1 Mass Balance Diagnostic Framework

1.1 Inputs

For each forced degradation experiment, we define:

- A = Remaining API (%)

- $D_1, D_2, \ldots$ = Individual degradant percentages

- $D = \sum_i D_i$ = Total degradants (%)

- σ = Analytical variability (%), assumed constant (e.g., $\sigma = 2.0$)

- Initial API = 100%

1.2 Core Metrics

1.2.1 Absolute Mass Balance (AMB)

$$\text{AMB} = A + D$$

This represents the total recovered material in the assay.

1.2.2 Relative Mass Balance (RMB)

$$\text{RMB} = \frac{D}{100 - A}$$

This measures how well the observed degradants explain the loss of API.

1.2.3 Z_{MB}: Uncertainty-Normalized Mass Balance Deviation

$$Z_{MB} = \frac{\text{AMB} - 100}{\sigma}$$

This expresses the mass balance deviation in units of analytical standard deviations.

1.2.4 Interpretation of Z_{MB}

$$|Z_{MB}| < 1 \Rightarrow Within analytical noise$$

$$1 \leq |Z_{MB}| < 2 \Rightarrow Borderline$$

$$|Z_{MB}| \geq 2 \Rightarrow Significant imbalance$$

1.3 AMB–RMB Diagnostic Map

A two-dimensional diagnostic map is constructed with:

- X-axis: AMB

- Y-axis: RMB

The following fixed diagnostic zones are defined:

Zone 1: Good / Consistent

$$98 \leq \text{AMB} \leq 102, \quad 0.8 \leq \text{RMB} \leq 1.2$$

Interpretation: Everything is consistent.

Zone 2: Missing Degradants

$$\text{AMB} < 98, \quad \text{RMB} < 0.8$$

Interpretation: API loss is observed, but degradants are not sufficiently detected.

Zone 3: Physical Loss

$$\text{AMB} < 98, \quad 0.8 \leq \text{RMB} \leq 1.2$$

Interpretation: Degradants explain API loss, but total mass is still missing (e.g., volatility, adsorption).

Zone 4: Overestimation / Noise

$$\text{AMB} > 102, \quad \text{RMB} > 1.2$$

Interpretation: Possible over-integration, co-elution, or analytical artifacts.

1.4 Final Decision Logic

Step 1: Significance Check

If:
$$|Z_{MB}| < 1$$

Then:
$$FinalResult : Within analytical variability (No action)$$

Step 2: If Significant ($|Z_{MB}| \geq 1$), Use Zone Classification

- Zone 1 $\Rightarrow$ Borderline but consistent, monitor

- Zone 2 $\Rightarrow$ Missing degradants $\Rightarrow$ improve detection

- Zone 3 $\Rightarrow$ Physical loss $\Rightarrow$ investigate volatility/adsorption

- Zone 4 $\Rightarrow$ Overestimation $\Rightarrow$ check method/peaks

1.5 Comparison with Traditional Criterion

Baseline (Conventional Rule)

$$If\, \text{AMB} < 98 \Rightarrow Investigate$$

$$Else \Rightarrow Pass$$

Limitation

This approach ignores:

- Analytical uncertainty

- Whether degradants explain API loss

- The diagnostic nature of the imbalance

Proposed Framework

The proposed method provides:

- Statistical significance via Z_{MB}

- Mechanistic interpretation via the AMB–RMB map

- Actionable diagnostic categories instead of a binary pass/fail