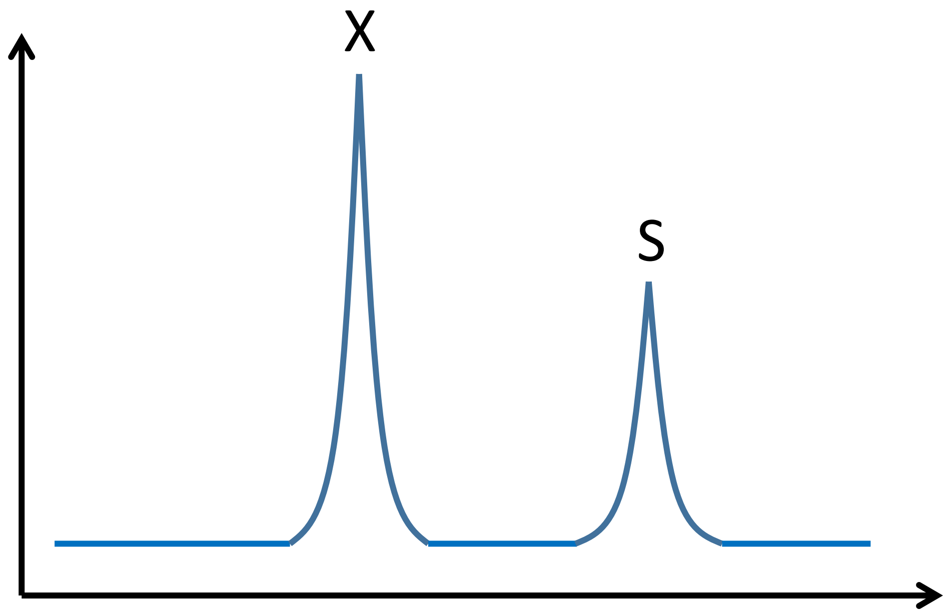
Internal Standard

- Internal standard is a known amount of compound, different from the analyte, that is added to an unknown sample.
- Signal from analyte is compared with signal from standard to quantify analyte.



Response Factor (F)

$$\frac{A_x}{C_x} = \mathrm{F} \times \frac{A_s}{C_s}$$

A: peak area   C: concentration

Step 1: determine F ⟶
- Internal standard (A_s, C_s)
- analyte with known concentration (A_x, C_x)

Step 2: determine the same analyte with unknown concentration

Practice

- In a chromatographic equipment, a solution containing 0.0837M X and 0.0666M S give peak area of A_x= 423 and A_s= 347.

  To analyze an unknown sample, 10.0mL of 0.146M S was added to 10.0mL of X, and the mixture was diluted to 25.0mL. This mixture gave a chromatography spectrum with area A_x= 553 and A_s= 582. Find C_x.

- Step 1: determine F

$$\frac{A_x}{C_x} = \mathrm{F} \times \frac{A_s}{C_s} \longrightarrow \frac{423}{0.0837M} = \mathrm{F} \times \frac{347}{0.0666M}$$

F = 0.970

- Step 2: determine C_x after dilution

$$\mathrm{C_s} = 0.146M \times \frac{10.0\,mL}{25.0\,mL} = 0.0584M$$

$$\frac{A_x}{C_x} = \mathrm{F} \times \frac{A_s}{C_s} \longrightarrow \frac{553}{C_x} = 0.970 \times \frac{582}{0.0584M} \longrightarrow C_x = 0.0572\mathrm{M}$$

- Step 3: determine C_x in unknown sample

$$\mathrm{C_s} = 0.0572M \times \frac{25.0\,mL}{10.0\,mL} = 0.143M$$

Standard Addition (Spiking)

- A known analyte with concentration gradient is added to the sample.
- Signal of this known analyte is measured to help us determine the concentration in the original sample.
- Key assumption: signal is proportional to the concentration of analyte.

$$\frac{X_i}{X_f + S_f} = \frac{I_x}{I_{x+s}}$$

X_i: unknown initial concentration of analyte (before adding standard) ⟶ I_x

X_f: unknown concentration of analyte (after adding standard)

S_f: known concentration of standard (after adding standard)

} ⟶ I_{x+s}

Dilution:

$$X_f = X_i \times \frac{V_0}{V}$$

$$S_f = S_i \times \frac{V_s}{V}$$

$$V = V_0 + V_s$$

V_0: initial volume of unknown sample

V_s: volume of standard added

Practice

- Ascorbic acid (Vitamin C) in a 50.0mL sample of orange juice was analyzed by an electrochemical to get a detecting current of 1.78μA. A standard addition of 0.400mL of 0.279M ascorbic acid increased the current to 3.35μA. Find the concentration of ascorbic acid in the juice.

$$V = V_0 + V_s = 50.0mL + 0.400mL = 50.4mL$$

$$X_f = X_i \times \frac{V_0}{V} \longrightarrow X_f = X_i \times \frac{50.0mL}{50.4mL}$$

$$S_f = S_i \times \frac{V_s}{V} \longrightarrow S_f = 0.279M \times \frac{0.400mL}{50.4mL} = 0.00221M$$

$$\frac{X_i}{X_f + S_f} = \frac{I_x}{I_{x+s}} \longrightarrow \frac{X_i}{X_i \times \frac{50.0mL}{50.4mL} + 0.00221M} = \frac{1.78\mu A}{3.35\mu A} \longrightarrow X_i = 0.00249M$$

- More accurate: more standards added to sample → Calibration Curve

$$\frac{X_i}{X_f + S_f} = \frac{I_x}{I_{x+s}} \quad \longrightarrow \quad \frac{X_i}{X_i \times \frac{V_0}{V} + S_i \times \frac{V_s}{V}} = \frac{I_x}{I_{x+s}}$$

$$X_f = X_i \times \frac{V_0}{V} \qquad S_f = S_i \times \frac{V_s}{V}$$

Rearrange

$$I_{x+s} \times X_i = I_x \times X_i \times \frac{V_0}{V} + I_x \times S_i \times \frac{V_s}{V}$$

Multiply by $\frac{V}{X_i \times V_0}$

$$I_{x+s} \times \frac{V}{V_0} = I_x + \frac{I_x}{X_i} \times S_i \times \frac{V_s}{V_0}$$

Y-axis

X-axis