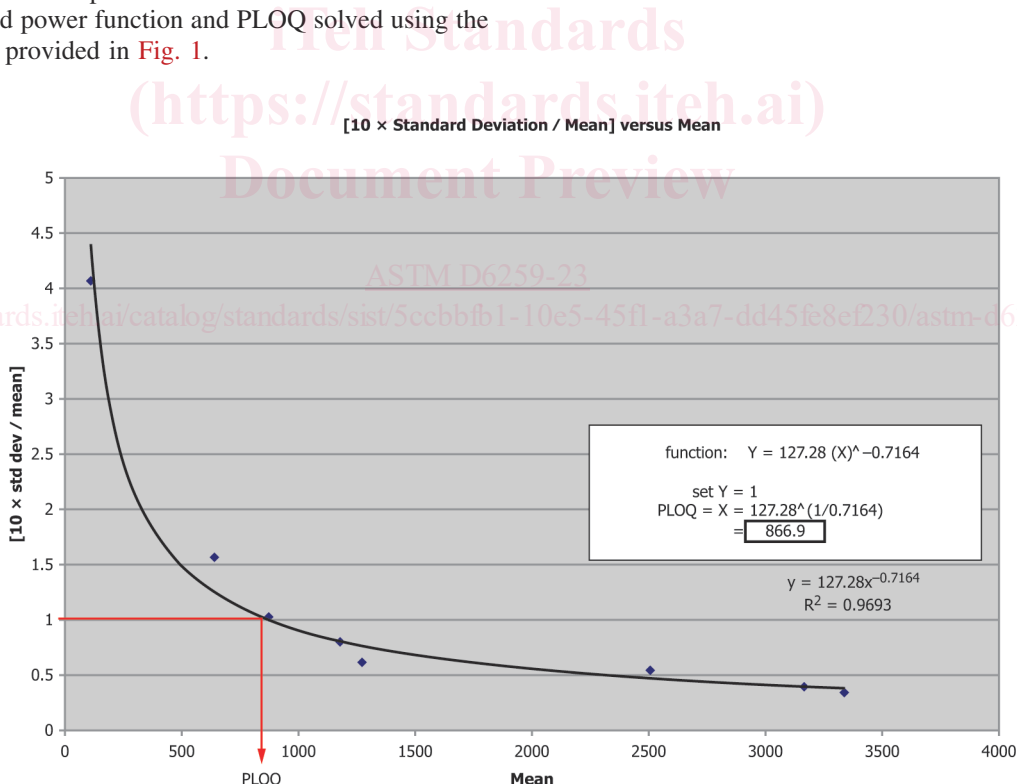PLOQ = $X = 127.28^{(1/0.7164)}$
= 866.9

$y = 127.28x^{-0.7164}$
$R^2 = 0.9693$

FIG. 1 Fitted Function and Solution for PLOQ Data from Table 1