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)

\[ \frac{A_x}{C_x} = F \times \frac{A_s}{C_s} \]

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 $F$

\[
\frac{A_x}{C_x} = F \times \frac{A_s}{C_s} \quad \rightarrow \quad \frac{423}{0.0837M} = F \times \frac{347}{0.0666M}
\]

$F = 0.970$

• Step 2: determine $C_x$ after dilution

\[
C_s = 0.146M \times \frac{10.0\text{ mL}}{25.0\text{ mL}} = 0.0584M
\]

\[
\frac{A_x}{C_x} = F \times \frac{A_s}{C_s} \quad \rightarrow \quad \frac{553}{C_x} = 0.970 \times \frac{582}{0.0584M}
\]

\[
C_x = 0.0572M
\]

• Step 3: determine $C_x$ in unknown sample

\[
C_s = 0.0572M \times \frac{25.0\text{ mL}}{10.0\text{ mL}} = 0.143M
\]
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) \(\rightarrow I_x\)

\(X_f\): unknown concentration of analyte (after adding standard)

\(S_f\): known concentration of standard (after adding standard) \(\rightarrow I_{x+s}\)

Dilution:

\[X_f = X_i \times \frac{V_0}{V}\]

\[V = V_0 + V_s\]

\[S_f = S_i \times \frac{V_s}{V}\]

\(V_0\): initial volume of unknown sample

\(V_s\): volume of standard added
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.
\[ V = V_0 + V_s = 50.0\text{mL} + 0.400\text{mL} = 50.4\text{mL} \]

\[ X_f = X_i \times \frac{V_0}{V} \quad \rightarrow \quad X_f = X_i \times \frac{50.0\text{mL}}{50.4\text{mL}} \]

\[ S_f = S_i \times \frac{V_s}{V} \quad \rightarrow \quad S_f = 0.279M \times \frac{0.400\text{mL}}{50.4\text{mL}} = 0.00221M \]

\[ \frac{X_i}{X_f + S_f} = \frac{I_x}{I_x} \quad \rightarrow \quad \frac{X_i}{X_i \times \frac{50.0\text{mL}}{50.4\text{mL}} + 0.00221M} = \frac{1.78\mu\text{A}}{3.35\mu\text{A}} \quad \rightarrow \quad X_i = 0.00249M \]
• More accurate: more standards added to sample $\quad$ Calibration Curve

\[
\frac{X_i}{X_f + S_f} = \frac{I_x}{I_{x-s}}
\]

\[
X_f = X_i \times \frac{V_0}{V} \quad S_f = S_i \times \frac{V_s}{V}
\]

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

Rearrange

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

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

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

$Y$-axis

$X$-axis

original concentration of analyte