Method Development and Validation: RMID for Regulated Industries

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Traditional time-consuming analytical techniques for Raw Material Identification and Verification (RMID) are being replaced by spectroscopic methods such as handheld Raman. In-situ RMID methods must accommodate new types of users, warehouse conditions, and faster sampling strategies without compromising the success of the method. This 4-part series guides Mira P users through method building, method validation and implementation.

Part III defines method validation and describes proper validation procedures for RMID verification methods with Mira P.

Part I: General Considerations
Part II: Method Development
Part III: Method Robustness and Validation
Part IV: Method Implementation

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Part III – Method Robustness and Validation

Validation

According to the International Conference on Harmonization (ICH) guidelines, «*the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose*». [1] This encompasses many aspects of a method:

- the ability of the analytical method to adequately assess the material, both inherently and discriminately
- robustness and reproducibility of the method
- whether the sampling technique is appropriate to the configuration of the sample (including packaging)
- whether the sampling technique will be successful in its intended environment
- operator ability/experience

RMID Part III will focus on system suitability tests and ensuring the robustness, reproducibility, and specificity of a method through proper validation procedures.

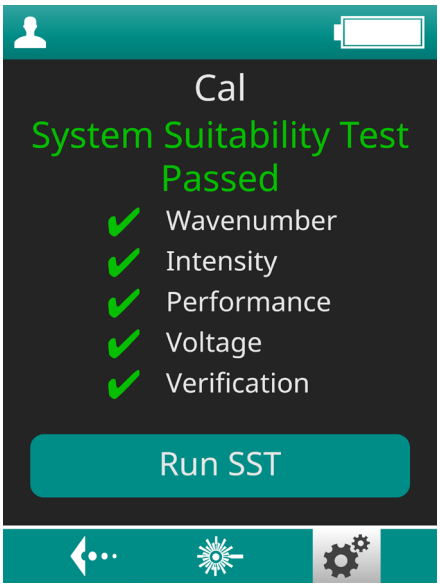
System Suitability

It must be reiterated that the first suitability question involves the ability of handheld Raman to analyze a specific material. Materials that fluoresce, highly colored materials/coatings/packaging, analytes present in low concentrations in a mixture (e.g. Vitamin D in a mineral oil matrix, or vanillin flavoring in a sugar base), and foil-wrapped samples will require another RMID technique.

The next step is to ensure system suitability. This involves instrument validation—confirming that an instrument is performing correctly and according to claims from the manufacturer. Below is the validation method adopted by Metrohm Raman:

- Confirms instrument performance over time
- Demonstrates that system calibration and verification is performed with suitable accuracy, sensitivity, and precision
- Confirms wavenumber calibration
- Adheres to standards required by EP 2.2.48 [2] and USP 1120 [3]

With CVA (Calibrate Verify Attachment), Metrohm Raman's two-sided standard, users perform system calibration with a certified 1:1 v/v mixture of Toluene:Acetonitrile, followed by a test of system suitability against a certified polystyrene standard. These are established standards for Raman spectrometers, according to The American Society for Testing and Materials (ASTM 1996) [4] and National Institute of Standards and Technology (NIST). [5]



It is recommended that the frequency of calibration be carefully considered for any RMID method with Mira P. Recommendations for this can be found in RMID Part II.

Validation Characteristics

There are three governing documents that define the validation characteristics that confirm capabilities of an analytical procedure:

- USP <1225> Validation of Compendial Procedures **[6]**
- USP <1120> Raman Spectroscopy (<858> and <1858> in the near future) **[3]**
- ICH Q2 (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) **[1]**

Although many validation characteristics are defined—accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range—*only the specificity test is required for identification methods*. **[1]** However, a good validation procedure for RMID with handheld Raman can also ensure precision and accuracy.

Robustness

Robustness should be established in the early stages of method development. As discussed in the first section of the current paper, a method is considered robust when it accounts for expected variance in method parameters. A robust method provides consistently reproducible results over time and under normal conditions, as encountered in the course of RMID.

Specificity

Specificity is defined as «*the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.*» **[1]**

Specificity testing is required for validating Mira P RMID methods. With handheld Raman, take extreme care when developing methods for materials that have very similar compositions and spectra, as they require high quality data to differentiate between them. Lower specificity may be observed with a handheld system than with a bench top unit, as handheld systems generally produce spectra with lower resolution, reduced spectral range, and increased noise. In practice, this results in decreased ability to distinguish very similar compounds and therefore increases the need for testing for false positives and false negatives.

For RMID, Mira P's ability to select between present, on-site compounds of closely related structure should be demonstrated. This requires obtaining PASS results from samples containing the analyte, and FAIL results from samples which do not contain the analyte, coupled with confirmation that a positive response is not obtained from any closely related samples.

Accuracy

Accuracy is the confirmation that a method can successfully be applied to a known analyte or reference. This is quite a basic validation characteristic that can provide assurance that Libraries/TS/OPs accurately reflect local samples.

Precision

Precision is a measure of the repeatability of a method. As discussed, a method is typically built by an administrator and the resulting method is applied by a routine user. Accurate RMID in these conditions absolutely relies on robustness and repeatability.

Validation Procedure

First, plan model development according to the range of products, packaging, suppliers, users, conditions, etc. that are expected. Proper definition of the limits of the model will also guide validation of the model.

Second, build the test set. These samples should be unique, different from those used in the Training Set for the model. For example, the TS should be built from a single batch, but validation of the model would be tested with a successive batch. The recommended test set will consist of:

Positives expected to PASS:

- 3 samples of the target analyte

Negatives expected to FAIL:

- 3 dissimilar samples that are expected to fail (selectivity)
- 3 similar samples that are expected to fail

Specificity Test (Positive)

Test samples as described for method development:

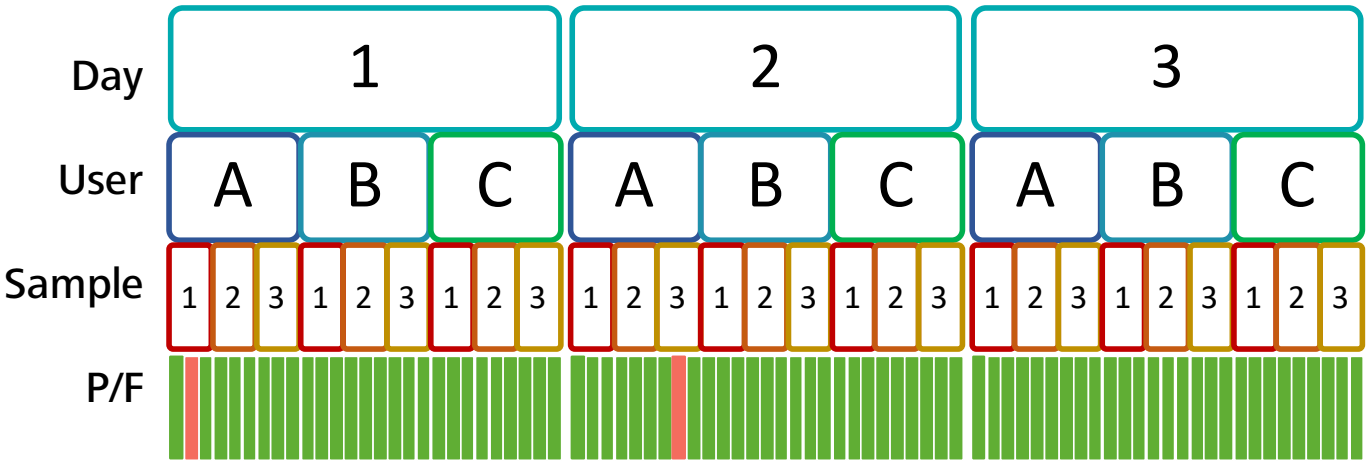
3 Days × 3 Users × 3 Samples × 3 Tests

The positive specificity test validates the model in its routine operation, including some normal variance. The negative specificity test confirms that other commonly received materials present on-site fail appropriately within the model. The three samples that are «similar» to the target analyte are closely related compounds, or starting materials (e.g. Caffeine ↔ Theophylline or Erythromycin ↔ Azithromycin). This is where the user's own knowledge is critical: every company's compounds that require specificity testing will be unique.

This validation procedure tests model robustness as well as specificity, accuracy, and precision. **Specificity** comes from the three sample types, **Accuracy** from confirming the target analyte, **Robustness** comes from the test conditions (three people over three days), and **Precision** comes from three tests per sample. These tests use the same Operating Procedure and Training Set that would be used in routine analysis.

The example below contains two false negatives to provide guidance in this scenario. The administrator will need to examine each spectrum for obvious outliers. In this case, it appears to be a case of human error, which can be solved by observing how User A is sampling.

It could be more informative to record p-values instead of the PASS/FAIL result. For example, if all p-values for Day 2 were lower than the rest, it may indicate that system calibration was not performed. Repeat testing in the validation procedure allows for careful evaluation of the performance of a model.



Specificity Test (Negative)

Negative specificity tests are designed to ensure that a model, its methods, and the instrumentation employed therein do not return false positives. There is a certain subtlety to determine whether a model fails appropriately. The classes of APIs and excipients may be very chemically similar, as in the case of chain length (Polysorbate 20/80), or identity of a cation. Sodium carbonate and lithium carbonate are *very* different salts, for example. Handheld Raman is a sensitive, specific technique, but such situations deserve special investigation to ensure that the method can adequately discriminate between such similar compounds.

If validation was unsuccessful, the model should be reviewed and expanded.

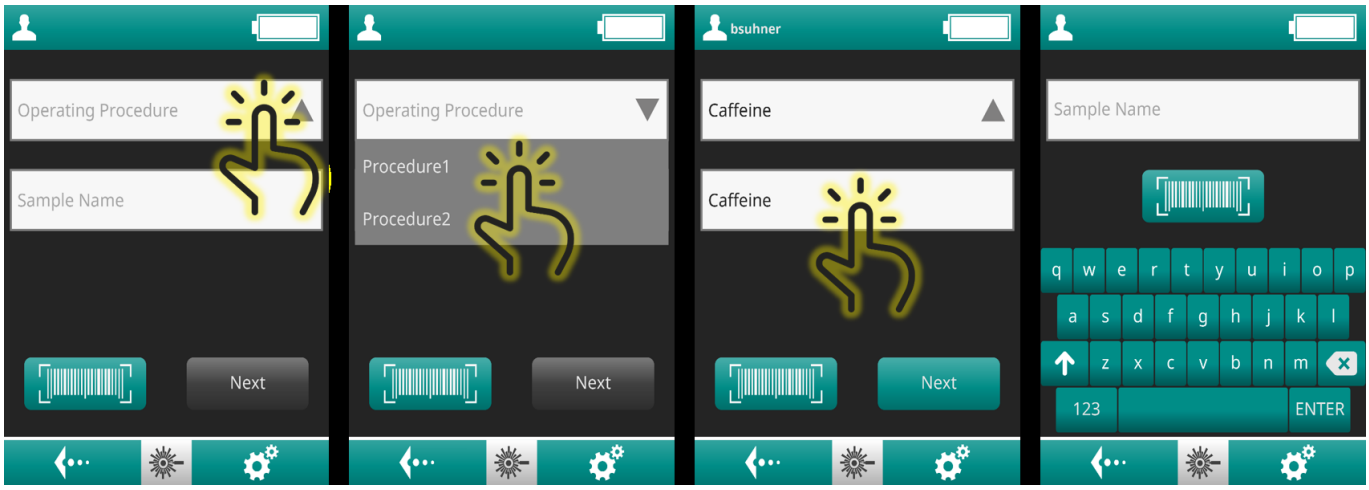
Labelling and Records

Mira P allows the user to rename a test on the device; this can be used to change the name of the sample to include information about each specific validation step for future reference (Lactose ⟶ Lactose Val 9.13.19 MJG S1 T2).

Conclusion

At this point, the model can be reviewed, approved, and signed. The Training Set and Operating Procedure, with specific versions noted, should be signed so no further changes are allowed.

After validation procedures are complete, you may use Mira P for RMID with confidence.



Procedure for renaming a test/spectrum on the device.

References

[1] ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2-r1-validation-analytical-procedures-text-and-methodology

[2] European Pharmacopoeia General Chapter 2.2.48 Raman Spectrometry

[3] USP 29 <1120> Raman Spectrophotometry
http://www.pharmacopeia.cn/v29240/usp29nf24s0_c1120.html

[4] *Standard Guide for Raman Shift Standards for Spectrometer Calibration*; ASTM E1840-96; ASTM International: West Conshohocken, PA (1996).
http://148.223.46.16/Normas/ASTM/E/E1840.PDF

[5] National Institute of Standards & Technology SRM 2241
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[6] USP 29 <1225> Validation of Compendial Procedures
http://www.uspbpep.com/usp29/v29240/usp29nf24s0_c1225.html

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