Calibration Methods: Regression & Correlation

Calibration – A series of standards run (in replicate fashion) over a given concentration range.

Standards – Comprised of analyte(s) of interest in a given matrix composition.

Matrix – Why is the composition of the matrix important?

Slope & Intercept – Explain the differences.

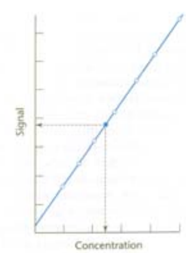


Figure 5.1 Calibration procedure in instrumental analysis: ○ calibration points; ● test sample.

*Figures used in slides from: Miller and Miller, 2005

1

Calibration Methods: Regression & Correlation

Key Questions:

1. Is the graph linear? If it is a curve, what is the form of the curve?
2. What is the best straight line on the curve? Do we force the line thought zero
3. Assuming linearity, what are the errors and confidence limits for the slope & intercept of the line. How do we show the errors?
4. When the calibration plot is used for the analysis of a test material, what are the errors and confidence limit for the determined concentration?
5. What is the limit of detection of the method? How do we determine?

2

Calibration Methods: Regression & Correlation

Determining Concentration from Calibration Curve

Basic steps:

(1) Make a series of dilutions of known concentration for the analyte.

(2) Analyze the known samples and record the results.

(3) Determine if the data is linear.

(4) Draw a line through the data and determine the line's slope and intercept.

(5) Test the unknown sample in duplicate or triplicate. Use the line equation to determine the concentration of the analyte: $y = mx + b$

$$\text{Conc}_{\text{analyte}} = \frac{\text{reading} - \text{intercept}}{\text{slope}}$$

3

Calibration Methods: Regression & Correlation

A closer look at linearity:

slope / intercept formula

$y = mx + b$

where m = slope, b = y-intercept, x = concentration of unknown.
What is y?

Points on the line:

(x_1, y_1) – normally the "blank" reading
$(x_2, y_2), (x_3, y_3)\ldots (x_i, y_i)$

The mean of the x-value is termed $\bar{x}$.
The mean of the y-value is termed $\bar{y}$.

$(\bar{x}, \bar{y})$ = centroid of all points

How is linearity assessed?

4

Calibration Methods: Regression & Correlation

Linearity is addressed by the product-moment correlation coefficient, *r*.
r is given by equation 5.2 and represented visually as *b*:

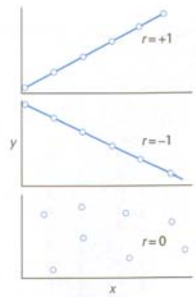


Figure 5.2 The product-moment correlation coefficient, *r*.

r-value of −1 = perfect negative correlation between *x* and *y*.
r-value of +1 = perfect positive correlation between *x* and *y*.
r-value of 0 = no correlation between *x* and *y*.

5

Calibration Methods: Regression & Correlation

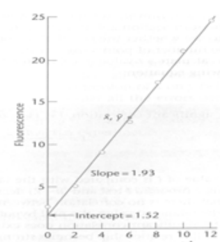


Figure 5.3 Calibration plot for the data in Example 5.3.1.

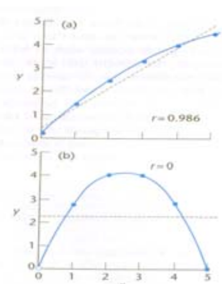


Figure 5.4 Misinterpretation of the correlation coefficient, *r*.

6

Calibration Methods: Regression & Correlation

The line of regression of *y* on *x*:

Deviations will occur – both negative and positive. We then need to minimize the sum of squares of the residuals. This explains the use of:

Slope of least squares line: $b = \dfrac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_i (x_i - \bar{x})^2}$

Intercept of least square line: $a = \bar{y} - b\bar{x}$

Example 5.4.1

- Interpretation of results
- See Figure 5.3 for visual representation

7

Calibration Methods: Regression & Correlation

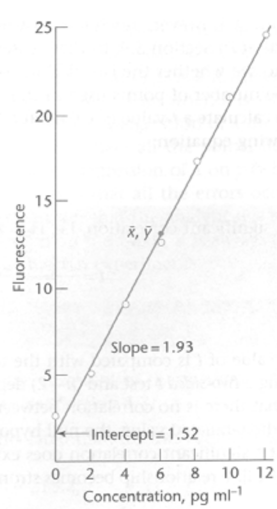


Figure 5.3 Calibration plot for the data in Example 5.3.1.

8

Calibration Methods: Regression & Correlation

Errors in the slope and intercept of the regression line.
Why important?

First calculate the statistic, $s_{y/x}$ which estimates the random errors in the y-direction:

$$s_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}} \qquad (5.6)$$

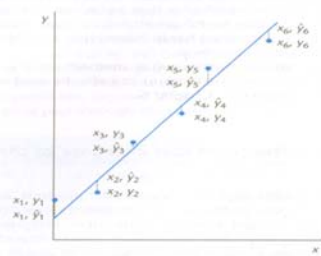


Figure 5.5 The y-residuals of a regression line.

9

Calibration Methods: Regression & Correlation

Then calculate:

$$s_b = \frac{s_{y/x}}{\sqrt{\sum_i (x_i - \bar{x})^2}}$$

$$s_a = s_{y/x}\sqrt{\frac{\sum_i x_i^2}{n\sum_i (x_i - \bar{x})^2}}$$

10

Calibration Methods: Regression & Correlation

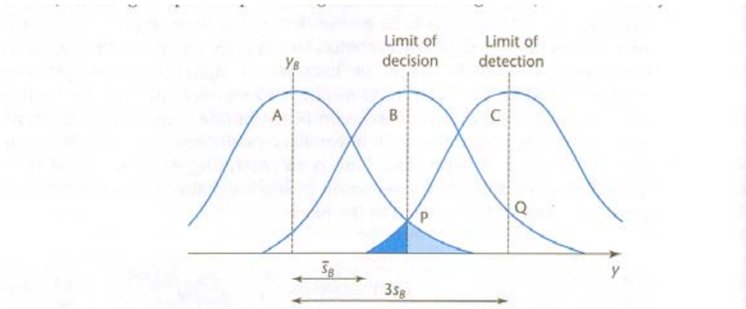


Figure 5.7 Definitions of the limit of decision and the limit of detection.

11

Calibration Methods: Regression & Correlation

Example 5.7.1

Estimate the limit of detection for the fluorescein determination studied in the previous sections.

We use equation (5.12) with the values of y_B(=a) and S_B(=$S_{y/x}$) previously calculated. The value of y at the limit of detection is found to be1.52 + 3 × 0.4329, i.e. 2.82. Use of the regression equation then yields a detection limit of 0.67 pg ml^{-1}. Figure 5.8 summarizes all the calculations performed on the fluorescein determination data.

12

Calibration Methods: Regression & Correlation

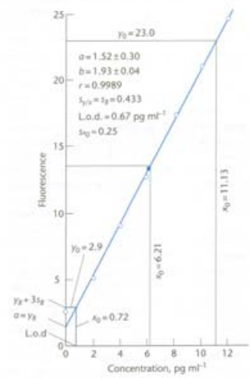


Figure 5.8 Summary of the calculations using the data in Example 5.3.1.

13

Calibration Methods: Regression & Correlation

<u>Example 5.3.1</u>

Standard aqueous solutions of fluorescein are examined in a fluorescence spectrometer and yield the following fluorescence intensities (in arbitrary units)?

Fluorescence intensities:

2.1	5.0	9.0	12.6	17.3	21.0	24.7

Concentration, pg ml^{-1}:

0	2	4	6	8	10	12

14

Calibration Methods: Regression & Correlation

	x_i	y_i	$xi-x$	$(x_i-x)^2$	y_i-y	$(y_i-y)^2$	$(x_i-x)(y_i-y)$
	0	2.1	-6	36	-11.0	121.00	66.0
	2	5.0	-4	16	-8.1	65.61	32.4
	4	9.0	-2	4	-4.1	16.81	8.2
	6	12.6	0	0	-0.5	0.25	0
	8	17.3	2	4	4.2	17.64	8.4
	10	21.0	4	16	7.9	62.41	31.6
	12	24.7	6	36	11.6	134.56	69.6
Sums:	42	91.7	0	112	0	418.28	216.2

The figures below the line at the foot of the columns are in each case the sums of the figures in the table: note that $\Sigma(x_i-\bar{x})$ and $\Sigma(y_i-\bar{y})$ are both zero. Using these totals in conjunction with equation (5.2), we have:

$$r = \frac{216.2}{\sqrt{112 \times 418.28}} = \frac{216.2}{216.44} = 0.9989$$

15

Calibration Methods: Regression & Correlation

Use of regression lines for comparing analytical methods

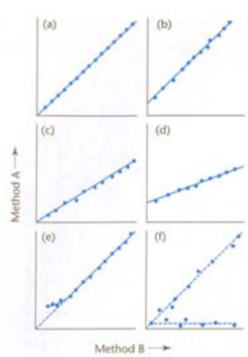


Figure 5.10 Use of a regression line to compare two analytical methods: (a) shows perfect agreement between the two methods for all the samples; (b)–(f) illustrate the results of various types of systematic error (see text).

16

Outliers

Treatment of Outliers

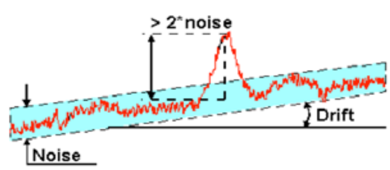
1. Re-examine for Gross Errors
2. Estimate Precision to be Expected
3. Repeat Analysis if Time and Sufficient Sample is Available
4. If Analysis can not be Repeated, Perform a Q-Test
5. If Q-Test Indicates Retention of Value, Consider Reporting the Median

17

Calibration Methods: Regression & Correlation

Limit of Detection (LOD) **-** lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

- mean of the blank sample plus 2 or 3 times the SD obtained on the blank sample (i.e., LOD = $mean_{blk} + Zs_{blk}$)



LOD calculation - alternative
Data required:
(1) calibration sensitivity = slope of line through the signals of the concentration standards including blank solution
(2) standard deviation for the analytical signal given by the blank solution

$$\text{LOD} = \frac{3x\ SD\ blank\ signals}{\text{slope of signals for std's}}$$

18