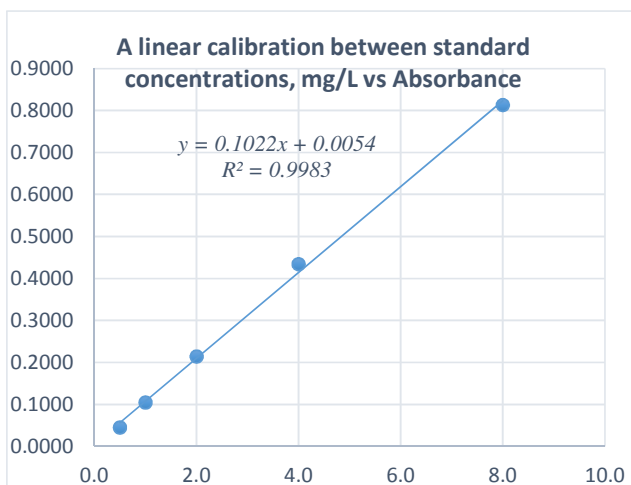# Minimum measurement error at the center of linear calibration curve

In any instrumental analysis, we use a series of working standards to calibrate the instrumental responses in terms of signals which can be in the form of absorbance, peak area or peak height.   To minimize calibration error, we always wish the calibration curve between the concentrations of the working standards ($x$'s) and the instrument responses ($y$'s) to be a straight line correlation in the form of $y = a + bx$ where $a$ is the $y$-intercept and $b$, the gradient of the linear regression. So, when we obtain an instrument response of an analyte from the sample prepared, we can calculate the amount of $x$ from the above linear equation.   We know that each instrument response is actually an average of many random signals with a normal distribution and hence the gradient and y-intercept of the linear calibration curve are having certain amounts of variation as well.

It is important to choose a dynamic range of the calibration curve which is right for your sample solution prepared for measurement.   Ideally the reading of the sample solution's instrument responses lie at about the center of the straight line curve, in order to have a better confidence interval. The following illustration will prove this point.

Suppose we have got a series of working analyte standard solutions ranging from 0.5 mg/L to 8.0 mg/L to calibrate a UV-Visible spectrometer. The data of standard concentrations vs color absorbance are summarized below:

| Conc. C, mg/L | Absorbance A |
|:---:|:---:|
| 0.5 | 0.045 |
| 1.0 | 0.105 |
| 2.0 | 0.214 |
| 4.0 | 0.434 |
| 8.0 | 0.813 |

**A linear calibration between standard concentrations, mg/L vs Absorbance**

$y = 0.1022x + 0.0054$
$R^2 = 0.9983$

To determine the confidence interval of the concentration of the analyte in the sample solution, $x_{meas}$ when its instrument response in terms of absorbance, $y_{meas}$ is measured, we use the following equation:

$$x_{meas} \pm t_{(n-2)} \frac{1}{b} SE(y) \sqrt{1 + \frac{1}{n} + \frac{(y_{meas} - \bar{y})^2}{b^2 \sum (x_i - \bar{x})^2}}$$

where

    $n$ is the number of calibration points $(x, y)$,

    $t_{(n-2)}$ is the Student's t-value at (n-2) degrees of freedom,

    $b$ is the gradient of the linear calibration curve, and,

    $SE(y)$ is the standard error of $y$ estimates, represented by:

$$SE(y) = \sqrt{\frac{\sum (y_{i,obs} - y_{i,cal})^2}{n-2}}$$

    $y_{i,obs}$ is the experimental observed absorbance at $i$th point,

    $y_{i,cal}$ is the calculated absorbance at $i$th point from the linear equation $y = a + bx$

In this example, the following data were obtained:

    the linear equation was    $y = 0.0054 + 0.1022x$

    $n = 5$,

    $SE(y) = 0.0147$,

    $t_{(n-1)} = t_4 = 2.78$, and,

    when $y_{meas}$ of a sample solution $= 0.368$, the $x_{meas} = 3.55$ mg/L $\pm$ 0.44 mg/L

Now, if we were to arbitrarily calculate a range of $x_{meas}$ based on a series of $y_{meas}$ and their respective confidence interval, we have:

| $y_{meas}$ | $x_{meas}$ | CI |
|------------|------------|-------|
| 0.050 | 0.436 | 0.471 |
| 0.100 | 0.926 | 0.460 |
| 0.200 | 1.904 | 0.445 |
| 0.300 | 2.883 | 0.438 |
| 0.400 | 3.861 | 0.440 |
| 0.500 | 4.840 | 0.452 |
| 0.600 | 5.819 | 0.472 |
| 0.700 | 6.797 | 0.500 |
| 0.800 | 7.776 | 0.534 |

From the above table, it is obvious that confidence intervals became narrower at the middle range of the calibration curve.    This is even clearer through plotting a graph of $x_{meas}$'s *vs* the respective confidence intervals *CI*:

A graph of $x_{meas}$ vs $CI$

$x_{meas} \rightarrow$