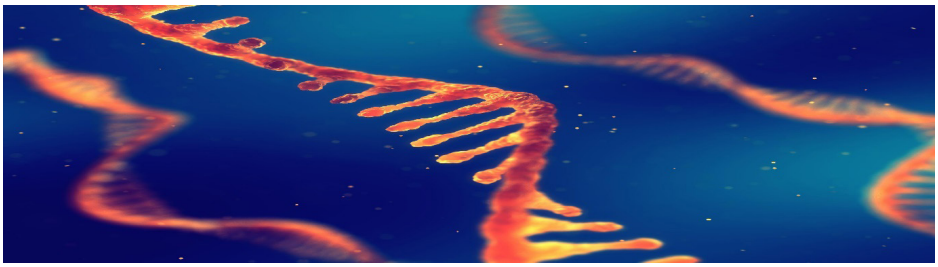
Application Note

Biopharma and Pharma

Agilent

Trusted Answers

Identification of Commercially Available Oligonucleotide Starting Materials Directly Through Containers



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Abstract

Handheld Raman spectrometers can selectively identify oligonucleotide starting materials, also known as phosphoramidites, directly through amber bottles. This application note discusses the theory and deployment of Agilent handheld Raman systems based on spatially offset Raman spectroscopy (SORS) for chemical ID verification of phosphoramidites in a good manufacturing practice (GMP) environment.

Introduction

During the COVID-19 pandemic, nonscientists grappled with well-known diagnostic methods like polymerase chain reaction (PCR) to understand how one could rapidly detect the presence of SARS-CoV-2. PCR tests leverage nucleotides or short RNA/DNA molecules synthesized using protected nucleosides called phosphoramidites as building blocks. The arrangement, chemical modification, and number of these building blocks confer specific binding affinity to target RNA/DNA sequences, with application in disease screening, biomedical research, and criminal forensics. As the number of applications calling for oligonucleotides has dramatically increased in the last few years, the supply chain is under strain to meet demand and quality requirements. Current GMP (cGMP)-mandated tests, such as raw material ID at receipt, maintain quality standards. They act as the first line of defense to prevent the introduction of incorrect, subpar or adulterated raw materials in an oligonucleotide manufacturing process.

In this application note, the Agilent Vaya Raman raw material identity verification system, a handheld Raman spectrometer, was used to analyze several commercially available phosphoramidites used in the synthesis of oligonucleotides. These oligonucleotides are usable as starting materials, as stated in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q11. The purpose of this analysis was to demonstrate that Raman spectroscopy is an effective solution for the identification/differentiation of biopharmaceutical materials through transparent and opaque containers in a cGMP manufacturing environment.

Raman spectroscopy as a compliant spectroscopy technique with most pharmacopeias for ID testing

Raman spectroscopy is an optical technique based on the recording and analysis of the inelastic scattering of incident monochromatic light by a specific analyte. It is described in detail in most pharmacopeias. In particular, the United States Pharmacopeia chapters USP<858> Raman Spectroscopy and USP <1858> Raman Spectroscopy – Theory and Practice, the European Pharmacopeia chapter EP 2.2.48, the Chinese Pharmacopeia General Rule Section 0421 and the Japanese Pharmacopeia (Supplement II, JP XVII) section 2.26. These pharmacopeias discuss its theory, main techniques, instrument performance requirements and associated applications to support pharmaceutical organizations in their quality control work. In these pharmacopeia chapters, Raman is described as an acceptable technique, equivalent to molecular spectroscopies like Mid-IR, for the identification test of raw materials and their subsequent release into production.[1-4] Recommendations on qualitative methods development and validation are also provided to ensure method robustness.[5]

SORS as a pharmacopeia-compliant subset of Raman spectroscopy for ID testing

Identification testing by handheld back-scattering Raman spectroscopy, also known as conventional Raman spectroscopy (CRS), is popular in many pharmaceutical warehouses. It enables the rapid identification of raw materials through transparent containers, such as polyethylene liners or glass bottles. However, the limitation of CRS analysis to work through only transparent barriers has contributed to the emergence of SORS, a pharmacopeia-approved methodology for analysis of raw materials directly through nontransparent containers. Such containers include multilayer paper sacks, white polyethylene tubs, and blue drums. For the ID of a raw material through a container, SORS typically takes two Raman measurements at the surface of the container. One measurement is called "zero", where the area of excitation coincides with the area of detection. The other is at an 'offset' position (i.e., where the area of excitation no longer coincides with the area of detection), typically a few millimeters away. The acquired container-rich zero spectrum is scalarly subtracted to the acquired content-rich offset spectrum. This obtains a container-free content spectrum, which is used for comparison against a library of known spectra. For example, lactose monohydrate in a multilayer paper sack can be identified without knowing the paper material or its relative signal contribution.

Fast and specific SORS for raw material ID

Agilent released the first handheld SORS spectrometer for use in a cGMP environment. The Vaya rationalizes and accelerates the identification of pharmaceutical raw materials at reception. Based on SORS technology, the Vaya supports warehouse personnel in the chemical ID of excipients, active pharmaceutical ingredients, and other starting materials directly through transparent and nontransparent containers (Figure 1). No opening container, sampling, unnecessary movement of containers nor sampling booth are required. With SORS, the Vaya probes the different vibrational modes of an analyte to obtain a unique chemical fingerprint and confirm its identity. It also differentiates the analyte from analogs, such as diastereomers; isomers of position; or polymorphs, with high confidence and no false positives. With Vaya, the warehouse staff and quality control group can rapidly verify incoming containers and release them into production in a matter of hours. In contrast, using Mid-IR, NIRS or CRS, this process would take days.

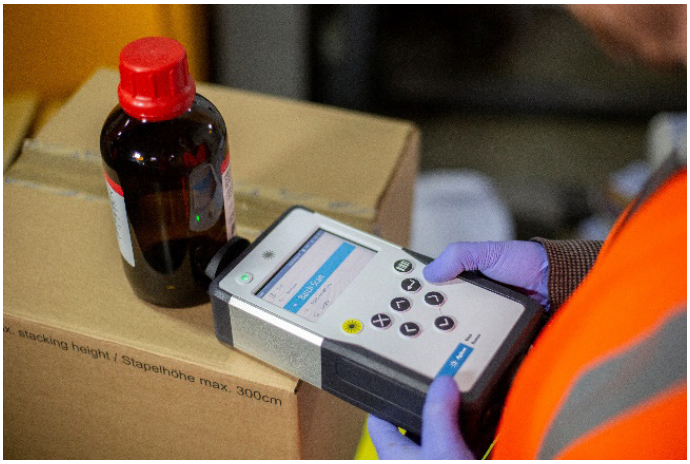

Figure 1. The Agilent Vaya Raman can verify the ID of oligonucleotide starting materials directly through amber bottles.

Easy deployment of the Vaya

With the popularity of fit-for-purpose, handheld solutions, there is now movement away from traditional molecular spectroscopy techniques, including Mid-IR and NIRS, to deploy alternative ID solutions, such as Vaya. The transition to a new, alternative ID technique follows a simple regulatory path. In most regions, one can expect to file a manufacturing change request to add an alternative methodology through an annual report (USA) or an electronic filing with response in 30 days (Europe). Evidence to be provided for filing are typical, i.e. testing results, a comparability protocol with old ID technique (optional), and/or comparative validation data. To support filing requirements, evidence development and subsequent deployment, the Vaya incorporates features supporting 21 Code of Federal Regulations (CFR) Part 11 and GMP requirements. The Vaya's wizard-based ID method development and validation modules enable advanced users to develop robust methods, which meet USP <1225> or ICH Q2 (R1).

In the validation module, a method under development can be cross checked against other existing methods in production to verify the absence of identification conflict (Figure 2).

In addition, the method's robustness and validity can be tested through positive and negative challenges (Figure 3). The Vaya invites the user to test different lots; different sampling spots of the correct raw material in container for positive testing; and closely related structures that can find their way into the warehouse for negative challenges. For cGMP and audit purposes, the validation unit includes automated reporting and audit trail traceability.

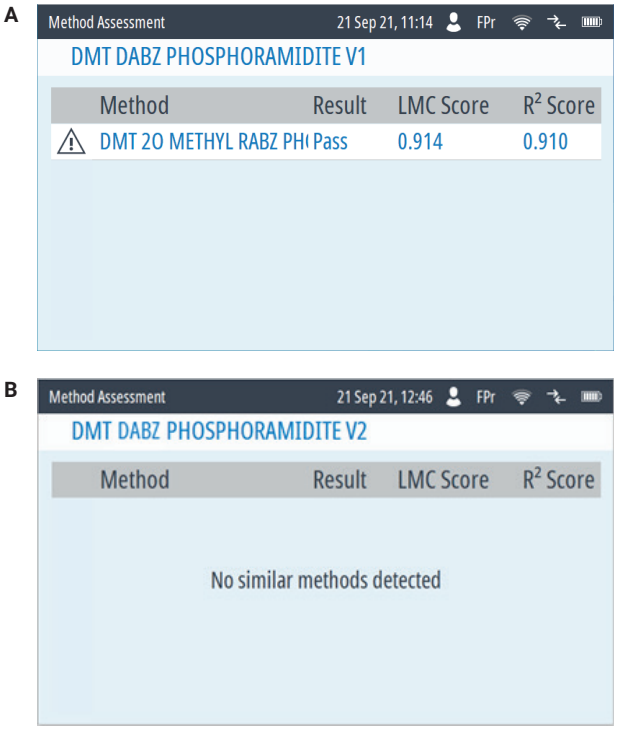

Figure 2. Method Assessment: a cross check against methods in production for DMT-dA(bz) Phosphoramidite (A) before the addition of analogous sample, and (B) after the addition of analogous samples (i.e., DMT-2'O-Methyl-rA(bz) Phosphoramidite).

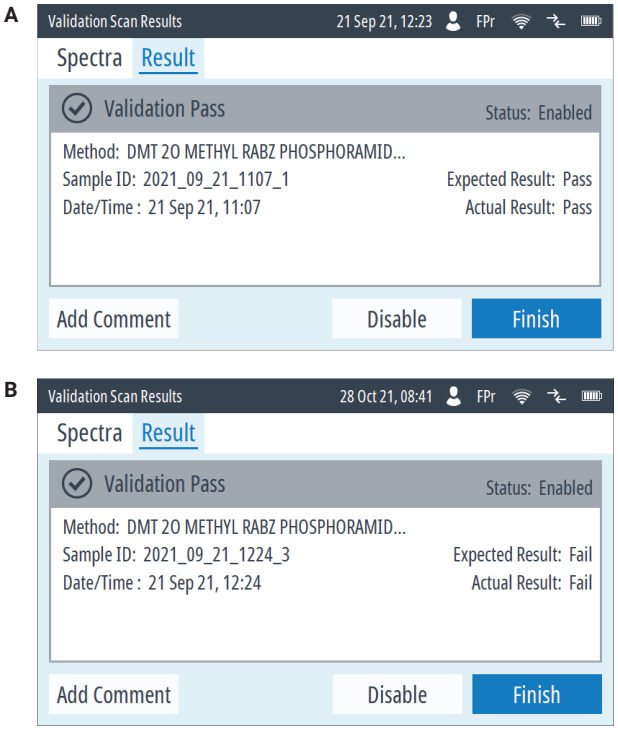

Figure 3. (A) Positive and (B) negative challenge scans for DMT-2'O-Methyl-rA(bz) Phosphoramidite.

Postdeployment, regular performance qualifications (PQ) of the hardware/software and methods are sufficient to demonstrate fitness for use. Vaya includes a System Check (Figure 4), which, in seconds, verifies the instrument performance against the photometric and wavelength accuracy; photometric precision; and laser power requirements set by USP <858>, EP 2.2.48.

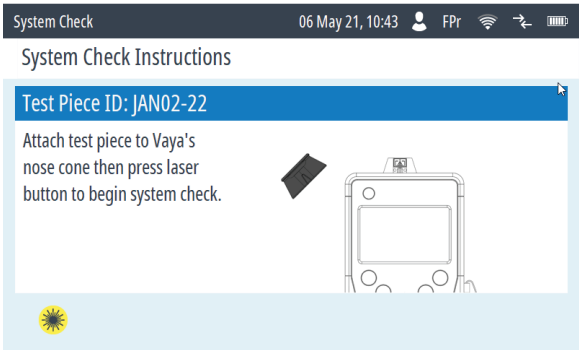

Figure 4. The performance of the Agilent Vaya Raman can easily be checked against USP and EP requirements using the System Check module.

The methods can easily be requalified by running a simple ID test using existing retains or standards. Method maintenance is not necessary as the SORS Raman spectrum/signal solely depends on the chemical make up of the analyte, as opposed to that of NIR, for example, which is also affected by the physical characteristics of the analyte.

In the warehouse, little-to-no training is necessary to perform the different tasks on Vaya. The ID verification test, known as a 'Batch Scan' (Figure 5), uses a rigorous dedicated workflow and delivers actionable answers, enabling nontechnical users to easily perform the analysis in a warehouse.

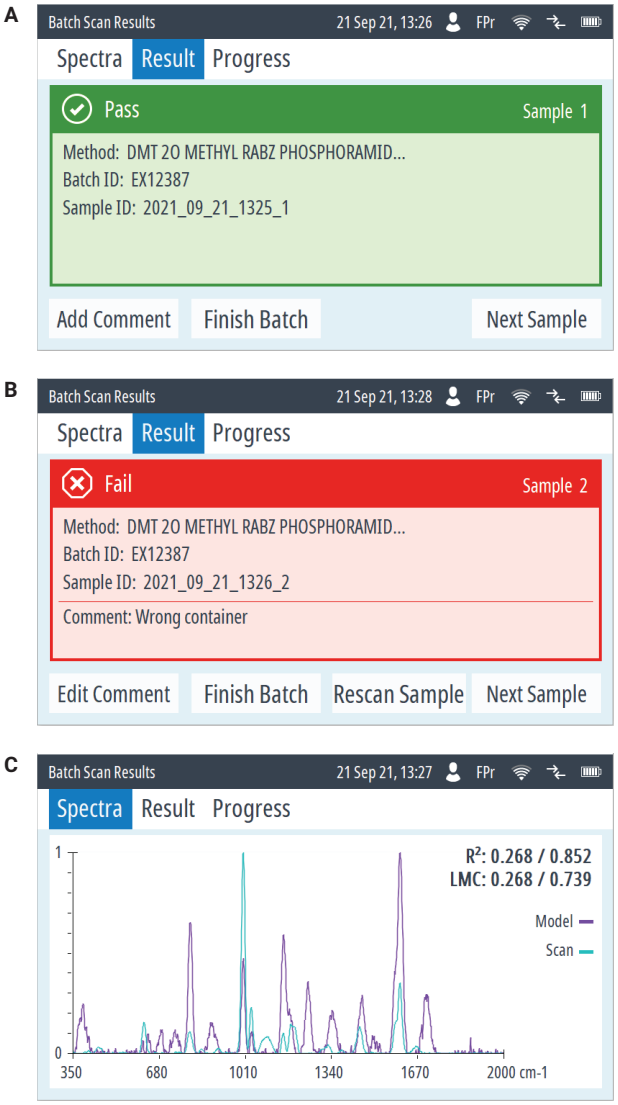

Figure 5. The Agilent Vaya Raman delivers a color-coded binary answer indicating a pass (A) or fail (B) for the verification of raw material ID. The spectrum of the analyte can be displayed against the reference model for further confirmation (C).

Experimental

All oligophosphoramidite building blocks were supplied from Sigma Aldrich. ID verification methods were created for each of the oligo building block materials. These were developed for ID verification through the manufacturer-supplied amber bottles. Several of the oligos tested had very similar spectra and therefore presented an illustrative showcase for the excellent selectivity of the technique. Each method was created from the mean of 10 scans of the material in container and the most similar materials spectroscopically were added to each method as analogous samples to enhance selectivity. The resultant model was then tested at the validation stage of development in terms of an expected pass scan (the subject material in container) and an expected fail scan (a different analogous material in container). Finally, to confirm selectivity of each model, each oligo was tested three times against each model.

The oligo samples were:

- DMT-2'O-Methyl-rA(bz) Phosphoramidite
- DMT-dA(bz)-CPG
- DMT-dG(ib) Phosphoramidite
- DMT-dT Phosphoramidite
- DMT-dA(bz) Phosphoramidite

Results and discussion

Figure 6 shows the spectral data obtained from the phosphoramidite materials acquired through the amber glass containers. DMT-dG(ib) Phosphoramidite and DMT-dA(bz)-CPG spectra present sufficient spectral differences in the 1,000 cm^{-1} and 1,500 cm^{-1} regions, to be easily differentiable from all four other oligo materials. However, the spectral overlay also indicates that three oligos (DMT-2'O-Methyl-rA(bz) Phosphoramidite, DMT-dT Phosphoramidite, and DMT-dA(bz) Phosphoramidite) have very similar spectra, presenting a challenge for developing selective and robust methods for their identification. These building blocks are likely to score above the ID two-decision criteria thresholds against more than one model, resulting in false positive identification results.

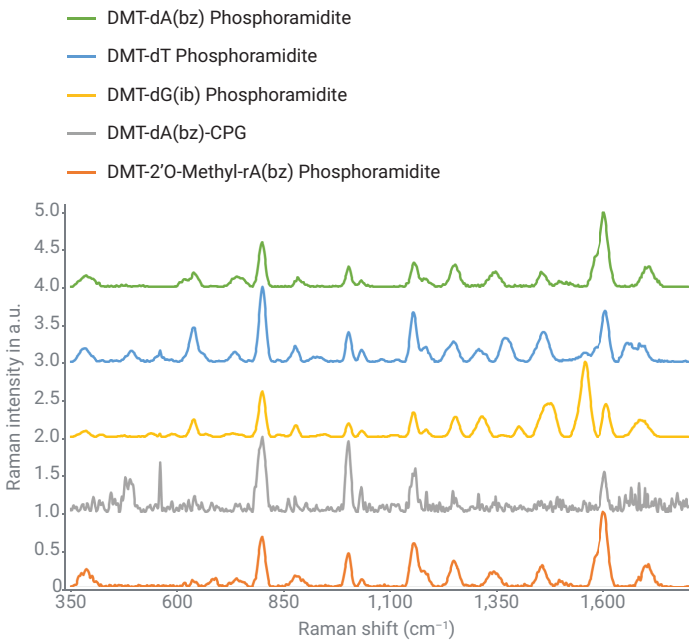

Figure 6. Overlay of Raman spectra for phosphoramidites acquired through amber glass.

The challenge matrix in Figure 7 graphically represents how Vaya differentiated the oligo spectra without the addition of analogous samples to the method. In a challenge matrix, an ID test is conducted for each of the analytes in the class using the ID verification method developed for each analyte in the same class. An ideal challenge matrix shall only have "pass rate scores", indicated by the green color along the matrix diagonal, indicating that the method recognizes its corresponding material perfectly. Off the diagonal, the ideal matrix should display only fail score rates, indicated by the tiles colored in red, indicating that the method correctly rejects incorrect materials. In this instance, the matrix is not perfect and false positive matches are evident with the three most similar materials. In these situations, where the chemistry and resultant spectra of a sample set are so similar as to make separation imperfect, Vaya permits the addition of spectra from analogous samples to the method build. By adding these additional data, the instrument algorithm can automatically adjust the scan score thresholds to achieve perfect identification without false positives.

An ideal matrix demonstrates that a group of methods has a very low level of false positive and can be deployed in a warehouse environment. Figure 8 shows the oligo building blocks challenge matrix, where the methods have been optimized with the addition of the respective analogs samples to reinforce the selectivity. It demonstrates that Vaya can easily differentiate all the phosphoramidites through amber glass.

Determination of a pass or fail result by Vaya

Vaya's selectivity power demonstrated in the previous section is partly derived from the use of a two-score decision engine based on a coefficient correlation (R^2) and a linear model coefficient (LMC). The two-score system is advantageous because the R^2 is a commonly used quantitative metric in Raman spectroscopy to ensure that the unknown sample spectrum is sufficiently similar to the model spectrum in the method. The LMC provides an extra check to enhance the decision engine specificity. It is based on the incorporation of additional spectra into the model, such as a challenge (i.e. analyte spectrum) and observing its influence over the LMC score.

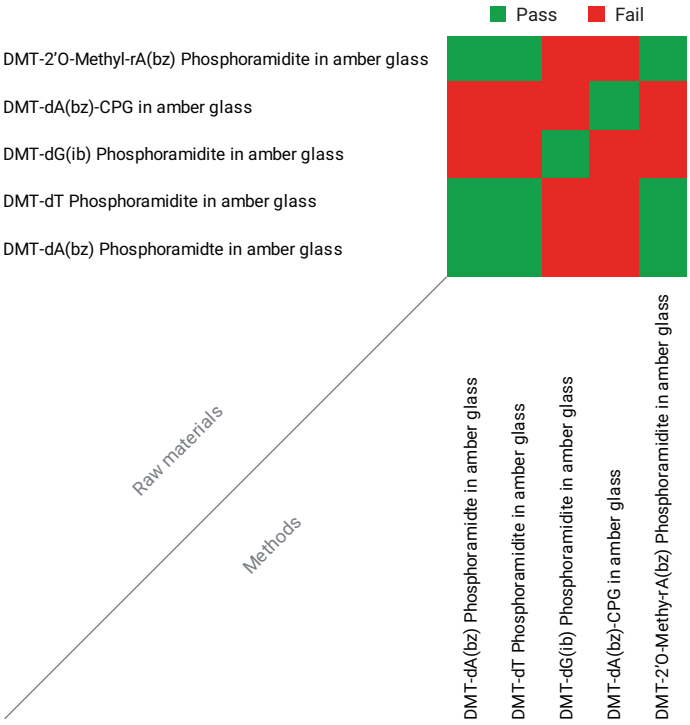

Figure 7. Challenge matrix for phosphoramidites without adding analogous samples to model.

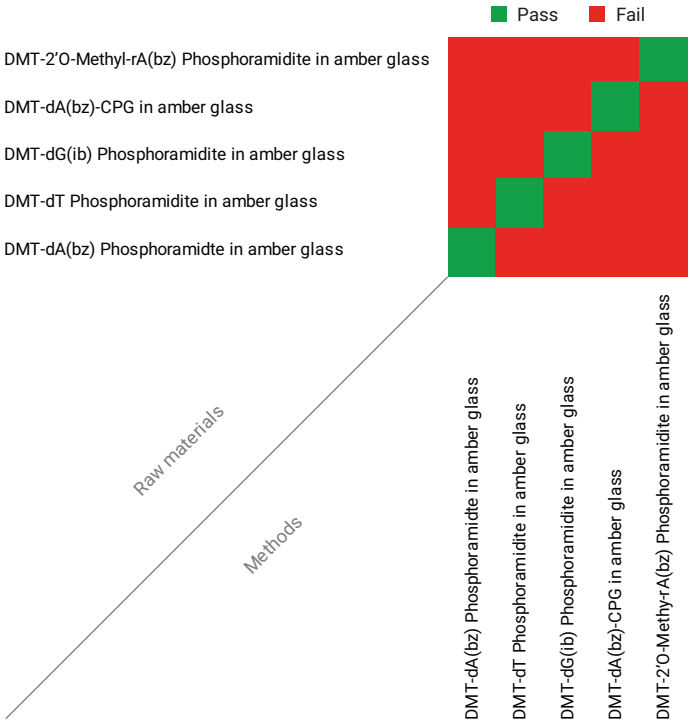

Figure 8. Challenge matrix for phosphoramidites, adding analogous samples to methods.

Conclusion

With the Agilent Vaya Raman system, it is now possible to verify oligo materials such as phosphoramidites when needed, without opening the container. Vaya can quickly evaluate raw materials and rapidly detect shipment errors, which are willful substitutions even with closely related, cheaper analogs. With Vaya, oligo and other biopharmaceutical raw materials can be verified in a matter of seconds.

References

1. USP <1858> Raman Spectroscopy- Theory and Practice: Quote from Page 2/8 Section Qualitative and Quantitative Raman Measurements/ Qualitative Raman Measurements: Qualitative Raman measurements yield spectral information about the molecular structure of the sample. Because the Raman spectrum is specific for a given compound, qualitative Raman measurements can be used as a compendial identification test. See Spectroscopic Identification Tests<197>.
2. European Pharmacopeia Chapter: EP 2.2.48 Raman Spectroscopy: When the Raman spectra of a sample and the Reference Standard of a substance to be identified are compared and both spectra exhibit similar scattering intensities at the same Raman shifts, the identity of those can be confirmed.
3. Chinese Pharmacopeia General Rule Section 0421: Raman spectrum can give accurate spectral information about the vibrational bands present in the sample. The contrast of the spectrum of the test sample with the standard sample collected in the same measurement conditions should be carried out, if the two spectra match in configurations and intensities, the two samples may be identical except stereoisomers.
4. Japanese Pharmacopeia (Supplement II, JP XVII) section 2.26/ 5.1: When the Raman spectra of a sample and the Reference Standard of a substance to be identified are compared and both spectra exhibit similar scattering intensities at the same Raman shifts, the identity of those can be confirmed.
5. European Pharmacopeia Chapter: EP 2.2.48: Record the spectra of a suitable number of materials which present typical variation (manufacturer, batch, particle size, impurity profile, etc.) and comply with the requirements of the monograph or established specifications. The number and selection of samples in the database depends on the specific application. Suitable mathematical pre-treatments of the Raman spectrum may facilitate qualitative comparison of spectra (e.g., baseline correction, normalization, derivatives, cosmic ray removal). The selectivity of the database that makes it possible to identify a given material and distinguish it adequately from other materials in the database is to be confirmed during the validation procedure. The spectra of the material to be examined can be compared with the spectral reference library using chemometric methods (e.g., similarity and distance measures, classification methods); these methods involve the set-up, assessment, and validation of chemometric model by the analyst.

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