Steps in a Typical Mass Spec Quantitative Analysis

1. Take aliquot
2. Add int. std.
3. Solubilize, etc.
4. Clean up, derivatize, concentrate
5. Inject into mass spec
6. Measure ion ratio of analyte v. internal standard

Sample

Intensity

analyte

int. std.

time
Effect of Adding An Isotopic Internal Standard

Case A: No Isotopic Overlap

Mass Spectrum

Selected-Ion-Monitoring Chromatogram

“Natural” Analyte

M: analyte

M+b: int. std.
Recommended Process for Achieving Fitness for Purpose

Examine what’s at stake
Set targets for measurement uncertainty and identification confidence

Define the Method

Assess Uncertainty
Describe the method’s measurement uncertainty and identification confidence

Targets met?
Yes
Fit for Purpose

No
Factors That Predict Method Transfer Success

Assumption: “Method worked in our lab so it should work in others”

- Method Development
- Method Validation
- Transfer & Validation
- Sample Analysis

- How Extensive?
- Repeatability
- Reproducibility
- Ruggedness
- Robustness

➢ How rugged, robust and reliable is the method to begin with?
How Much Information Do You Want With Your Regression Line?

Limited results with Excel:

\[ y = 0.925x + 0.247 \quad R^2 = 0.959 \]

Or regression results with error bars:

\[ \hat{y} = 0.25 \pm 0.49 + (0.925 \pm 0.095)x \]

~10% relative error in the intercept

~200% relative error in the slope
Method Validation for Endogenous Compounds

- We normally make a **standard curve** of analyte versus internal standard and measure the MS ion ratio.
- A linear relationship validates the method.
- But, what about in a real matrix?

- Prepare a **2nd standard curve by adding known amounts to a real matrix** to validate linearity.
  - Slopes of the 2 curves should be the same.