Steps in a Typical Mass Spec Quantitative Analysis

Sample

(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

Intensity

analyte

int. std.

time

1
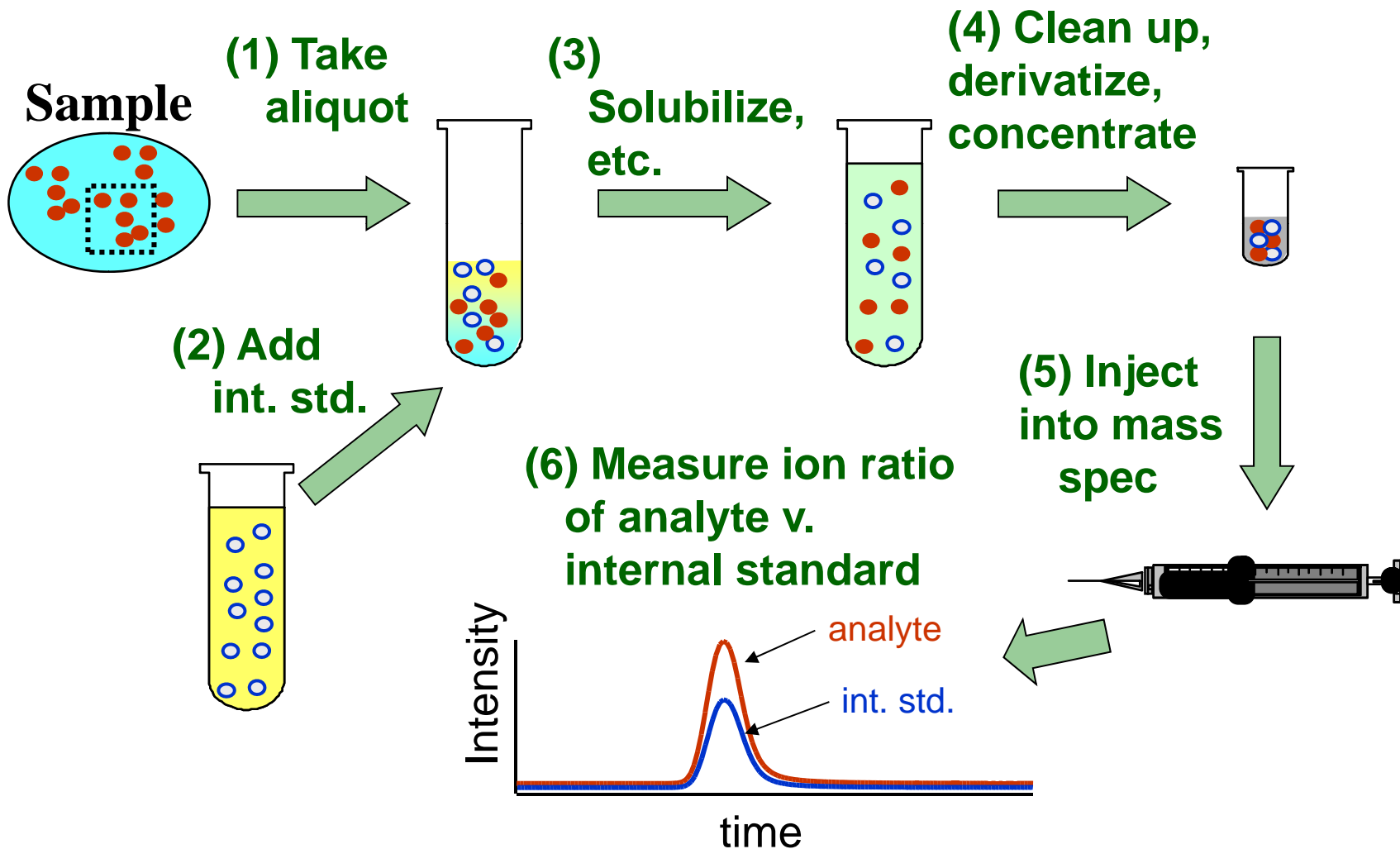
Effect of Adding An Isotopic Internal Standard
Case A: No Isotopic Overlap

Mass Spectrum

Selected-Ion-Monitoring Chromatogram

Intensity

M

"Natural" Analyte

m/z

Intensity

M

time

Intensity

M

M+b   +Int Std

0   6

m/z

Intensity

M: analyte

M+b: int. std.

time
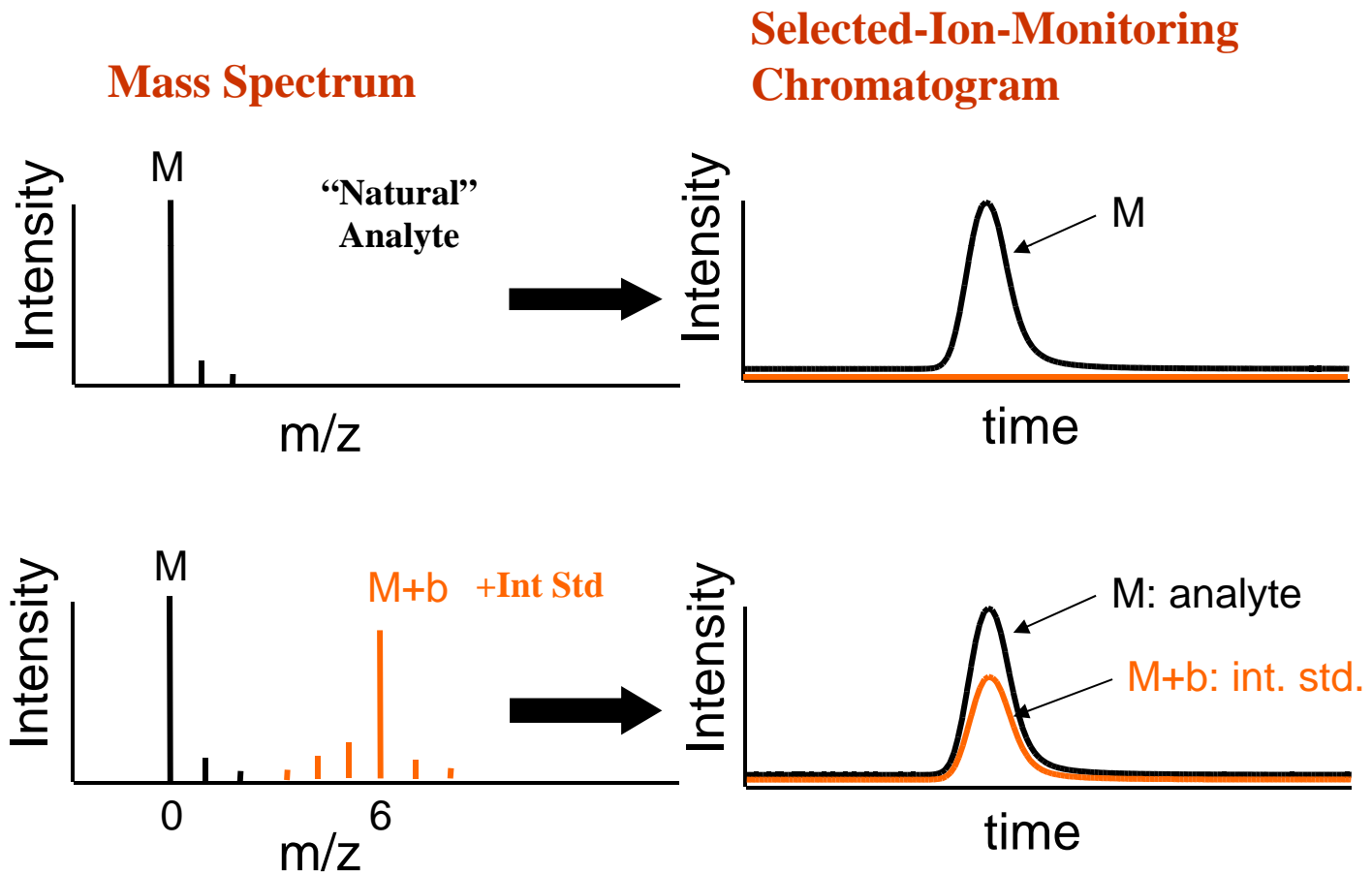
Recommended Process for Achieving Fitness for Purpose

```
┌─────────────────────────────────────────┐
│  Examine what's at stake                 │
│                                          │
│  Set targets for measurement uncertainty │
│       and identification confidence      │
└─────────────────────────────────────────┘
                    │
                    ▼
        ┌───────────────────────────┐          No          ◇ Targets met? ◇
        │    Define the Method       │ ◄─────────────────
        └───────────────────────────┘
                    │
                    ▼                                              │ Yes
┌─────────────────────────────────────────┐                      ▼
│     Assess Uncertainty                   │ - - - ►   ┌──────────────────────┐
│                                          │           │   Fit for Purpose     │
│  Describe the method's measurement       │           └──────────────────────┘
│  uncertainty and identification confidence│
└─────────────────────────────────────────┘
```
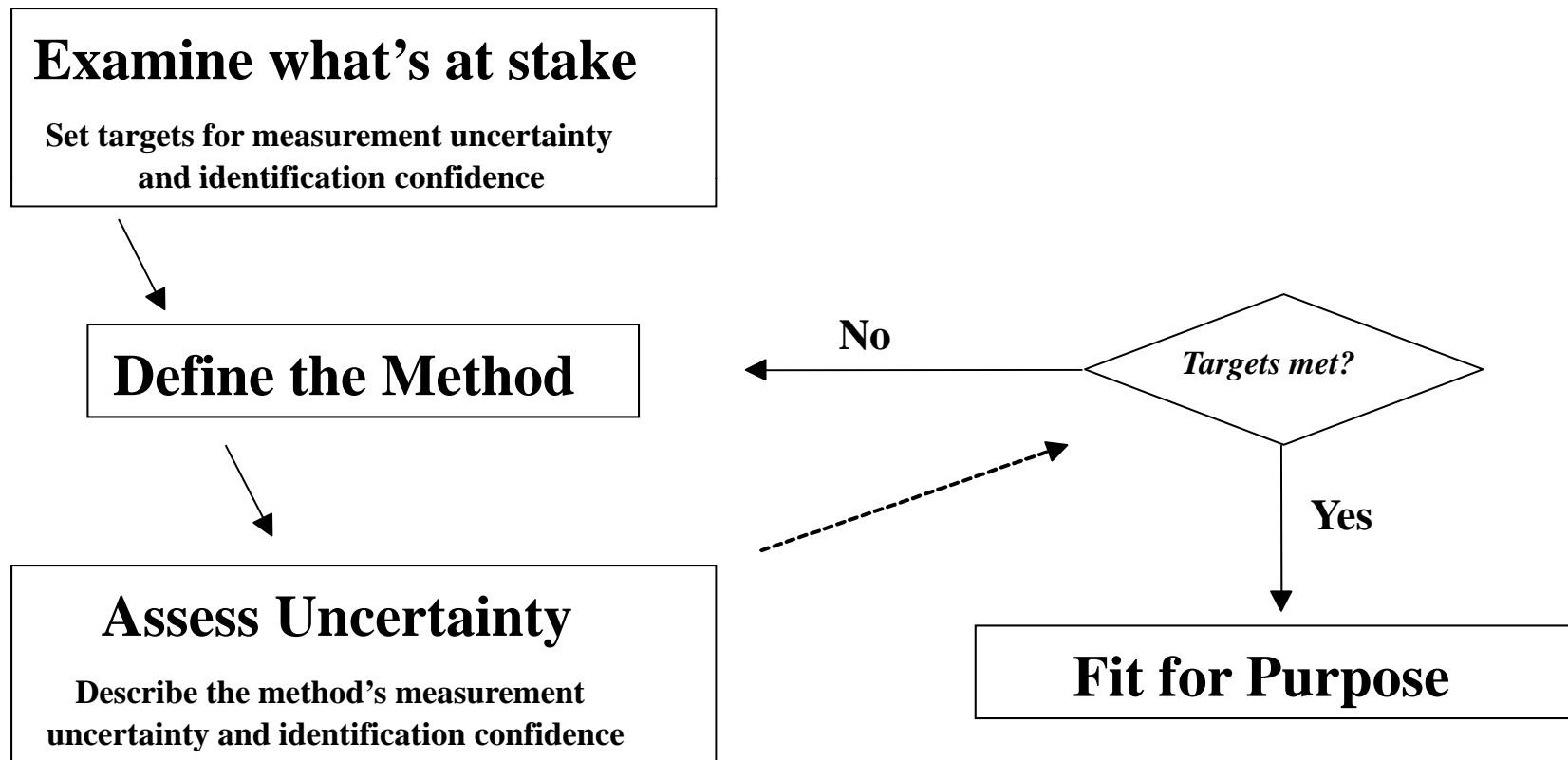
Factors That Predict Method Transfer Success

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
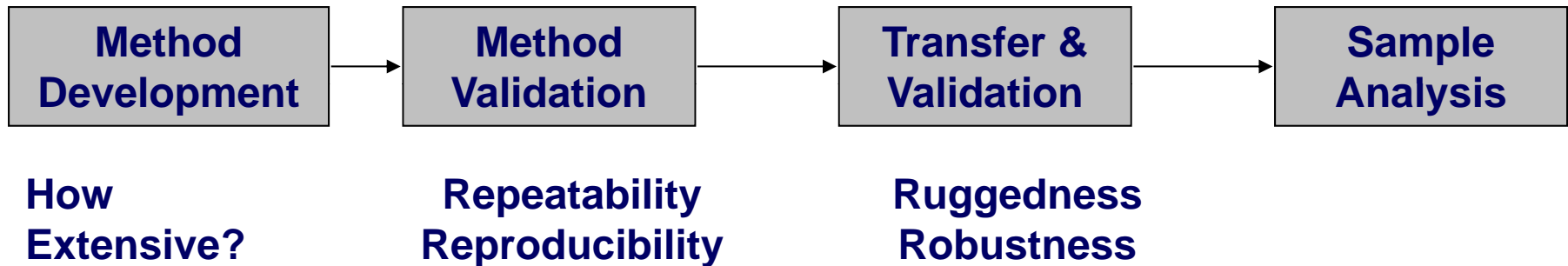
➢ How rugged, robust and reliable is the method to begin with?

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:*

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

MS *"observed"* measurements

Expected values of standards

~200% relative error in the intercept

~10% relative error in the slope

5
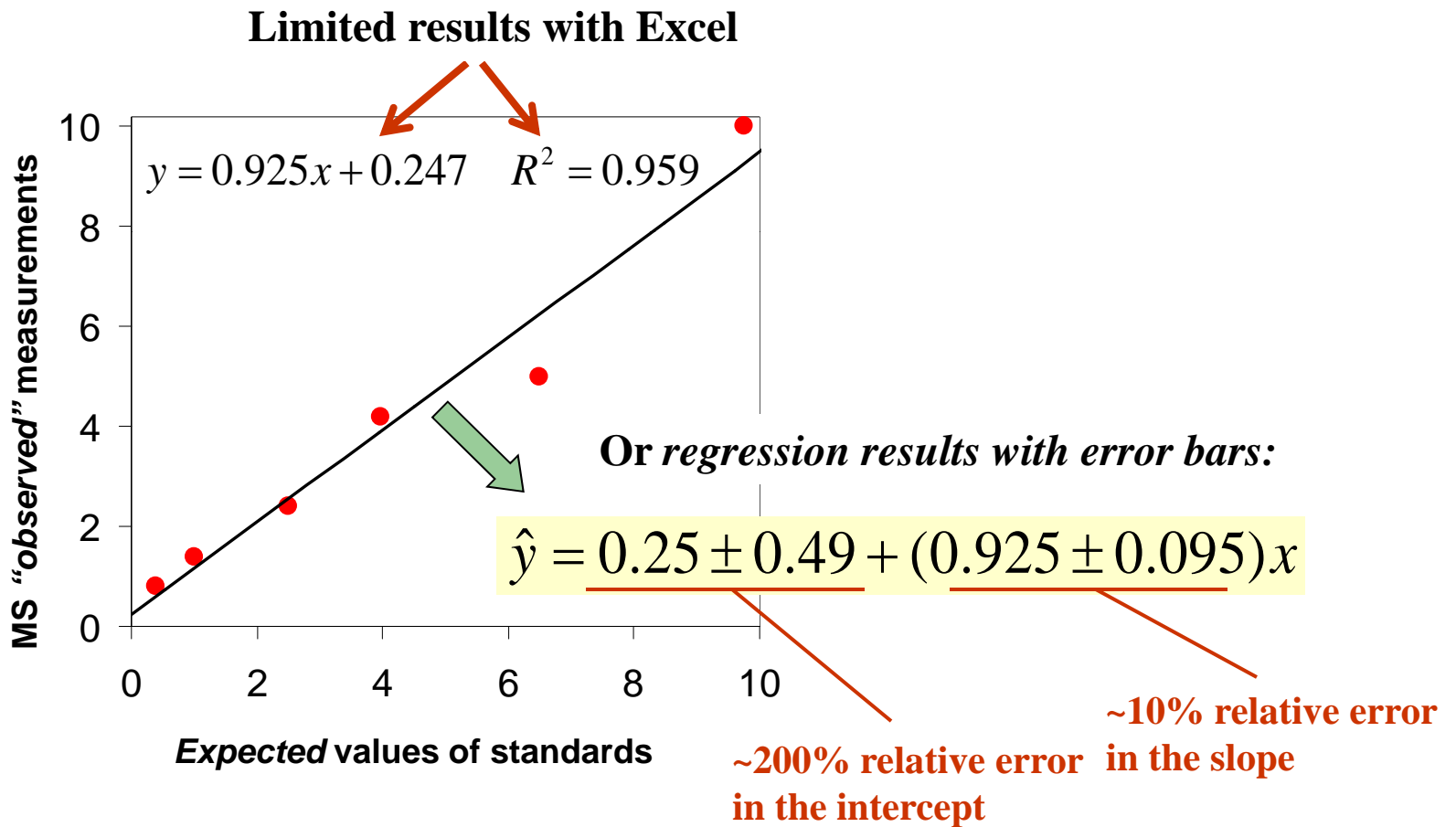
Method Validation for Endogenous Compounds

◆ 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

Real matrix

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