TNI Guidance on Instrument Calibration

GUID-3-110-Rev0

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This document was prepared to provide guidance on the instrument calibration section (1.7) the 2016 TNI Standard Volume 1Module4 (V1M4) Quality Systems for Chemical Testing. This document focuses primarily on those parts of section 1.7 which have changed substantially with the 2016 TNI Standard. This document is not intended to be an official interpretation of the Standard, nor is it to be used in place of the standard. This document is only intended to help users of the standard better understand and implement the standard in their laboratory. If there are questions regarding the use and interpretation of the Standard, submit a Standard Interpretation Request (SIR) for an official interpretation using the process on the TNI website. Note: Language quoted from the standard is shown in grey text boxes.

1.0   Introduction

The 2016 Standard contains several significant changes from the 2009 standard, including:
- removal and replacement of calibration points;
- minimum number of standards;
- relative error;
- single point calibration and linear range methods; and
- continuing calibration acceptance criteria.

Each of these changes is described in more detail in the sections below. In all cases, more stringent standards and criteria required by a mandated test method or regulation take precedence over this Standard.

2.0   Section 1.7.1.1 e) - Removal and Replacement of Calibration Standards

2.1   Need for Written Procedure

A laboratory must have a written procedure that addresses all the requirements in Section 1.7.1.1 e) (Sections 2.2 through 2.6 of this guidance). The laboratory has several options for the location of this procedure: it can be in the form of a Standard Operating Procedure (SOP) or within the Quality Manual. If in an SOP it can be in a general calibration SOP or within the applicable test method SOPs. This procedure should also be addressed/discussed within the Data Integrity program and training (if not already done).

If the laboratory decides to allow removal of calibration points from a curve, the SOP must specify the circumstances under which points may be removed, and the specific concentration levels that may be removed. Section 2.2 specifies the calibration points (or levels) that may or not be removed from a calibration. When points are removed, you must adjust the LOQ or Reporting level (2.3) to be consistent with the remaining calibration points. If you do not replace a calibration level by analyzing a replacement standard (see requirements in 2.5) or you remove multiple standard levels, you must ensure that the remaining number of standards meet the requirements outlined in 2.4. Finally, a standard may be removed from the interior of the standard curve if it meets certain requirements conditions. These conditions are discussed in 2.2 and 2.6.

2.2   Removal of Calibration Levels

> 1.7.1.1 e) i) The laboratory may remove individual analyte calibration levels from the lowest and/or highest levels of the curve. Multiple levels may be removed, but removal of interior levels is not permitted.

Whether a single analyte curve (e.g., NO_3) or a multi-analyte curve (e.g., volatile organics) the lowest and/or highest calibration standard can be removed (dropped), and such removal may be performed multiple times. This is done on an analyte specific basis. For example, this is sometimes necessary when strongly and poorly responding analytes are in the same standard mixture at the same concentration level.   If such a standard(s) is/are removed, the calibration range will need adjustment.

> 1.7.1.1 e) ii) The laboratory may remove an entire single standard calibration level from the interior of the calibration curve when the instrument response demonstrates that the standard was not properly introduced to the instrument, or an incorrect standard was analyzed. A laboratory that chooses to remove a calibration standard from the interior of the calibration shall remove that particular standard calibration level for all analytes. Removal of calibration points from the interior of the curve is not to be used to compensate for lack of maintenance or repair to the instrument.

An interior (e.g., mid-level) calibration standard, i.e., one between the lowest and highest calibration standards, cannot be selectively removed in order to pass calibration criteria. This helps prevent "cherry-picking" of calibration standards.

The intent is to allow a laboratory to provide a good and sound documented technical reason for the rare instance of removal of a standard from the interior of the curve. Examples of this could include bad injection; leaking purge vessel; the extract/standard spilled; or the bottle number was incorrectly transcribed. Standard removal or replacement is only to be allowed in the documented case of gross errors. It is not intended to allow removal or replacement of an interior calibration standard to improve

curve fitting. For multi-analyte methods (e.g., volatile organics) if a level is removed for one analyte, it must be removed for all analytes.

Examples of appropriate and inappropriate practices are shown in Appendix 1.

> 1.7.1.1 e) iii) The laboratory shall adjust the LOQ/reporting limit and quantitation range of the calibration based on the concentration of the remaining high and low calibration standards.

2.3 Adjust LOQ/Reporting Level

If the lowest calibration standard is removed the LOQ or reporting level must be adjusted; in most cases this will mean raising the LOQ or reporting level. Data reported below the lowest calibration standard concentration must be qualified. If the highest calibration standard is removed the quantitation range decreases. Sample dilutions may be required and data qualified if reporting above the quantitation range. See the example in Appendix 1.

2.4 Sufficient Number of Standards

> 1.7.1.1 e) iv) The laboratory shall ensure that the remaining initial calibration standards are sufficient to meet the minimum requirements for number of initial calibration points as mandated by this Standard, the method, or regulatory requirements.

See the guidance on Calibration, 1.7.1.1 f), minimum number of standards (Section 3 of this guidance) for more details and an example in Appendix 1.

2.5 Replacement of Calibration Levels

> 1.7.1.1 e) v) The laboratory may replace a calibration standard provided that
>
> a. the laboratory analyzes the replacement standard within twenty-four (24) hours of the original calibration standard analysis for that particular calibration level;
>
> b. the laboratory replaces all analytes of the replacement calibration standard if a standard within the interior of the calibration is replaced; **and**
>
> c. the laboratory limits the replacement of calibration standards to one calibration standard concentration.

Replacement means removing a standard under the conditions allowed in sub-section ii, and replacing it with a standard at the same concentration. This may be done under the following conditions:
- The replacement standard must be re-run within 24-hoursof the original calibration curve and inserted into the original calibration.
- The entire standard level (i.e., all analytes) must be replaced.
- Only one standard concentration level is replaced.

The replacement of a calibration standard including the reason(s) for replacement (see 2.6 below) must be documented, e.g., in the run log. Once the one calibration standard has been replaced evaluate the

calibration curve. If the calibration curve still fails to meet criteria, then corrective action needs to be taken and the whole calibration redone/reanalyzed.

2.6   Technically Valid Reason

1.7.1.1 e) vi) The laboratory shall document a technically valid reason for either removal or replacement of any interior calibration point.

Replacement of a standard cannot be performed solely in order to pass calibration criteria, calibration verification or quality control criteria, nor to compensate for lack of maintenance or repair to the instrument. The criteria used by the laboratory shall be addressed in a written procedure and appropriately documented. See Section 2.2 above for examples.

3.0   Section 1.7.1.1 f) - Minimum Number of Standards in Calibration

1.7.1.1 f) for regression or average response/calibration factor calibrations the minimum number of non-zero calibration standards shall be as specified in the table below;

Type of Calibration Curve	Minimum Number of Calibration Standards[b]
Threshold Testing[a]	1
Average Response	4
Linear Fit	5
Quadratic Fit	6

[a]The initial one-point calibration shall be at the project specified threshold level.
[b]Fewer calibration standards may be used only if equipment firmware or software cannot accommodate the specified number of standards. Documentation detailing that limitation shall be maintained by the laboratory.

Section 1.7.1.1 f) specifies the minimum number of standards for some of the most commonly used calibration models in the analytical chemistry laboratory. Note that Section 1.7.1.1 (l) (See Section 5.1) has an exception for procedures that use a zero point and single calibration standard.

The footnotes expand upon these specifications.
- Threshold testing is an analysis in which a sample is compared to a single point check standard, where the standard shall be at the threshold level required by project or regulation. This ensures that the greatest accuracy is at the action level and the best determination of whether a result is above or below that level can be made.
- In order to not place an undue burden on laboratories using equipment that cannot process the stated number of standards, there is provision for fewer calibration levels to be used when the automated instrument software is incapable of accommodating the required number. This is not a common occurrence and laboratories should attempt to upgrade their instrument with software capable of complying with this standard.

These requirements were chosen for a number of reasons. There is a statistical basis in that they ensure a minimum of three degrees of freedom for the calibration. Degrees of freedom are the number of values

in a calculation that are allowed to vary. For example, when a linear calibration, ax + b, is used two parameters, the slope and the intercept are defined. Therefore, for k number of standards, there are k-2 degrees of freedom. The more degrees of freedom there are, the less uncertainty there is in the regression. Three degrees of freedom ensures an acceptable level of uncertainty and thus in the example above, 5 standards are required.

Table 1. Degrees of Freedom with the Number of Calibration Standards

Type of Calibration Curve	Minimum Number of Calibration Standards	Number of Parameters	Degrees of Freedom
Threshold Testing	1	Not Applicable	Not Applicable
Average Response	4	1	3
Linear Fit	5	2	3
Quadratic Fit	6	3	3

These requirements are consistent with current calibration requirements of the EPA. For instance, they are generally consistent with current EPA SW-846 methods (e.g., EPA 8000D requires at least five standards for a linear regression and six for quadratic). The updates to the EPA 600 series methods either recommend (EPA 608.3) or require (EPA 624.1 and 625.1) five standards for a linear fit or six for a quadratic fit.

4.0   Section 1.7.1.1 k) - Relative Error

> 1.7.1.1 k) the laboratory shall use and document a measure of relative error in the calibration.

The laboratory must have a procedure to determine the relative error in the calibration. The use of this procedure must be documented. Several different calculations can be used for meet this requirement and are discussed in 4.1 and 4.2 below. The laboratory must ensure that every method that requires a standard curve is also evaluated for relative error. The procedure may be written into each method, or may be a stand-alone document, which identifies the type of calibration and the procedure (mathematical formula) that will be used to evaluate relative error.

4.1   Curves evaluated with %RSD

> 1.7.1.1 k) i) for calibrations evaluated using an average response factor, the determination of the relative standard deviation (RSD) is the measure of the relative error;

For initial calibrations, the widely used % relative standard deviation (RSD) is a measure of relative error. If the initial calibration is evaluated using %RSD, no further measure of relative error is required.

$$\text{Relative standard deviation (RSD)} = (100)*(S/\bar{x})$$

$\bar{x}$ = the arithmetic mean of the i measurements
S = the square root of the variance of i measurements

An example of this calculation is shown in Appendix 2.

4.2 Curves evaluated with r or r^2

If an initial calibration utilizes correlation coefficient (r) or coefficient of determination (r^2) the laboratory must determine relative error since correlation coefficient (r) and coefficient of determination (r^2) are NOT measures of relative error.

Two options are provided for measures of relative error – the laboratory may choose either and the procedure used must be documented in the SOP.

4.2.1   Option 1: Relative Error

Note that this is exactly the same equation used to calculate the % drift for a continuing calibration. The procedure used is to quantitate the low and mid-level calibration levels against the curve, and to calculate the %RE using the equation above.

%RE is measured at the lowest calibration level and at a point near the mid-level of the calibration (the continuing calibration verification level is recommended).

The %RE determined is evaluated based on criteria in the SOP. Most methods include continuing calibration criteria to evaluate the acceptability of the curve at the mid-level. The method may or may not include criteria for the low level of the calibration (more recent methods tend to include these criteria). If

the method does not include criteria, the criteria to be used must be determined and documented the SOP. In general, the criteria for the low-level standard would be expected to be somewhat but not dramatically wider than the mid-level. For example, if the criterion for the mid-level is +/- 30%, then the low-level might be +/- 50%.

In order for a standard curve to be acceptable, the correlation coefficient/coefficient of determination criterion specified in the method must be met **and** both the low-level and mid-level %RE measures must meet the acceptance criteria

4.2.2   Option 2: Relative Standard Error

1.7.1.1 k) ii)

b. Measurement of the relative Standard Error (%RSE)

 Relative Standard Error is calculated using the following equation:

$$\% \text{ RSE} = 100 \times \sqrt{\sum_{i=1}^{n} \left[\frac{x_i' - x_i}{x_i} \right]^2 \Big/ (n - p)}$$

x_i = True value of the calibration level i.
x'_i = Measured concentration of calibration level i.
p = Number of terms in the fitting equation. (average = 1, linear = 2, quadratic = 3).
n = Number of calibration points.

The RSE shall meet the criterion specified in the method. If no criterion is specified in the method, the maximum allowable RSE shall be numerically identical to the requirement for RSD in the method. If there is no specification for RSE or RSD in the method, then the RSE shall be specified in the laboratory SOP.

Relative standard error is analogous to %RSD (and is numerically identical to %RSD for the average RF type curve). %RSE is applicable to any type of curve (linear, quadratic, weighted or unweighted) but %RSD can only be applied to curves developed using average RF.

%RSE is included as an option in the latest version of method 8000 and in 40 CFR Part 136. One significant advantage of %RSE is that is gives one number characterizing the quality of the curve fit that can be used to compare all different potential curve fit types. %RSE is not required if (a) the curve type is average, evaluated by %RSD or (b) %RE has been used to satisfy the requirement to have a measure of Relative Error.

Consistent with method 8000 and Part 136, %RSE may be used in place of the correlation coefficient/coefficient of determination which are commonly used to evaluate linear and quadratic curve fits.

The criterion for %RSE is the same as the criterion for %RSD in the method. If the method does not include a %RSD requirement, then the laboratory must determine a limit and document it in their SOP.

Examples of both options are shown in Appendix 3 and Appendix 4 contains an Excel template calculator.

5.0   Sections 1.7.1.1 l), m), and p) - Single Point Calibration, Aroclor Calibration and Linear Range Verification

These three subsections contain special requirements for certain methods.

5.1   Section 1.7.1.1 l) - Single Point Calibration

1.7.1.1 l) when test procedures are employed that specify calibration with a single calibration standard and a zero point (blank or zero, however specified by the method), the following shall occur:

i The zero point and single calibration standard within the linear range shall be analyzed at least daily and used to establish the slope of the calibration.

ii To verify adequate sensitivity a standard shall be analyzed at or below the lowest concentration for which quantitative data are to be reported without qualification. This standard shall be analyzed prior to sample analysis with each calibration and shall meet the quantitation limit criteria established by the method. If no criteria exist the laboratory shall specify criteria in the SOP;

A laboratory may use a single standard calibration curve when the analytical method allows the use of a single calibration standard and a zero point is used (e.g., ICP methods). No new requirement was included. However, some clarification was provided. For each analytical batch:

- If using a 2-points daily calibration, the slope of the calibration is established using one calibration standard and one zero point.
- Labs must check sensitivity prior to sample analysis. This is done by analyzing one calibration check at (or below) the reporting level. If not provided by the method, quantitative acceptance criteria have to be provided in the Lab SOP.

See examples in Appendix 5.

Section 1.7.1.1 p) - Linear Range Verification

1.7.1.1 p) some methods allow data within the linear range of the instrument, but above the daily calibration, to be reported without qualification. For these methods, the laboratory shall establish the upper reporting limit through analysis of a series of standards. The upper reporting limit is equal to the concentration of the highest standard meeting the method limits for accuracy. The laboratory shall establish linearity annually and check it at least quarterly with a standard at the top of the linear working range, or at the frequency defined by the method. The laboratory shall dilute samples with results above the linear calibration range, or qualify the over-range results as estimated values.

Some methods (such as ICP) allow data above the daily calibration range but below the upper reporting limit to be reported without qualification:

Unless defined by the method, the upper reporting limit must be established annually;

The upper reporting limit must be within the linear range of the instrument/method, and to establish this limit, analyze a series of standards as follows:

- concentrations beyond the daily calibration range must be included;
- one of the concentrations should be at or below the LOQ;
- determine the number of standards to analyze, use the table in 1.7.1.1 f) as guidance.

The upper reporting limit is the highest point that meets the method requirements for accuracy. The method requirements for linearity must be met throughout the range.

Linearity must be checked quarterly (or at the frequency defined by the method).

- Analyze a standard at the upper reporting limit. This is to check that the reporting range is still within the linear range.
- The method requirement for accuracy (usually provided for the annual requirement) shall be met.

For routine batches:

- any result above the daily initial calibration range but at or below the upper reporting limit may be reported without any qualification;
- any result above the upper reporting limit must be reported as an estimated value or, to be reported without qualification the sample must be diluted and analyzed at a concentration below the upper reporting limit. (Note: EPA Method 200.7 requires dilution and reanalysis if the calculated results in great than 90% of the linear range.).

Examples are provided in Appendix 6.

Section 1.7.1.1 m) - Aroclor calibration

> 1.7.1.1 m) for analysis of Aroclors which use a linear through origin model (or average response factor) the minimum requirement is to perform an initial multi-point calibration for a subset of Aroclors (e.g., a mixture of 1016/1260) and to use a one-point initial calibration to determine the calibration factor and pattern recognition for the remaining Aroclors;

A multi point calibration of a mixture of 1016/1260 should be sufficient to demonstrate linearity without the need to perform multi point calibration on the other Aroclors because the 1016/1260 mix includes many of the peaks found in the other five Aroclors.

6.0 Section 1.7.1.2 f) - Continuing Calibration Acceptance Criteria

> 1.7.1.2 f) Criteria for the acceptance of a continuing instrument calibration verification shall be established. If the continuing instrument calibration verification results obtained are outside the established acceptance criteria, the following steps shall be taken:

At the frequency established by the method or SOPs, a continuing instrument calibration verification (aka continuing calibration verification or CCV) must be performed. The laboratory must have acceptance criteria to determine the continuing use of the calibration curve based on the acceptability of the CCV. When a CCV fails, steps must be taken before the analysis of further samples can continue. 6.1, .2 and .3 below outline the steps that must be taken to resolve the failure. Note that 1.7.2.1 (d) does not require a closing CCV if an internal standard is used.

6.1 Obvious Cause

> 1.7.1.2 f) i) If a cause for the calibration verification failure is identified that impacts **only** the calibration verification sample (e.g. a missed autosampler injection), then analysis may proceed if a second calibration verification sample is analyzed immediately and the result is within acceptance criteria. Samples analyzed previously shall be considered valid if bracketed by a passing calibration verification sample (refer to Section 1.7.1.2 d)). The cause for the failure of the first calibration verification result shall be documented.

When the CCV fails, examine the run to determine if the cause of the failure only only affects the failed CCV. Examples of this type of failure could include: missed autosampler injection, low/no internal standard (IS) in the CCV, or CCV spiked at an incorrect concentration. In this case, another CCV, which is analyzed immediately (before analysis of further samples) can be run to verify the curve. If the second CCV passes, then analysis may resume. Data prior to a failing CCV is considered valid if this second CCV passes. The use of a second CCV is only applicable if the failure can be identified and only affects the failed CCV. The cause of the failure must be documented if a second CCV is run. If the failure cannot be identified or documentation is not performed, the samples preceding the failure back to the last passing verification is not considered valid.

6.2 No Obvious Cause and/or Potential Impact

> 1.7.1.2 f) ii) if the cause for the calibration verification failure is **not identifiable** or has **impacted other samples**, then corrective action shall be performed and documented. Prior to analyzing samples, the laboratory shall demonstrate acceptable performance after corrective action with calibration verification or a new initial calibration shall be performed. Samples analyzed prior to the calibration verification failure shall be **reanalyzed** or the results **qualified** if calibration verification bracketing is required (refer to 1.7.1.2 d))

If a reason for the failure cannot be determined or if the failure could have possibly impacted other samples a second CCV cannot be run. Corrective action can be performed to determine the cause. Once the issue has been resolved, all samples which preceded the failure and followed the last passing verification must be reanalyzed, or qualified (see next section). Examples of failures of this type could include: poor peak shape, poor response, and incorrect IS concentration. The corrective action must be documented and a passing CCV or new calibration must be run.

6.3 Qualification of Results

> 1.7.1.2 f) iii) Data associated with an unacceptable calibration verification shall qualified if reported, and shall not be reported if prohibited by the client, a regulatory program or regulation. Data associated with calibration verifications that fail under the following special conditions shall still be qualified, but may use a different qualifier.

If samples are analyzed using a system on which the calibration has not been verified, the results shall be qualified. Data associated with an unacceptable calibration verification may be fully useable when reported under the following special conditions, unless prohibited by the client, a regulatory program or regulation:

> 1.7.1.2 f) iii) a. when the acceptance criteria for the continuing calibration verification are exceeded high (i.e., high bias) and there are associated samples that are non-detects, then those non-detects may be reported. Otherwise the samples affected by the unacceptable calibration verification shall be re-analyzed after a new calibration curve has been established, evaluated and accepted; or
>
> 1.7.1.2 f) iii) b. when the acceptance criteria for the continuing calibration verification are exceeded low (i.e., low bias), those sample results may be reported if they exceed a maximum regulatory limit/decision level. Otherwise the samples affected by the unacceptable verification shall be re-analyzed after a new calibration curve has been established, evaluated and accepted.

- When a verification fails high, then there may be a high bias in the sample results. This would not affect samples where the reported results are below the detection and/or quantitation limit as the result would only be lowered and samples that are non-detects may be considered usable data.
- The opposite is true when a verification fails low. If the sample exceeds an action level of some kind (e.g. MCL), the bias would only make the result higher and samples with values that exceed the action level may be considered as usable data.

    In both cases, the data must be reported with the appropriate qualifier which may be different from the qualifier that is used to indicate data associated with an unacceptable calibration.

If neither of these scenarios are applicable, then the data cannot be used, and the samples must be reanalyzed after an acceptable calibration curve has been established or reported with an appropriate qualifier.

<p>**Appendix 1. Removal of Calibration Levels**</p>

A. Removal of Low or High points

Consider this calibration data:

	A	B	C
	Concentration, ug/L	Area	Response Factor
3	0.05	1097075	21941500
4	0.5	12858983	25717966
5	2.5	67621646	27048658
6	5	1.43E+08	28600000
7	10	3.02E+08	30200000

The percent relative standard deviation, in Excel, (=(STDEV(C3:C7))/AVERAGE(C3:C7) is 11.8%. If the method specified a criterion of 10% in order to use the average response factor, this curve does not meet that criteria. By dropping the low point, the %RSD changes to 6.95 and is acceptable, but the laboratory would have to reduce its calibration range to 0.5 to 10 ug/L, raise the reporting limit to 0,5 ug/L, or qualify below 0.5 ug/L. The remaining data points would need to meet the requirement to have at least 4 calibration levels to use average response factor.

B. Removal of interior points: Not Acceptable
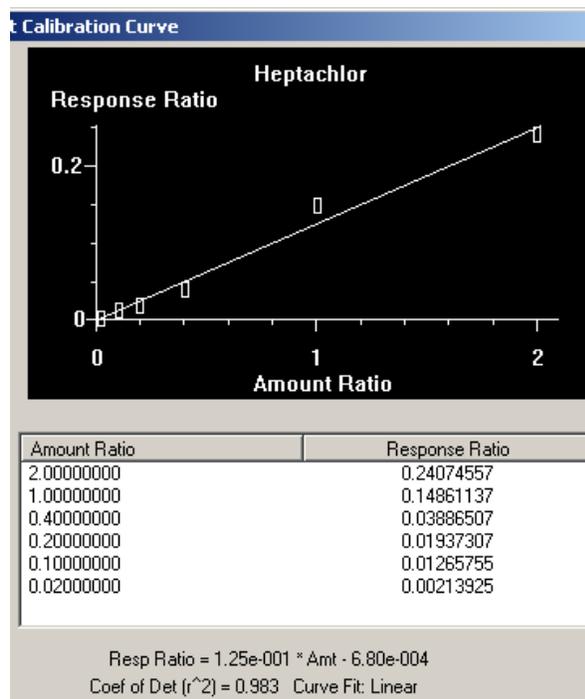
With 1.0 Standard
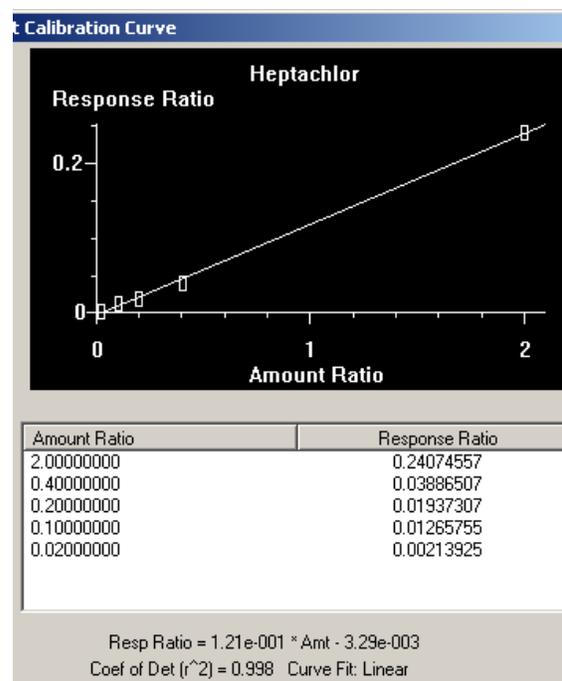
Without 1.0 Standard

By dropping the 1.0 standard, the coefficient of determination, r^2, went from .983 to .998. There was no justifiable reason for dropping this point other than to achieve a higher r^2.

C. Removal of interior points: Not Acceptable

```
                        *RESPONSE FACTOR REPORT GC MS #2

   Method Path : C:\MSDchem\1\METHODS\
   Method File : 032702.M
   Title       : method 525
   Last Update : Wed Mar 27 14:03:40 2002
   Response Via : *INITIAL CALIBRATION

*  CALIBRATION FILES
   1   =CAL1.D   2   =CAL2.D   3   =CAL3.D   4   =CAL4.D   5   =CAL5.D   6   =CAL6.D

*        COMPOUND            1      2      3      4      5      6    *  AVG      %RSD
   ------------------------------------------------------------------------------

   1) I  p-Terphenyl-d14     ----------------ISTD---------------------

   2)    Acenapthene-d10     ----------------ISTD---------------------
   3)    Hexachlorocycl...  0.331 [    ] 0.267 0.248 0.242 0.226 0.263    15.56
   4)    Propachlor         0.623 0.559 0.510 0.462 0.454 0.528 0.523    12.12
   5)    Hexachlorobenzene  0.510 0.493 0.501 0.507 0.494 0.546 0.509     3.87

   6) I  Chrysene-d10        ----------------ISTD---------------------
   7)    Simazine           0.285 0.277 [    ] 0.140 0.153 0.113 0.194    42.01
   8)    Atrazine           0.418 0.446 0.299 0.292 0.359 0.282 0.349    20.01
   9)    Pentachlorophenol  0.202 0.164 0.078 0.040 0.037 0.028 0.092    80.85
  10)    Lindane            0.250 0.272 0.208 0.211 0.292 0.256 0.248    13.51
  11)    Metribuzin         0.261 0.278 0.176 0.128 0.141 0.101 0.181    40.35
  12)    Alachlor           0.209 [    ] 0.177 0.172 0.209 0.166 0.187    11.18
  13)    Heptachlor         0.120 [    ] 0.097 0.097 0.127 0.107 0.110    12.31
  14)    Metalochlor        0.618 0.680 0.504 0.468 0.549 0.489 0.552    14.97
  15)    Aldrin             0.122 0.146 0.119 0.125 0.171 0.142 0.137    14.45
  16)    Heptachlor Epo...  0.087 [    ] 0.084 0.089 0.116 0.114 0.098    15.76
  17)    Butachlor          0.273 0.286 0.207 0.190 0.200 0.161 0.219    22.48
  18)    Nonachlor          0.140 [    ] 0.128 0.136 0.180 0.153 0.148    13.87
  19)    4,4-DDE            0.234 0.257 0.221 0.222 0.285 0.315 0.256    14.84
  20)    Dieldrin           0.140 0.150 0.143 0.148 0.190 0.202 0.162    16.49
  21)    Endrin             0.042 [    ] 0.034 0.032 0.039 0.037 0.037    10.53
```

In the calibration file above, it is clear the laboratory selectively removed interior calibration levels.

D. Removal of interior points: Example of an Acceptable Removal

Standard Concentration	Area
0.02	213925
0.1	1265755
0.2	1937307
0.4	3886507
1	1486113
2	24074557
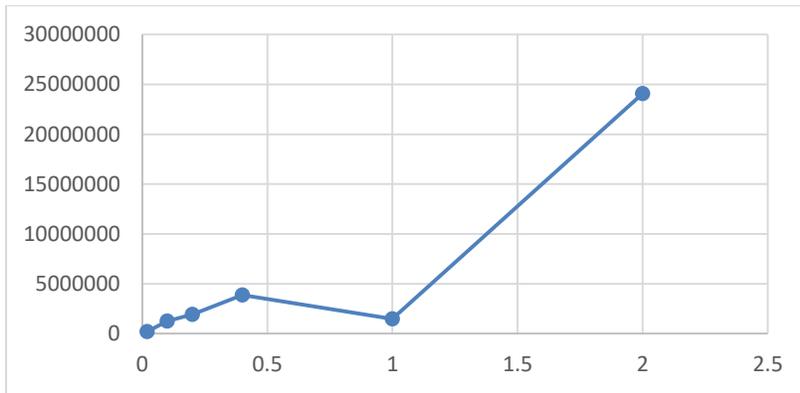
It is obvious something is wrong with the 1.0 standard. It has an area ~ 10 times lower than it should be. This could be because a wrong dilution was made, or the wrong standard analyzed. A fresh standard was reanalyzed and gave this area: 13852107. The new graph shows the problem was corrected.
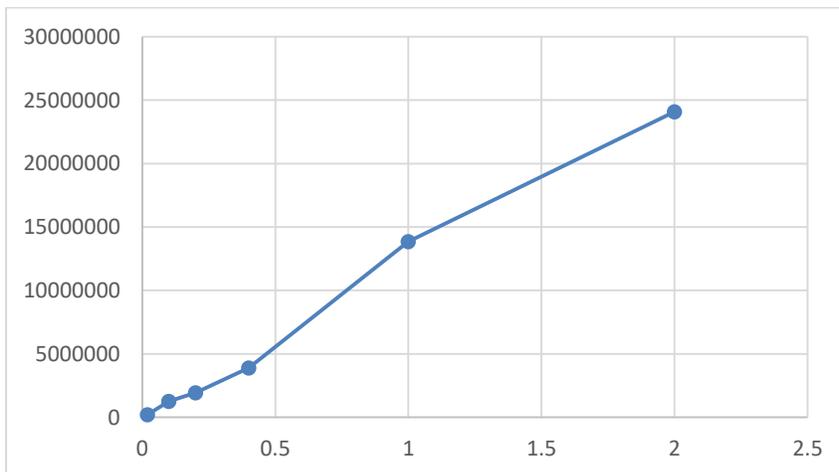
Appendix 2. Example of a Relative Standard Deviation Calculation

	A	B	C
	Concentration	Area	Average RF
3	0.05	1497075	29941500
4	0.5	12858983	25717966
5	2.5	67621646	27048658
6	5	1.43E+08	28600000
7	10	3.02E+08	30200000

Percent Relative Standard Deviation =(STDEV(C3:C7))/AVERAGE(C3:C7) = 6.8%

Appendix 3. Example of Relative Error Calculations (Fluoride)

Below is an example of a typical calibration curve. This is for fluoride by ion chromatography, but almost any method could illustrate the same properties. In this case, the relative error using three different calibration curve fits – unweighted, weighted by 1/concentration, and weighted by 1/(concentration)^2 are described. The criterion for curve acceptance in method 300 is not well defined, but the laboratory could be using a criterion of r > 0.995 – no EPA methods have more stringent limits for the correlation coefficient. In this case, all three curve fits easily meet the minimum criterion for the correlation coefficient. The curve with the "best" (highest number) correlation coefficient is the linear unweighted, and so presumably the laboratory would select this fit. However, that would be a very bad choice because of the large relative error at the low end of the curve – a sample with a response equal to the low standard at 0.05 would return a result of 0.133. Either of the weighted curves is acceptable, but the 1/concentration^2 curve is best, despite having the "worst" correlation coefficient. Using either of the new TNI requirements for measuring relative error makes the choice clear. If using the option of calculating the relative error at the low point, the concentration squared weighted curve shows an error of only 0.78%. If using the RSE option, then the lowest RSE is given by the concentration squared curve.

		Relative Error, %		
Conc.	Response	Linear Unweighted	Linear 1/x	Linear 1/X^2
0.05	1497075	266.11%	16.43%	0.78%
0.5	12858983	13.30%	-12.09%	-9.10%
2.5	67621646	-6.11%	-7.83%	-3.19%
5	1.43E+08	-3.50%	-2.47%	2.14%
10	3.02E+08	1.13%	3.35%	7.80%
	R	0.9994	0.9990	0.9979
	RSE	152.00%	12.47%	7.24%

Appendix 4. Basic Calculator for Relative Error and Percent Relative Error

This is available as a downloadable spreadsheet on the Chemistry Committee page on the TNI website in the Documents folder (**https://nelac-institute.org/committee/chemistry**). Two versions of the table are shown, one with example data filled in and the other showing the Excel formulas.

n, Number of points	5

p, Number of terms	1

True Value	Measured Value	((Measured-True)/True)2	Column d/(n-p)	%RE
0.05	0.058215	0.02699449	0.006748623	16.43%
0.5	0.43955	0.01461681	0.003654203	-12.09%
2.5	2.30425	0.00613089	0.001532723	-7.83%
5	4.8765	0.00061009	0.000152523	-2.47%
10	10.335	0.00112225	0.000280563	3.35%

Sum		0.012368633	
Square root		0.1112	
%RSE		11%	

n, Number of points	B5

p, Number of terms	B7

| True Value	Measured Value	((Measured-True)/True)2	Column C/(n-p)	%RE
A11	B11	=POWER((A11-B11)/A11,2)	=D13/(B5-B7)	=(B11-A11)/A11*100
A12	B12	=POWER((A12-B12)/A12,2)	=D13/(B5-B7)	=(B12-A12)/A12*100
A13	B13	=POWER((A13-B13)/A13,2)	=D13/(B5-B7)	=(B13-A13)/A13*100
A14	B14	=POWER((A14-B14)/A14,2)	=D13/(B5-B7)	=(B14-A14)/A14*100
A14	B15	=POWER((A15-B15)/A15,2)	=D13/(B5-B7)	=(B15-A15)/A15*100

Sum		=SUM(E13:E17)	0.012368633
Square root		=SQRT(E21)	0.1112
%RSE		=E22*100	11%

Note: For the Number of terms, use 1 for average response factor, 2 for linear regression and 3 for quadratic.

Appendix 5. Example of a Daily Calibration for Linear Range Methods

The zero point (1 in the graph below) and single calibration standard within the linear range (3 in the graph below) is analyzed daily and used to establish the slope of the calibration.

A standard is analyzed at or below the lowest concentration for which quantitative data are to be reported without qualification (2 in the graph below) is analyzed prior to sample analysis
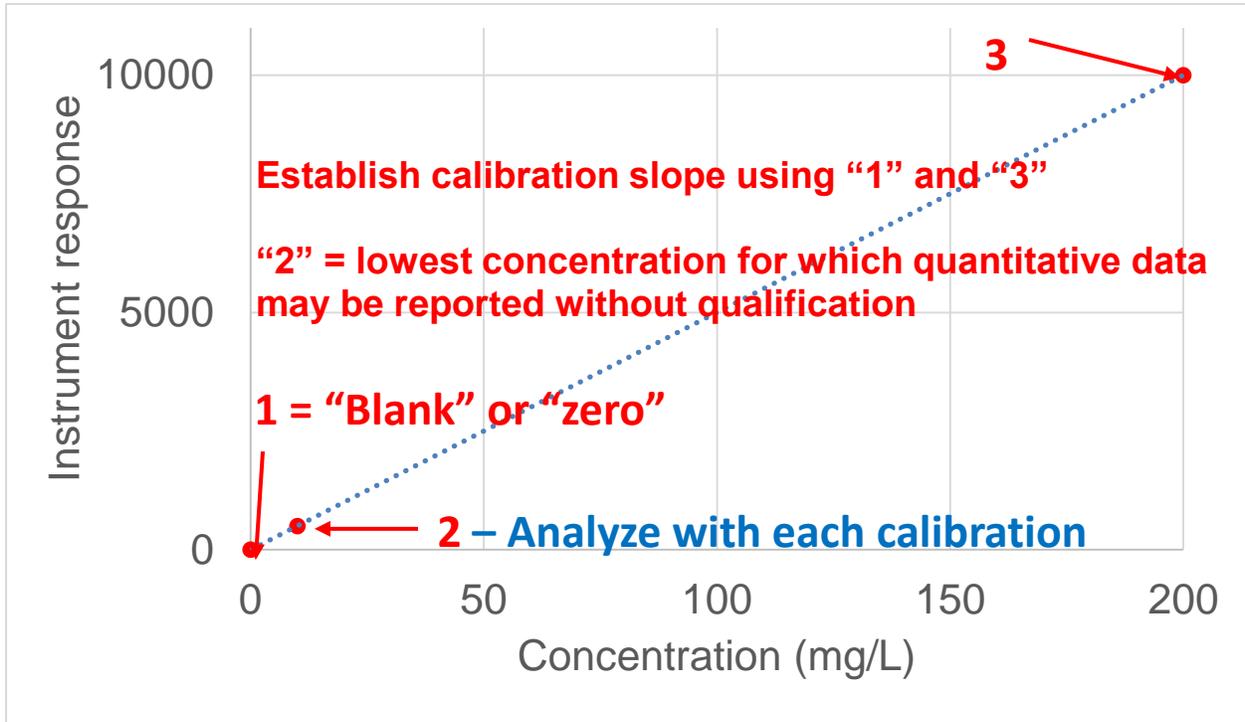
Appendix 6. Example of an Annual and Quarterly Linear Range Verification

Standards 1-3 are analyzed daily as shown in Appendix 5. Standards 4-7 are analyzed once per year to verify linearity. Standard 7 is the highest concentration that met the linearity requirements and is analyzed quarterly.