Quality Assurance and Calibration Methods

Chapter 5

Range and Robustness

Range: concentration interval over which linearity, accuracy, and precision are all acceptable

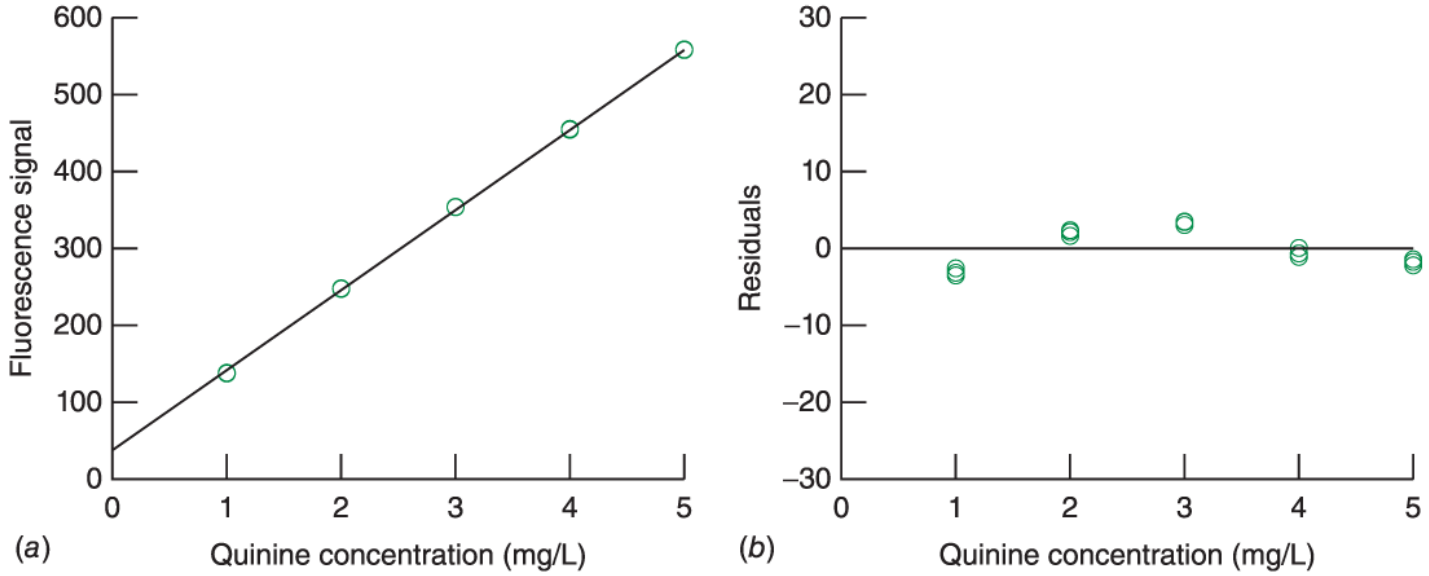
- **Linear range**: concentration range over which calibration curve is linear
- **Dynamic range:** concentration range over which there is measurable response

Robustness: ability of an analytical method to be unaffected by small, deliberate changes in operating parameters

Example: Fitness for Purpose of Linear Calibration (1 of 3)

A plot of the 1- to 5-mg/L quinine standards measured using fluorescence spectroscopy in Figure 5-2 yields the linear equation $y = 104.8x + 36.2$ with $R^2 = 0.999\ 7$.

Is this linear calibration fit for purpose for the determination of samples containing about 3 mg/L quinine if 1.50- and 4.50-mg/L quinine spikes of a blank solution yield signals of 194.0 and 504.8?



Example: Fitness for Purpose of Linear Calibration (2 of 3)

Solution: R^2 is greater than the 0.999 criterion. The residual plot shows much reduced residuals compared to those for the 1- to 10-mg/L calibration in Figure 5-2.

The most important determinant of fitness for purpose is accuracy, as determined by spike recovery. The quinine concentration corresponding to a reading of 194.0 is

$$x = \frac{y - 36.2}{104.8} = \frac{194.0 - 36.2}{104.8} = 1.506 \text{ mg/L}$$

for a spike recovery of 100.4%. For the 4.50-mg/L spike, the concentration is 4.47 mg/L and recovery is 99.3%. Both recoveries are within 100 ± 2%. The 1- to 5-mg/L standards include less than 0.5 times and more than 1.5 times the expected concentration. The linear calibration from 1 to 5 mg/L is fit for purpose.

Example: Fitness for Purpose of Linear Calibration (3 of 3)

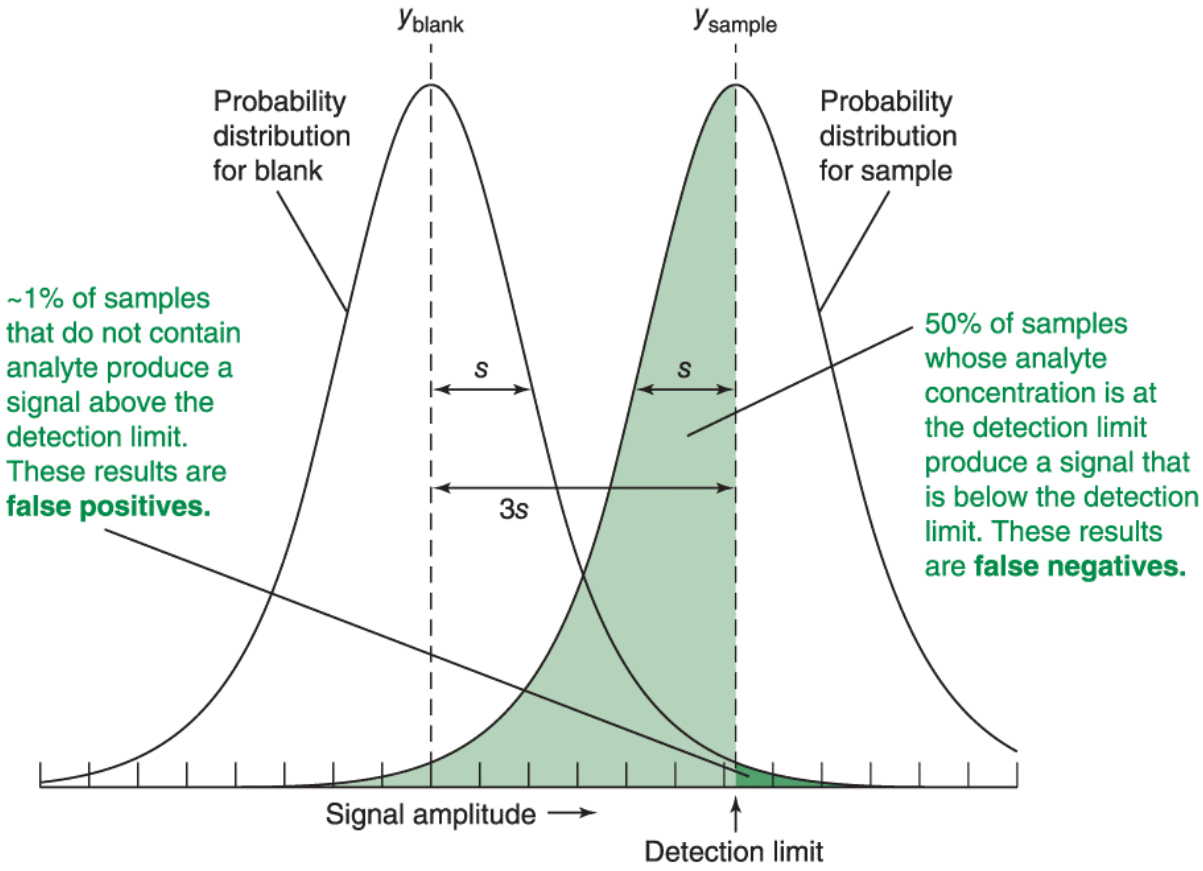
Test Yourself: If a blank spiked to 3.00 mg/L quinine gave a signal of 354.4, what is the predicted quinine concentration and its recovery?

Limits of Detection and Quantitation

Detection limit (lower limit of detection): smallest quantity of analyte that is *significantly different* from the blank

Quantitation limit (lower limit of quantitation): smallest quantity of analyte that can be measured with reasonable accuracy

Figure 5-3



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Detection Limit (1 of 2)

1. After estimating the detection limit from previous experience with the method, prepare a sample whose concentration is ~1 to 5 times the detection limit.
2. Measure the signal from n replicate samples ($n \geq 7$).
3. Compare the standard deviation (s) of the n measurements.
4. Measure the signal from n blanks (containing no analyte) and find the mean value, y_{blank}.
5. The minimum detectable signal, y_{dl}, is defined as

   Signal detection limit: $\mathbf{y_{dl} = y_{blank} + 3s}$

6. The corrected signal, $y_{sample} - y_{blank}$, is proportional to the sample concentration:

   Calibration line: $\mathbf{y_{sample} - y_{blank} = m \times \text{sample concentration}}$

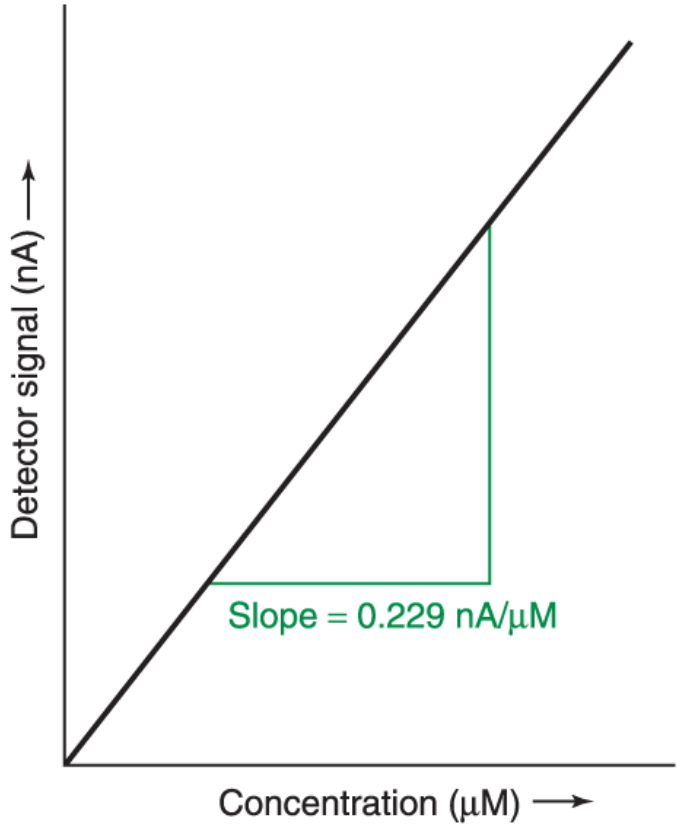
   where y_{sample} is the signal observed for the sample and m is the slope of the linear calibration curve. The *minimum detectable concentration* is obtained by substituting y_{dl} for y_{sample} to get

   Detection limit: $\mathbf{\text{minimum detectable concentration} \equiv \frac{3s}{m}}$

Detection Limit (2 of 2)

Another common way to define detection limit is from the least-squares equation of a calibration curve.

- **Detection limit (LOD)** $\equiv \frac{3s}{m}$
- **Quantitation limit (LOQ)** $\equiv \frac{10s}{m}$



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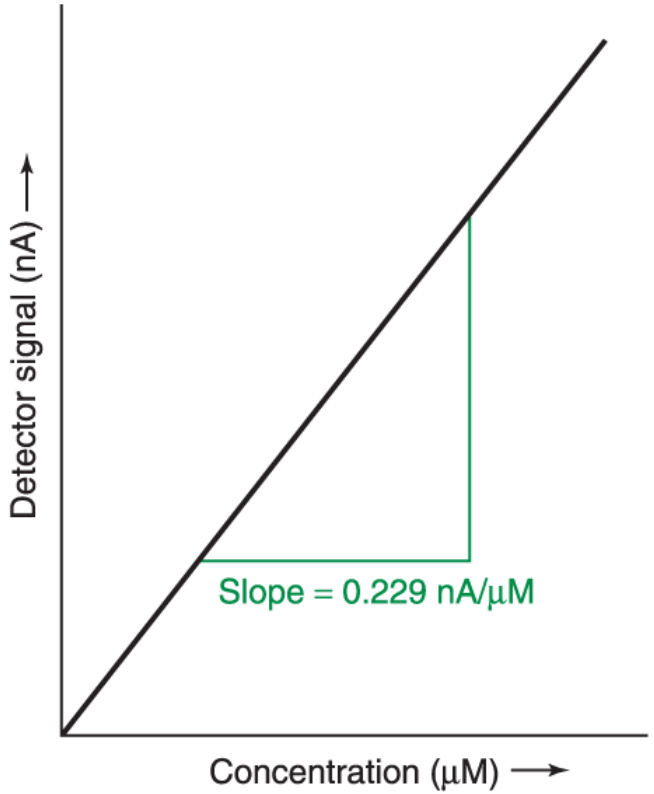
Example: Detection Limit (1 of 4)

From previous measurements of a low concentration of analyte, the signal detection limit was estimated to be in the low nanoampere range. Signals from seven replicate samples with a concentration about 3 times the detection limit were 5.0, 5.0, 5.2, 4.2, 4.6, 6.0, and 4.9 nA. Reagent blanks gave values of 1.4, 2.2, 1.7, 0.9, 0.4, 1.5, and 0.7 nA.

The slope of the calibration curve for higher concentrations is m = 0.229 nA/μM. **(a)** Find the signal detection limit and the minimum detectable concentration. **(b)** What is the concentration of analyte in a sample that gave a signal of 7.0 nA?



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Example: Detection Limit (2 of 4)

Solution: **(a)** First, compute the mean for the blanks and the standard deviation of the samples. Retain extra, insignificant digits to reduce round-off errors.

Blank: Average = $y_{blank} = 1.2_6$ nA

Sample: Standard deviation = $s = 0.5_6$ nA

The signal detection limit from Equation 5-4 is

$$y_{dl} = y_{blank} + 3s = 1.2_6 \text{ nA} + 3(0.5_6 \text{ nA}) = 2.9_4 \text{ nA}$$

The minimum detectable concentration is obtained from Equation 5-6:

$$\text{Detection limit} = \frac{3s}{m} = \frac{3(0.5_6 \text{ nA})}{0.229 \text{ nA}/\mu\text{M}} = 7._3 \ \mu\text{M} = 7 \ \mu\text{M}$$

Example: Detection Limit (3 of 4)

Solution: **(b)** To find the concentration of a sample whose signal is 7.0 nA, use Equation 5-5:

$$y_{\text{sample}} - y_{\text{blank}} = m \times \text{concentration}$$

$$\Rightarrow \text{concentration} = \frac{y_{\text{sample}} - y_{\text{blank}}}{m} = \frac{7.0 \text{ nA} - 1.2_6 \text{ nA}}{0.229 \text{ nA}/\mu\text{M}} = 25._1 \ \mu\text{M}$$

Example: Detection Limit (4 of 4)

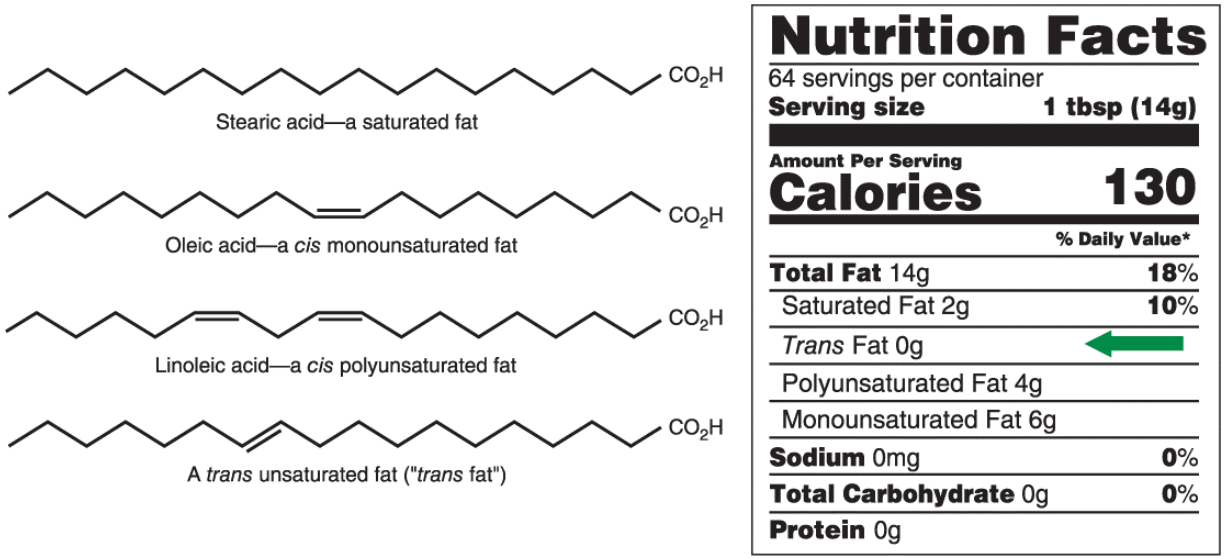
Test Yourself: Find the minimum detectable concentration if the average of the blanks is 1.0_5 nA and $s = 0.6_3$ nA.

Reporting Limit

Reporting limit is the concentration below which regulations say an analyte is "not detected."

- *It does not mean* that analyte is not observed. It means the analyte is below the prescribed level.
- The reporting limit is set at least 5 to 10 times higher than the detection limit.

Figure 5-4



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Section 5-3
Standard Addition

Standard Addition (1 of 3)

Standard addition: known quantities of the analyte added to the unknown

- Increase in signal indicates how much analyte was in the original unknown.
- This method requires a linear response to analyte concentration.
- Higher precision can be achieved when standards are added by mass instead of volume.

Especially appropriate when sample composition unknown, complex, or affects the analytical signal

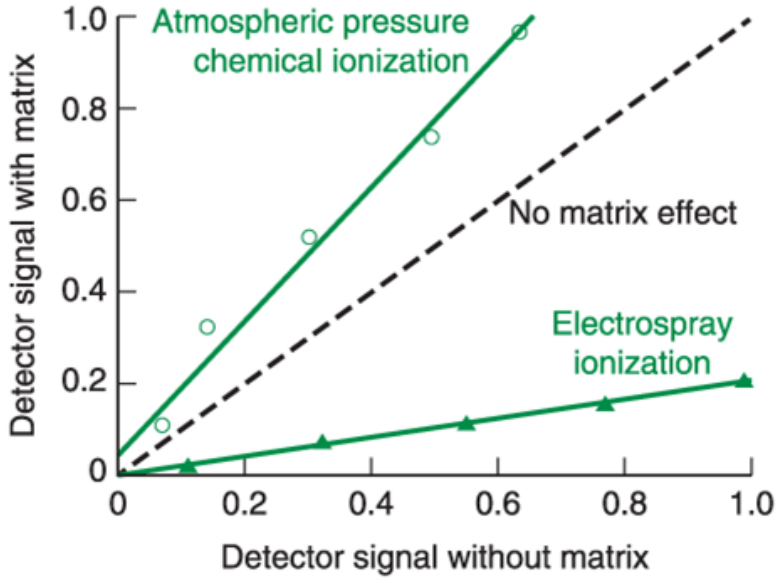
Standard Addition (2 of 3)

Matrix effect: change in analytical sensitivity caused by something in the sample other than analyte

- Sample composition affects the analytical signal.
- It is difficult to create standards and blanks whose composition matches that of the sample.
- Standards and blanks do not match the composition of the unknown.

Makes traditional calibration curves unreliable

Figure 5-5



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Standard Addition (3 of 3)

Signal is directly proportional to analyte concentration, so

$$\frac{\text{Concentration of analyte in inital solutions}}{\text{Concentration of analyte + standard in final solution}} = \frac{\text{signal from initial solution}}{\text{signal from final solution}}$$

$$\frac{[X]_i}{[X]_f + [S]_f} = \frac{I_X}{I_{X+S}}$$

$$[X]_f = [X]_i \left(\frac{V_i}{V_i + V_S} \right) \qquad [S]_f = [S]_i \left(\frac{V_S}{V_i + V_S} \right)$$

Example: Standard Addition (1 of 3)

Serum containing Na^+ gave a signal of 4.41 mV in an atomic emission analysis. Then 5.00 mL of 2.08 M NaCl were added to 95.0 mL of serum. This spiked serum gave a signal of 7.82 mV. Find the original concentration of Na^+ in the serum.

Example: Standard Addition (2 of 3)

Solution: From Equation 5-9, the final concentration of Na^+ after slight dilution by the spike of standard is $[X]_f = [X]_i\left(V_i/(V_i+V_s)\right) = [X]_i\left(95.0\text{ mL}/100.0\text{ mL}\right)$. The final concentration of added standard is $[S]_f = [S]_i\left(V_s/(V_i+V_s)\right) = (2.08\text{ M})\left(5.00\text{ mL}/100.0\text{ mL}\right) = 0.104\text{ M}$.

Equation 5-8 becomes

$$\frac{[Na^+]_i}{0.950[Na^+]_i + 0.104\text{ M}} = \frac{4.41\text{ mV}}{7.82\text{ mV}} \Rightarrow [Na^+]_i = 0.126\text{ M}$$

Example: Standard Addition (3 of 3)

Test Yourself: If spiked serum gave a signal of 7.09 mV, what was the original molarity of Na^+?

Graphical Procedure for Standard Addition to Single Solution

For successive standard additions to one solution:

$$\underbrace{I_{X+S}\left(\frac{V_i + V_S}{V_i}\right)}_{\text{Function to plot on } y\text{-axis}} = I_x + \frac{I_x}{[X]_i}\underbrace{[S]_i\left(\frac{V_S}{V_i}\right)}_{\text{Function to plot on } x\text{-axis}}$$

Figure 5-7

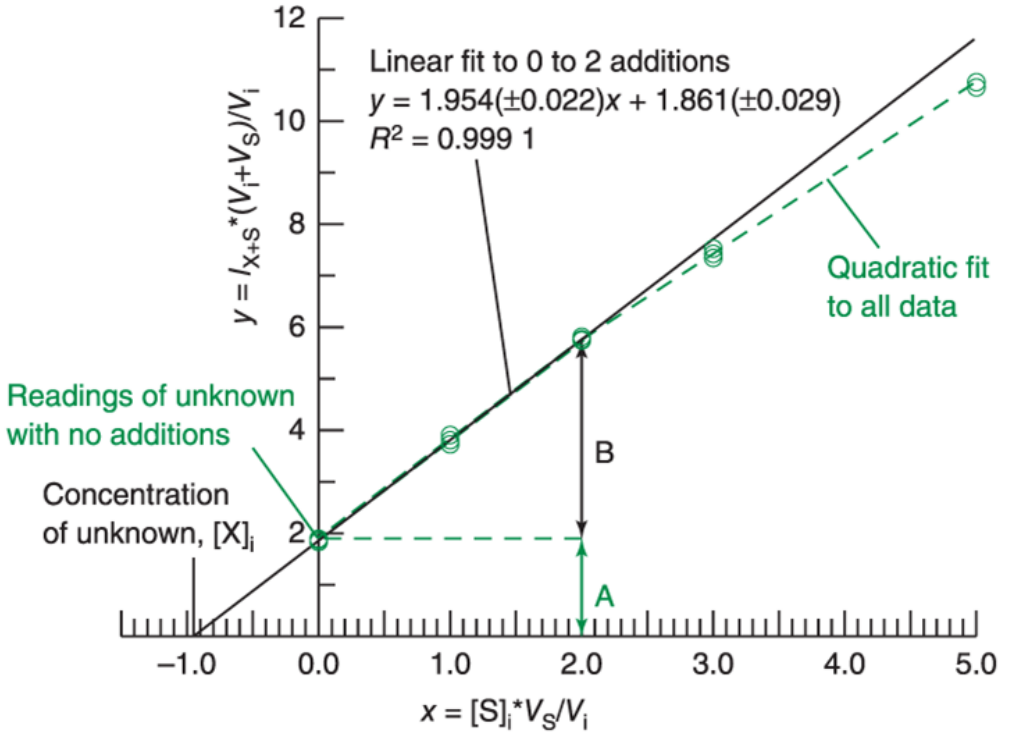

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Figure 5-6

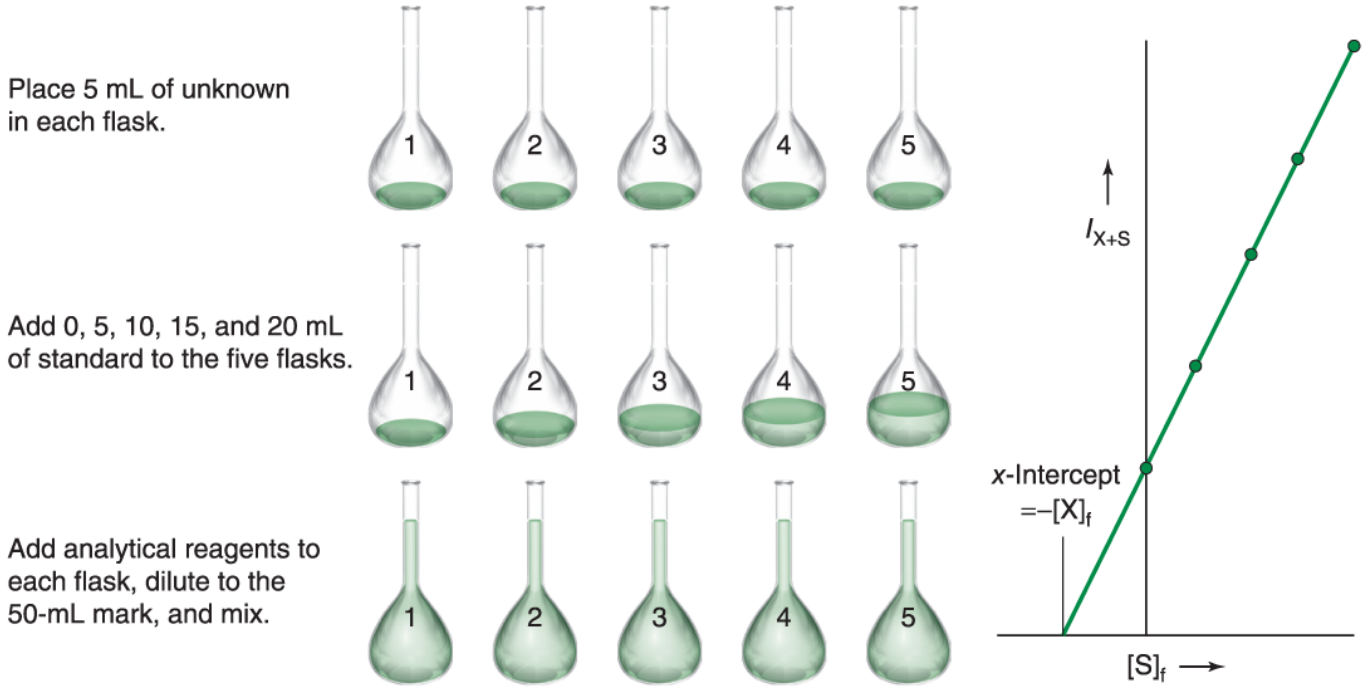
Data for standard addition experiment with variable total volume

	A	B	C	D	E
1	Arsenic standard addition experiment: Add 1000 ppb arsenic to 10.0 mL of water				
2					
3	V_i (mL) =	V_S = mL arsenic	I_{X+S} =	x-axis function	y-axis function
4	10.0	added	signal (μA)	S_i*V_S/V_i	$I_{X+S}*(V_i+V_S)/V_i$
5	$[S]_i$ (ppb) =	0.000	1.89	0.000	1.890
6	1000	0.000	1.87	0.000	1.870
7		0.000	1.83	0.000	1.830
8		0.010	3.90	1.000	3.904
9		0.010	3.72	1.000	3.724
10		0.010	3.80	1.000	3.804
11		0.020	5.75	2.000	5.762
12		0.020	5.80	2.000	5.812
13		0.020	5.73	2.000	5.741
14		0.030	7.40	3.000	7.422
15		0.030	7.50	3.000	7.523
16		0.030	7.32	3.000	7.342
17		0.050	10.70	5.000	10.754
18		0.050	10.60	5.000	10.653
19		0.050	10.70	5.000	10.754
20		D5 = $A$6*B5/$A$4		E5 = C5*($A$4+B5)/$A$4	

Graphical Procedure for Multiple Solutions with Constant Volume

- The equation of a line is $y = mx + b$.
- The *x*-intercept is obtained by setting $y = 0$:
  - $0 = mx + b$
  - *x*-intercept = $-b/m$
- The **magnitude of the *x*-intercept** is the final concentration of unknown in the *tested* sample.
- The initial concentration of unknown, $[X]_i$, is the final concentration of unknown, $[X]_f$, divided by the dilution factor.

Figure 5-8



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Example: Standard Addition with Constant Total Volume (1 of 3)

In Figure 5-8, 5.00 mL of unknown in each flask are diluted to 50.00 mL.

If the *x*-intercept is −0.235 mM, what is the original concentration of analyte in the unknown?

Figure 5-8

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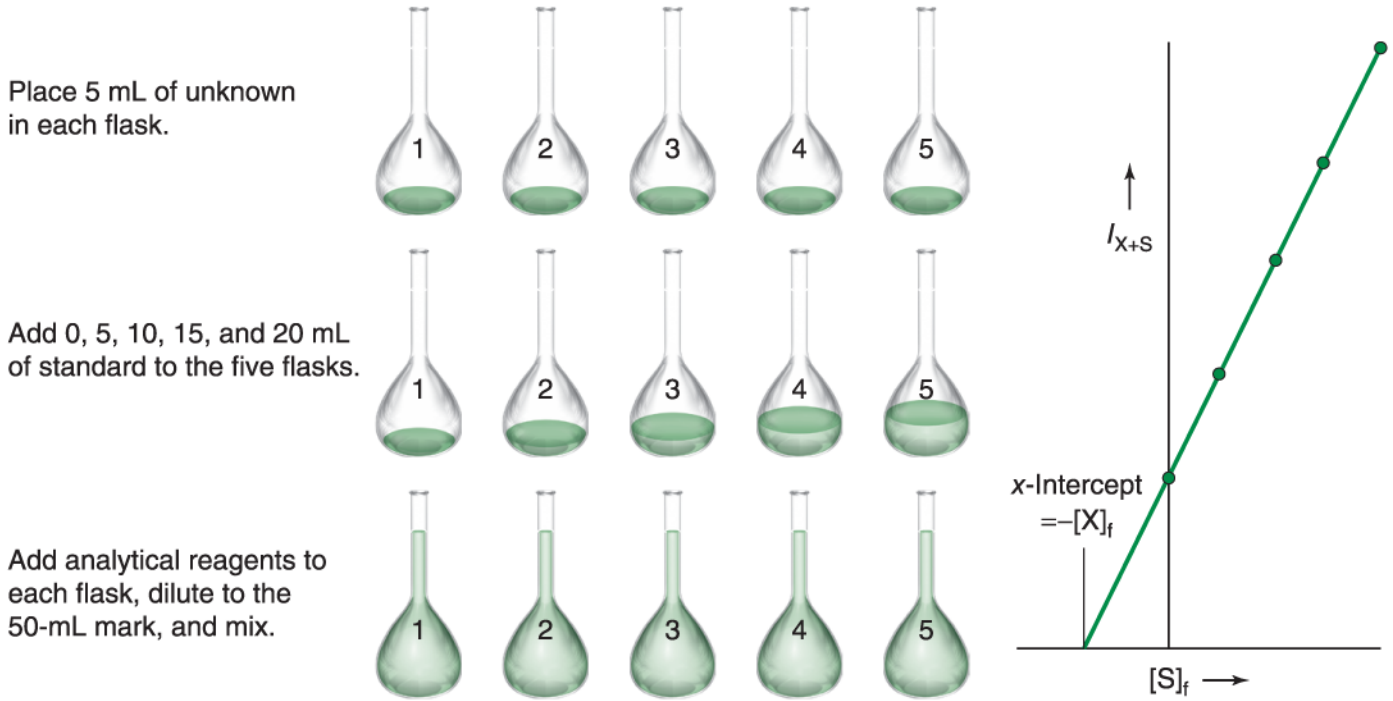
Example: Standard Addition with Constant Total Volume (2 of 3)

Solution: The dilution factor for the analyte is 5.00 mL/50.00 mL = 0.100. The magnitude of the *x*-intercept is the final concentration of diluted analyte, $[X]_f$. The original concentration was 1/0.100 = 10.00 times greater = 2.35 mM.

Example: Standard Addition with Constant Total Volume (3 of 3)

Test Yourself: In each flask of a standard addition experiment like Figure 5-8, 1.00 mL of blood serum was diluted to 25.00 mL to measure a hormone with a molecular mass of 373 g/mol. The *x*-intercept of the graph was −4.2 ppb (parts per billion). Find the hormone concentration in the serum and express your answer in ppb and molarity. Assume that the density of serum and all solutions is close to 1.00 g/mL.

Figure 5-8



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Section 5-4
Internal Standards

Standards

Standard addition: known amount of a compound—*same substance as analyte*—added to the unknown

Internal standards: known amount of a compound—*different from analyte*—added to the unknown

External standards: solutions with known concentrations of analyte used to prepare a calibration curve