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 I discusses basic applications of Raman, including sampling considerations and evaluation types, to help users design a robust RMID method.

Part I: General Considerations

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Introduction

Raw material identification (RMID) methods must accommodate several factors to create the most accurate and robust solution for the task. Specific consideration must be given to:

- **Sampling strategy:** how to collect the best quality data, given specific conditions
- **Presentation of the sample:** morphology, packaging, chemical nature
- **Suitability of the model:** inclusion of all relevant variation
- **Selectivity of the method of choice:** is handheld Raman an appropriate technique?

A successful methodology with Metrohm's Mira P handheld Raman system will consider each of these collectively. This document presents guidelines and best practices regarding development of methods with Mira P for successful implementation in a RMID scheme.

Chemical Suitability for Raman Analysis

- Typically, most molecules with covalent bonds are Raman active; however, the nature and intensity of their signal can vary.
- Increasing the number and conjugation of bonds generally increases Raman activity.
- Some salts, ionic compounds and metals are not well-suited to Raman analysis. (Specific applications developed for Mira can be found in the References section. **[1–5]**)
- Raman is an ideal measurement technique for aqueous solutions, unlike Fourier-transform infrared spectroscopy (FTIR) and near-infrared spectroscopy (NIRS). Water does not interfere with the Raman signal of the solute.
- Fluorescence is one of the biggest challenges for analysis with Raman, as it can overwhelm the signal from Raman scattering. Highly fluorescent materials are not well-suited for analysis with 785 nm Raman.
- It is estimated that 80% of common active pharmaceutical ingredients (APIs) and excipients are well-suited to RMID with handheld Raman (**Fig. 1**).

General Considerations

Each sample must be considered independently to design the correct sampling strategy for Mira P method development. For example, in the simplest circumstance, a clear glass vial of a liquid (e.g. an alcohol) could be placed into the vial holder on the system. This would allow for the effortless collection of high quality, low interference Raman spectra of a sample with a strong Raman signal.

Conversely, one may encounter a combination of factors that can negatively affect the quality and reproducibility of a spectrum, such as multiple layers of packaging, materials which are weak Raman scatterers, and fluorescence.

As an example, Metrohm Raman has been successful with sampling microcrystalline cellulose, a common excipient with a low signal. The Raman signal can be occluded by both fluorescence and interference from the container, in addition to complications from particle size and sample purity. A material like this requires a higher level of method development to provide the necessary assurance for a material identification method.

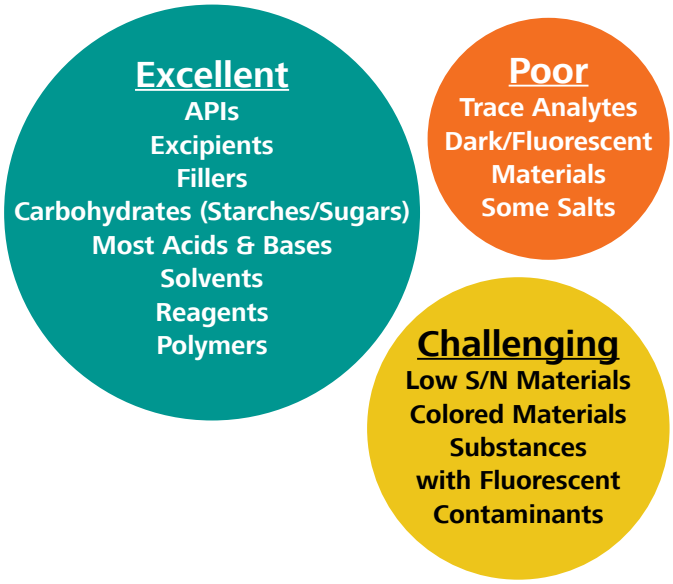

Figure 1. Best suggested uses for Raman spectroscopy.

Testing Considerations

When approaching a new material for RMID with Mira P, a number of questions should be asked:

- How will the material be tested with Mira P?
- How can the method account for changing «field conditions»?
- How many vendors of the material are expected?
- Is there a certified reference material available?
- What are the properties of the material (i.e., synthetic or natural; solid or liquid)?
- How many batches/lots of the material are available?
- Which packaging materials may be encountered, and is there consistency across vendors?
- Is this material common within the industry?

Each question must be carefully investigated when developing a method for RMID. A further discussion of each point follows.

Material Testing

Several questions arise at the beginning of this process. *What is the current practice for material testing?* It is assumed here that materials are being sampled in non-laboratory conditions, a common practice with handheld Raman instruments. *Can the material be assessed through its container, or is an aliquot being removed? Is the aliquot taken from the top of the container? Can a probe be used to assess the contents in the middle of a container?*

Accounting for other types of «field conditions» is essential, since warehouse conditions are less controlled than those in laboratories. Such conditions include: variance in ambient light, temperature, container material, and sample heterogeneity. Method robustness results from such built-in variance and lends reliability to the method during normal usage.

Number of Vendors

Robust method development is based on inclusion of as much variance across the sample as possible. In the instance of multiple vendors, data from all possible vendors is needed to ensure the inclusion of manufacturer specific features in the Raman spectrum.

Reference Material Availability

Reference material is considered to be representative of tested material and, in most cases, is a highly pure (>99%) raw material. Material with low-levels of contaminants can be considered a reference as long as the signal is not compromised (i.e. lactose anhydrous with 5% lactose monohydrate).

Material Source

Generally, any synthetic material will have lower variance in Raman spectra due to the consistent chemical nature and purity of the material. In such cases, the best practice is simply to use known, proven samples when building libraries.

The sampling of natural materials must permit for more chemical variance and, therefore, Raman spectral variance. As with the number of vendors, efforts must ensure that as many samples as possible are included in the training set to account for chemical, composition, and purity differences.

Material Presentation

The form of a sample can affect spectral acquisition and consistency. Familiarity with the utility of each Smart Tip will help the user choose the ideal sampling optic for a sample.

For example, sampling through multiple layers of polyethylene may require an Extra Long Working Distance (XLWD) Attachment. The Vial Holder provides the most consistent sampling condition, but requires that sample aliquots be taken. Liquids may exhibit better spectral consistency than solids with some handheld Raman systems, but ORS™ (Orbital Raster Sampling) in Mira P effectively samples solids, even mixtures, with a large area of interrogation. More information about ORS™ is found in the References section. **[6–8]**

Number of Batches/Lots

Attention must be paid to ensure that variance across multiple batches or lots is accounted for in an operating procedure. Materials from at least three different batches or lots should be scanned to overcome issues in this case.

Packaging Materials

Most RMID methods for Mira P will involve sampling through some type of thin enclosure. This enclosure might include glass bottles (clear and tinted), plastic bags (clear, translucent, colored, coated), and foils.

The impact of packaging material needs to be accounted for when developing a method. This must be extended to instances where packaging materials become folded or doubled up. Generally, with foil materials, Raman sampling will not be possible as the reflective nature of the packaging prevents light from penetrating into the sample.

To account for spectral effects from packaging materials, compare spectra of the material both with and without packaging. Check the spectral quality, and adjust the method as needed to get the best possible spectrum.

Commonality of the Sample

If it is a common material within an industry, there is a high probability that its spectrum exists within a commercial Raman spectral library (such as the onboard USP library). Spectra from these commercially available libraries can be used for comparison, when determining whether a method is collecting the best quality data and can be traced back to a reference library.

Note that the existence of a spectrum in a commercial library does not preclude verification of the sample's performance, as this may be impacted by a number of the factors mentioned above.

Types of Evaluation

When developing RMID methods, a number of evaluations of collected spectra can be performed. Mira P Cal software offers three levels of evaluation:

Verification is based on collected, proprietary Training Set data. Verification is used in a central receiving organization as part of a procedure check for incoming quality control (QC). It affirms that the claimed material identity is correct with principal component analysis (PCA), which compares each new spectrum within a training set comprised of spectra associated with the material. A positive verification returns a PASS result.

Identification is determined by the correlation comparison of new spectra with a Spectral Library. Identification involves determination of the identity of a material in question, reporting possible matches along with statistics showing the level of similarity.

Mixture Matching is an extension of identification, wherein Mira P can automatically detect and report up to three separate components in a mixture.

Verification is the most important RMID method discussed herein. Metrohm Raman has previously published a white paper which provides more information about evaluation types. [9]

Definitions

An **Operating Procedure (OP)** is the final method for the identification of unknowns or verification of samples. An OP defines all parameters for the acquisition of spectra (laser power, integration time, averaging of spectra, and choice of Smart Tip), the evaluation type (e.g. Identification, Verification, and/or Mixture Matching) and barcode population of sample fields. If verification is the purpose of the method, the appropriate training set and confidence interval will also be defined. In the case of identification, a library and corresponding match score threshold will be specified.

A **Training Set (TS)** is a compilation of spectra, all representative of a single product **(Fig. 2)**. In contrast to libraries, a training set contains standard spectra with potentially relevant variances describing one single material. Therefore, the number of training sets corresponds to the number of products being evaluated.

Training Sets are used for Verification methods. An evaluation is achieved on the basis of null hypothesis testing with a PCA model. The critical result of verification is the p-value, which must be above (1- threshold value) for a «Pass» and below (1- threshold value) for a «Fail». The threshold value is specified in the OP. Normally, a threshold value of 0.95 is chosen, corresponding to a 95% confidence interval, but this can differ depending on the application.

A **Library** is a compilation of spectra, each representing a single product. The number of spectra corresponds to the number of products. Libraries are used in Identification Mode, with results reflecting a correlation value, also known as a HQI (hit quality index) or match score. This value describes the square of Pearson's correlation between two spectra. Values between 0 (low) and 1 (high) describe the degree of similarity of the two spectra.

The correlation value of an unknown sample spectrum with every member of the library is calculated, and the three hits with highest correlation above a defined threshold value (match score limit) are reported on the instrument and in the software. A match threshold value of 0.85, specified in the OP, is suggested for the purposes described here.

Conclusion

Once it is determined that handheld Raman is a suitable method for RMID at the receiving point, method building may begin. The next tutorial, RMID Part II, will inform the user about specific sampling recommendations, and provide a workflow for method building with Mira Cal P and data acquisition with Mira P.

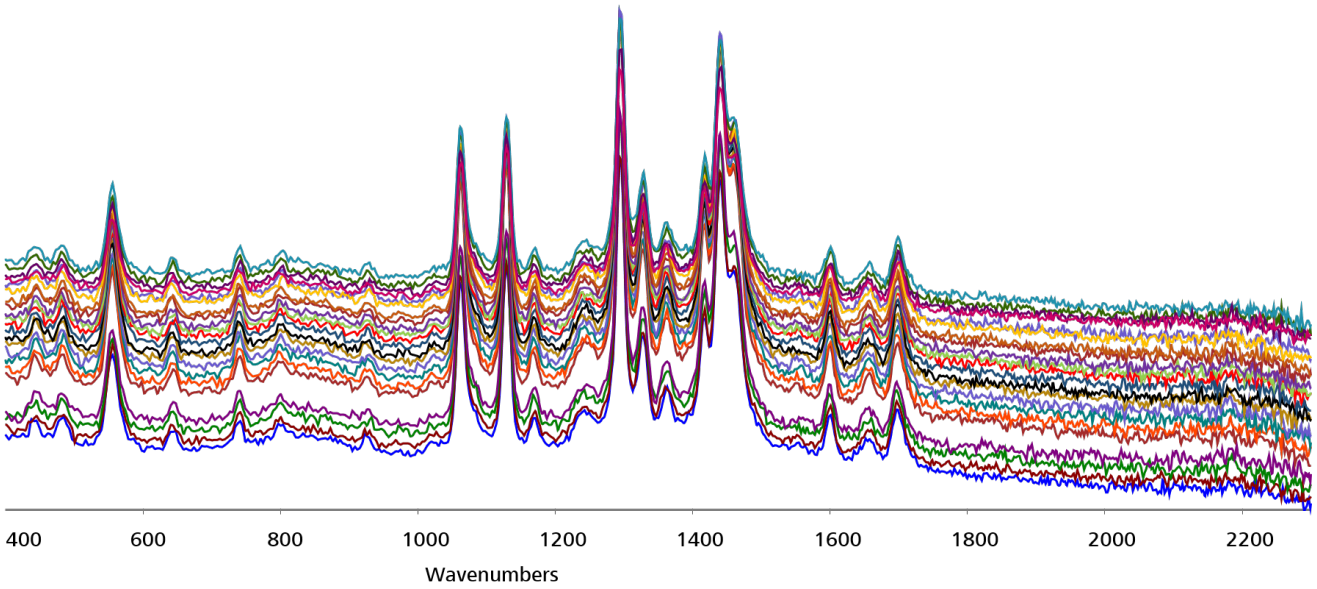

Figure 2. Example Training Set.

References

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[2] Metrohm Application Note **RS-005** Differentiation of inorganic salts using Raman spectroscopy
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