Calibration Examples

DIRECT CALIBRATION (single point)

A standard containing 1.0 ppm of NO_3-N was treated with colourizing agents to give an absorbance of 0.20 at 507 nm. A sample treated in the same manner, gave an absorbance of 0.15 at the same wavelength. Determine the concentration of nitrate-nitrogen in this sample.

$A_x = 0.15 \qquad A_s = 0.20$

$A_x \propto [x]$ & $A_s \propto [S]$

$$\therefore \frac{[x]}{[S]} = \frac{A_x}{A_s} \qquad \therefore [x] = \left(\frac{0.15}{0.20}\right)[1.0]$$

$$= 0.75 \text{ ppm } NO_3\text{-N}$$

DIRECT CALIBRATION (multiple point)

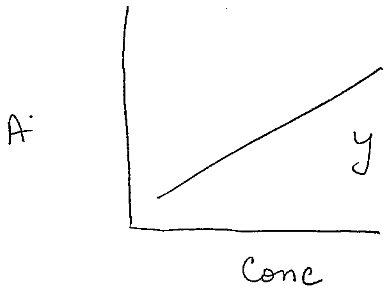
A calibration curve for the analysis of a protein is established using five calibration standards and found to yield a straight line over the range of 0 to 20 μg of protein. The equation of the calibration curve when the corrected absorbance is plotted versus the mass of protein in the sample is given by;

$$Y = 0.01630\ (\pm 0.00022)\ X + 0.0047\ (\pm 0.0026)$$

Find the quantity of protein for a sample that gives a measured absorbance of 0.264 when a blank has an absorbance of 0.095.

A_x = corrected absorbance for unknown sample $= 0.264 - 0.095$

$$= 0.169$$



$$y = 0.0163\,x + 0.0047$$

$$\therefore x = \frac{y - b}{m}$$

$$= \frac{0.169 - 0.0047}{0.01630}$$

$$= 10.1\ \mu g \text{ protein}$$

STANDARD ADDITION (with dilution)

An unknown sample of Cu^{2+} gave an absorbance of 0.262 in an atomic absorption analysis. Then 1.00 mL of solution containing 100.0 ppm Cu^{2+} was mixed with 95.0 mL of unknown and the mixture was diluted to 100.00 mL in a volumetric flask. The absorbance of the new solution was 0.500.

a) Denoting the initial unknown concentration as $[Cu^{2+}]_i$, write an expression for the final concentration $[Cu^{2+}]_f$ after dilution in units of concentration of ppm.

b) In a similar manner, write the final concentration of added standard, designated as $[S]_f$.

c) Find $[Cu^{2+}]_i$ in the unknown.

a) recall $C_1 V_1 = C_2 V_2$ for dilutions

$$\therefore \quad [Cu^{2+}]_f = [Cu^{2+}]_i \times \frac{V_i}{V_f} = [Cu^{2+}]_i \left(\frac{95}{100}\right)$$

b)

$$[S]_f = [S]_i \cdot \frac{V_i^{sp}}{V_f^{sp}} = (100.0 \text{ ppm})\left(\frac{1.00 \text{ mL}}{100. \text{ mL}}\right) = 1.00 \text{ ppm } Cu^{2+}$$

c)

$$A_x \propto [Cu^{2+}]_{\text{inital}}$$

$$A_{x+s} \propto [Cu^{2+}]_{\text{after spike}}$$

$$[Cu^{2+}]_{\text{after spike}} = [Cu^{2+}]_{\text{inital}} \cdot \frac{V_i}{V_f} + [Cu^{2+}]_{\text{spike}} \cdot \frac{V_i^{sp}}{V_f^{sp}}$$

$$= 0.95\,[Cu^{2+}]_i + 1.00 \text{ ppm } Cu^{2+}$$

$$\frac{A_x}{A_{x+s}} = \frac{[Cu^{2+}]_i}{0.95\,[Cu^{2+}]_i + 1.00 \text{ ppm}} = \frac{0.262}{0.500} = 0.524$$

$$\therefore [Cu^{2+}]_i = 0.497_8\,[Cu^{2+}]_i + 0.524 \text{ ppm}$$

$$0.502_2\,[Cu^{2+}]_i = 0.524 \text{ ppm}$$

$$[Cu^{2+}]_i = \frac{0.524}{0.502_2} = 1.04 \text{ ppm}$$

INTERNAL STANDARD (with dilution)

A solution containing 3.46 mM X (analyte) and 1.72 mM S (standard) gave peak areas of 3473 and 10,222, respectively, in a chromatographic analysis. Then 1.00 mL of 8.47 mM S was added to 5.00 mL of unknown X and the mixture was diluted to 10.0 mL. This solution gave peak areas of 5428 and 4431 for X and S, respectively.

a) Calculate the response factor for the analyte.
b) Find the concentration of S (mM) in the 10.0 mL of mixed solution.
c) Find the concentration of X (mM) in the 10.0 mL of mixed solution.
d) Find the concentration of X in the original unknown solution.

a) response factor, $F = \dfrac{A_x/[X]}{A_s/[S]}$

$$= \frac{(3473/3.47\ mM)}{(10{,}222/1.72\ mM)} = 0.168_3$$

b) $[S]_f = [S]_i \cdot \dfrac{V_i^{sp}}{V_f^{sp}}$

$$= (8.47\ mM)\left(\frac{1.00\ mL}{10.0\ mL}\right)$$

$$= 0.847\ mM$$

c) $\dfrac{A_x}{[X]_f} = F\left(\dfrac{A_s}{[S]_f}\right)$

$$\therefore [X]_f = \frac{A_x}{F} \cdot \left(\frac{[S]_f}{A_s}\right) = \frac{5428}{0.168_3} \cdot \frac{0.847\ mM}{4431}$$

$$= 6.61_5\ mM$$

d) $[X]_i = [X]_f \cdot \dfrac{V_f}{V_i} = (6.61_5\ mM) \cdot \dfrac{10.0\ mL}{5.00\ mL}$

$$= 12.3_3\ mM$$