

# 1 Mass Balance Diagnostic Framework — Core Methodology

## 1.1 Inputs

For each forced degradation experiment, the following quantities are defined:

- $A$  = Remaining API assay (%)
- $D_1, D_2, \dots$  = Individual degradation product levels (%)
- $D = \sum_i D_i$  = Total degradants (%)
- $\sigma$  = Analytical variability (%), derived from method precision
- Initial API content = 100%

The analytical variability term  $\sigma$  is obtained from intermediate precision studies in accordance with ICH Q2(R1), calculated as the pooled standard deviation of API assay and degradant recovery across replicate measurements ( $n \geq 6$ ). In early development phases where full validation data are unavailable, conservative or literature-informed estimates of  $\sigma$  may be applied without altering the qualitative diagnostic logic.

## 1.2 Core Metrics

### 1.2.1 Absolute Mass Balance (AMB)

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

AMB represents the total recovered drug-related material following stress exposure.

### 1.2.2 Relative Mass Balance (RMB)

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

RMB measures how effectively observed degradants account for the measured loss of API.

### 1.2.3 $Z_{\text{MB}}$ : Uncertainty-Normalized Mass Balance Deviation

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

$Z_{\text{MB}}$  expresses mass balance deviation in units of analytical standard deviation, enabling statistical assessment of significance.

#### 1.2.4 Interpretation of $Z_{MB}$

$|Z_{MB}| < 1 \Rightarrow$  Analytically insignificant (expected noise)

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

$|Z_{MB}| \geq 2 \Rightarrow$  Statistically significant imbalance (95% confidence)

Thresholds of  $|Z_{MB}| \geq 3$  may be used for high-confidence investigation triggers (99% confidence), depending on development stage and risk tolerance.

### 1.3 AMB–RMB Diagnostic Map

A two-dimensional diagnostic space is defined with:

- X-axis: Absolute Mass Balance (AMB)
- Y-axis: Relative Mass Balance (RMB)

Joint interpretation of AMB and RMB enables mechanistic classification of mass balance behavior beyond single-value thresholds.

#### Zone 1: Consistent Recovery

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

Interpretation: Observed deviation is consistent with analytical variability.

#### Zone 2: Missing or Non-Chromophoric Degradants

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

Interpretation: API loss is observed, but degradants are not sufficiently detected (e.g., non-UV-active species, co-elution).

#### Zone 3: Physical Loss Mechanisms

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

Interpretation: Degradants explain API loss, but total mass is missing, suggesting adsorption, volatility, or sample handling losses.

#### Zone 4: Analytical Overestimation

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

Interpretation: Potential over-integration, co-elution, or incorrect relative response factors (RRF).

## 1.4 Systematic Bias and Response Factor Assessment

Consistent directional deviation of  $Z_{MB}$  across multiple stress conditions or time points suggests a systematic analytical bias rather than random recovery error. Such behavior is indicative of mismatched relative response factors between the API and degradants and warrants targeted RRF verification rather than broad method redevelopment.

## 1.5 Final Decision Logic

### Step 1: Statistical Significance Check

If:

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

Then:

Final Result: Within analytical variability (No action required)

### Step 2: Diagnostic Classification (If $|Z_{MB}| \geq 1$ )

- Zone 1  $\Rightarrow$  Borderline but consistent; monitor trend
- Zone 2  $\Rightarrow$  Missing degradants; apply orthogonal detection (e.g., LC–MS)
- Zone 3  $\Rightarrow$  Physical loss; investigate adsorption or volatility
- Zone 4  $\Rightarrow$  Overestimation; verify RRF and integration parameters

## 1.6 Comparison with Traditional Criterion

### Conventional Rule

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

### Limitation

This binary approach ignores analytical uncertainty, degradant recovery behavior, and mechanistic diagnosis, often leading to false-positive investigations.

### Proposed Diagnostic Approach

The framework replaces rigid pass/fail logic with:

- Statistical significance assessment via  $Z_{MB}$
- Mechanistic insight via AMB–RMB joint interpretation
- Actionable, risk-based recommendations

This enables consistent, regulator-aligned, and scientifically justified mass balance interpretation.