

APPENDIX A: INSTRUCTIONS FOR CALIBRATION WORKBOOK

1. ReadMe Spreadsheet

Purpose

This worksheet supports linear, quadratic, cubic, and quartic models:

Linear: $y = \beta_0 + \beta_1x + \epsilon$

Quadratic: $y = \beta_0 + \beta_1x + \beta_2x^2 + \epsilon$

Cubic: $y = \beta_0 + \beta_1x + \beta_2x^2 + \beta_3x^3 + \epsilon$

Quartic: $y = \beta_0 + \beta_1x + \beta_2x^2 + \beta_3x^3 + \beta_4x^4 + \epsilon$

y = response

x = concentration

The worksheet estimates the coefficients (β s) and the variance of the error term, ϵ . The workbook then performs the following functions:

- determine which model (linear, quadratic, etc.) is better
- determine the replication of unknowns needed for uncertainty control
- determine whether zero and span responses are acceptable
- estimate the concentration and 95% uncertainty of candidate standards analyzed on the same day as the initial calibration or a subsequent day.

Organization

The workbook consists of several worksheets, which are displayed as tabs at the bottom of the screen. The functions of these worksheets are described below:

ReadMe	describes the workbook, explaining how to use the worksheets
Measurement Data	allows for user input of calibration and other analytical data and includes statistical calculations for polynomial regression
Curves 1	displays the calibration data, the best-fit line, and its confidence bands
Residuals 1	displays the difference between the observed responses and those estimated by the best-fit calibration line
Curves 2	displays the calibration data, the best-fit quadratic curve, and its confidence bands
Residuals 2	displays the difference between the observed responses and those estimated by the quadratic regression line
Curves 3	displays the calibration data, the best-fit cubic curve, and its confidence bands
Residuals 3	displays the difference between the observed responses and those estimated by the best fit cubic regression line
Curves 4	displays the calibration data, the best-fit cubic curve, and its confidence bands
Residuals 4	displays the difference between the observed responses and those estimated by the best-fit quartic regression line

Chart Data includes the data used to create the Curves and Residuals charts.

Conventions

The **Measurement Data** worksheet includes instructions that guide the user through the steps in its use. The worksheet is also color coded to simplify use. Shaded cells that are bordered in blue lines are for input of data. These cells are unprotected, but all other cells of the **Measurement Data** worksheet are protected. The only other unprotected cell in the workbook is cell **F4** of the **Chart Data** worksheet. That cell controls the width of the confidence bands plotted in the **Curves 1** and **Curves 2** charts.

Derived values and statements are colored red. These cells contain formulas and are protected to prevent alteration.

Spreadsheet background colors indicate the order of the polynomial supported by the calculations in the area.

Light green is used for the linear model.
Yellow is used for the quadratic model.
Gray is used for the cubic model
Light blue is used for the quartic model.

Use

The Measurement Data worksheet guides the user through six steps.

STEP 1 Enter Calibration Data

In this step, up to 50 calibration points may be entered. Each calibration point has two parts—the certified concentration of the calibration gas standard and the instrument response when testing the standard. These values are entered in two columns. The spreadsheet performs computations in columns I through P (linear), Q through X (quadratic), Y through AZ (cubic), and BA and above (quartic).

STEP 2 Review the Parameter Estimates

In this step, the user reviews the estimates of the intercepts (b_0), slopes (b_1) and other coefficients (b_2 , b_3 , and b_4) for the four models, examines their confidence intervals and the residual error variances (s^2). The result of an F test indicates which of the models is best. The linear model is recommended unless the quadratic or higher-order model significantly reduces the residual error.

STEP 3 Review the Charts

In this step, the user reviews the charts named **Curves 1**, **Residuals 1**, **Curves 2**, **Residuals 2**, etc. These charts help the user understand why one model performs better than the other.

STEP 4 Assess Magnitude of Uncertainty

In this step, the user enters the assumed concentration of a candidate standard and selects a

replication number, r . Based on the calibration results, the worksheet estimates the 95% uncertainty that would result from measuring such a standard r times. The user can use this as a guide for deciding whether to proceed with analysis, to produce additional calibration points, or to take some corrective action.

STEP 5 Assay Candidate Standard on Same Day

In this step, the user enters the responses to a candidate standard that is tested on the same day as the calibration of STEP 1. The worksheet provides an estimate of the candidate's concentration and its 95% uncertainty. The worksheet also indicates whether the variability in responses is larger than expected (unacceptable).

STEP 6 Assay Candidate Standard on Different Day from Initial Calibration

In this step, the user enters the responses to a candidate standard that is tested on a different day from the calibration of STEP 1. The worksheet first assesses the zero and span responses. If the zero and span responses are acceptable, the user proceeds to enter the results from testing a candidate standard. The results include those for zero and nonzero reference standards. (The quadratic model requires the use of two different nonzero standards.)

The spreadsheet determines whether the regression curve has changed since the initial calibration. The data are corrected for any change and the estimated concentration of the candidate standard is provided together with its 95% uncertainty.

The spreadsheet also determines whether the standard error of the mean response is acceptable (<1% of the mean response). This additional check is meant to guard against hysteresis or other errors that are not corrected by the spreadsheet's adjustments.

2. Measurement Data Spreadsheet

STEP 1 Enter Calibration Data

Enter the calibration data in the shaded spaces below. The first column (I) simply counts the calibration points that you enter. The second column (X) is for the certified concentrations of the calibration gas standards. The third column (Y) is for the instrument responses corresponding to the calibration standards. The number of points cannot exceed 50.

I	X_i	Y_i	Color Code
1	0.000	0.2194	
2	0.500	0.7141	red = derived value (protected)
3	1.000	1.2885	
4	1.500	1.9132	blue = entered value (unprotected)
5	2.000	2.5910	
6	2.500	3.2866	black = fixed text (protected)
7	3.000	4.1078	
8	3.500	4.9446	
9	4.000	5.8145	
10	4.500	6.7230	
11	5.000	7.7284	
12	5.500	8.7566	
13	6.000	9.8013	
14	6.500	10.8818	
15	7.000	12.0982	
16	7.500	13.3122	
17	8.000	14.5840	
18	8.500	15.9238	
19	9.000	17.3271	

STEP 2 Review the Parameter Estimates

Review the estimates of the coefficients (\mathbf{b}_0 , (\mathbf{b}_1, \dots)) for the linear and quadratic models, their confidence, and the residual error variances (\mathbf{s}^2).

Linear Model		95% Confidence Limits	
Parameter	Estimate	Lower	Upper
$b_0 =$	-1.0778	-1.7351	-0.4204
$b_1 =$	1.9005	1.7757	2.0253
$s^2 =$	0.4986	0.2807	1.1205
$s =$	0.7061	0.5298	1.0585
$df =$	17		
$t =$	2.1098		

Quadratic Model		95% Confidence Limits	
Parameter	Estimate	Lower	Upper
$b_0 =$	0.1964	0.1960	0.1968
$b_1 =$	1.0011	1.0010	1.0012
$b_2 =$	0.0999	0.0999	0.0999
$s^2 =$	0.0005	0.0003	0.0011
$s =$	0.0220	0.0164	0.0335
$df =$	16		
$t =$	2.1199		

Comparing the two models:

$$F_{\text{ratio}} = 1027$$

$$F_{\text{critical}} = 2.3167 \quad (5\% \text{ significance level})$$

The quadratic model produces a significantly smaller error variance. The quadratic model appears to be the better choice.

If cubic or quartic models are supported by compelling scientific theory or data, then review the following estimates for those models. Otherwise, **go to Step 3**.

Cubic Model		95% Confidence Limits	
Parameter	Estimate	Lower	Upper
$b_0 =$	0.1952	0.1593	0.2310
$b_1 =$	1.0030	0.9676	1.0385
$b_2 =$	0.0994	0.0901	0.1087
$b_3 =$	0.0000	-0.0006	0.0007
$s^2 =$	0.0005	0.0003	0.0012
$s =$	0.0227	0.0168	0.0352
$df =$	15		
$t =$	2.1315		

Comparing quadratic and cubic models:

$$F_{\text{ratio}} = 0.9385$$

$$F_{\text{critical}} = 2.3849 \text{ (5\% significance level)}$$

The error variances are not significantly different at the 5% level. The quadratic model appears to be a better choice than cubic.

Quartic Model		95% Confidence Limits	
Parameter	Estimate	Lower	Upper
$b_0 =$	0.2206	0.1900	0.2512
$b_1 =$	0.9285	0.8786	0.9783
$b_2 =$	0.1390	0.1156	0.1625
$b_3 =$	-0.0069	-0.0109	-0.0030
$b_4 =$	0.0004	0.0002	0.0006
$s^2 =$	0.0003	0.0001	0.0007
$s =$	0.0165	0.0121	0.0261
$df =$	14		
$t =$	2.1448		

Comparing quadratic and cubic models:

$$F_{\text{ratio}} = 1.8954$$

$$F_{\text{critical}} = 2.4630 \text{ (5\% significance level)}$$

The error variances are not significantly different at the 5% level. The cubic model appears to be a better choice than quartic.

STEP 3 Review the Charts

View the charts named **Curves 1** and **Residuals 1**. **Curves 1** shows confidence bands for the estimated regression. Compare these bands with those of the quadratic regression, **Curves 2**. (Note: You can change the width of the confidence band interval by changing the "p-value" in cell F4 of the worksheet named **Chart Data**.) **Residuals 1** shows how the calibration points deviated from the calibration line. Look for a simple pattern (such as a quadratic curve) in the chart. If such a pattern appears, the quadratic model may be better. View **Residual 2**, the deviations from the best-fit quadratic curve. If **Residual 2** effectively removes the simple pattern observed in **Residual 1** and if the magnitude of the deviations has been significantly reduced (as evidenced by a reduction in the estimate s^2), then the quadratic model is superior. An F-test can be run to determine if the two error variances are significantly different.

$$F = 1026.764 \qquad \text{Prob. of greater } F = 4.51\text{E-}21$$

The quadratic model produces a significantly smaller error variance. The quadratic model appears to be the better choice.

STEP 4 Assess Magnitude of Uncertainty

Enter the concentration at which you would like to evaluate the uncertainty of estimation and prediction. Also enter r, the number of assays to be performed. Increasing r tends to reduce the prediction uncertainty, but with diminishing effect.

$$\text{Concentration} = 6$$

$$r = 3$$

Review the estimated mean response (estimate that only takes into account the calibration uncertainty), and the confidence intervals. Review the predicted mean response and its confidence intervals. To satisfy the EPA protocol requirements, the 95% confidence limits for the concentration should be $\leq \pm 1\%$ of the concentration.

Estimates below are based on the quadratic model. Tab-Right to view estimates based on the other model.

	95% Confidence Limits		
	Estimate	Lower	Upper
Instrument Response =	9.8006	9.7858	9.8155

	95% Confidence Limits		
	Prediction	Lower	Upper
Instrument Response =	9.8006	9.7698	9.8314
Concentration =	6.0000	5.9860	6.0140
95% uncertainty in prediction =		0.23%	

STEP 5 *Assay Candidate Standard on Same Day*

Proceed with the analysis of candidate standards if their 95% uncertainties, as estimated above, are <1%. Enter the responses from the repeated analyses of an individual candidate standard in the spaces provided below.

Note: This step applies only to candidate standards that are assayed on the same day as the calibration.

Enter the instrument responses for up to 10 repeated analyses of a single candidate standard below.

Analysis Number	Response	Estimated Concentration
1	4.500	3.2466
2	4.501	3.2473
3	4.499	3.2460
4		
5		
6		
7		
8		
9		
10		

NOTE: For Cubic and Quartic Model estimates, view the Calculations in the spreadsheet's shaded regions.

mean = 3.2466
standard deviation = 0.0006
df = 2
F = 0.0008
F sig? = FALSE (The sample variance is acceptable.)
Pr(>F) = 0.9992
95% Uncertainty = 0.58%
23.97% = portion of uncertainty² due to calibration

STEP 6 Assay Candidate Standard on Different Day from Initial Calibration

This step applies to candidate standards that are assayed on a different day than the initial calibration. Before candidate standards are run, the measurement system is challenged with zero and span checks. Three or more discrete checks of the zero gas and three or more checks of the span gas are made. Enter the results below:

Response to Zero gas	Span conc.	Response to Span
0.000	9.000	16.010
0.001	9.000	16.000
-0.001	9.000	15.990
n = 3		n = 3
mean = 0.000		mean = 16.000
s = 0.001		s = 0.010
Cal. Resp.= 0.196	Cal. Response =	17.301

	Zero Gas Results	Span Gas Results
Std. Error = s/\sqrt{n} =	0.0006	0.0058
Rrs/100=	0.1600	0.1600
	Std. Error is okay	Std. Error is okay
Relative Difference (RD) =	1.14%	-7.52%
	RD is okay	RD is excessive

Following successful completion of the zero and span checks, the candidate standard is measured together with reference standards. While the candidate standard is normally interspersed with the reference standards, the analysis conducted in this sheet requires that the results be entered separately. There are two ways to do this. One way is to enter an analysis set (one candidate standard response and the responses from its zero and nonzero standard analyses) below. Another approach is to enter all of the responses (multiple sets) below. Enter zero and reference standard responses in the area to the left and enter the responses to a single candidate standard in the area to the right, below.

More than one nonzero reference standard is needed for the quadratic and higher-order models.

Estimates below are based on the quadratic model. Tab-Right to view estimates based on the other model.

Reference Standards (Enter 0 for Zero Concentration)					Candidate Standard	
Conc.	Response	Conc. ²	Conc. ³	Conc. ⁴	Observed Response	Estimated Conc.
0.000	0.218	0.000	0.000	0.000	4.010	2.9437
0.000	0.219	0.000	0.000	0.000	4.000	2.9374
0.000	1.220	0.000	0.000	0.000	3.990	2.9311
4.500	6.693	20.250	91.125	410.063		
4.500	6.723	20.250	91.125	410.063	nnn =	3
4.500	6.773	20.250	91.125	410.063	mean =	4.000 2.9374
9.000	17.317	81.000	729.000	6561.000	stdev =	0.0063
9.000	17.327	81.000	729.000	6561.000	std error =	0.12%
9.000	17.337	81.000	729.000	6561.000	df =	2
					F =	0.1128
					F sig? =	FALSE
					Pr{>F}	0.8938
nn=	9					The standard error is okay.

Coefficient are not significantly different.
 Consider including the new data as part of original calibration (Step 1).

Estimated Concentration of Candidate Standard
 2.9374

95% Uncertainty Portion of uncertainty² due to calibration uncertainty
 0.66% 45.68%

95% Confidence Limits for Candidate Standard Concentration

Lower **Upper**
 2.9181 2.9567

These upper and lower limits are compared with the corresponding limits estimated on different assay dates to establish that the candidate standard has not drifted.