Application Note

Pharma and Biopharma

# Verification of Common Stearates Without Complex Chemometrics with Agilent Vaya SORS

## Fast, effective, through-plastic-liner identification of similar compounds using a handheld Raman spectrometer and onboard software tools


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## Abstract

Magnesium, calcium, or zinc stearates are commonly used in pharmaceutical drug manufacturing. While these metal stearates exhibit similar chemical properties, they are not necessarily interchangeable in manufacturing processes. It is critical therefore that they are identified and differentiated at receipt in the warehouse to avoid process disruptions. Accurately differentiating stearate analogs at receipt by Raman spectroscopy has historically been challenging. Given the similarities of the spectra of the compounds, sophisticated chemometric software is often needed to build stearate models that are then used to identify them.

This study shows that the Agilent Vaya handheld Raman spectrometer with Spatially Offset Raman Spectroscopy (SORS) can identify metal stearates in their original primary packaging, without the need for complex chemometric software packages. The handheld Vaya Raman enables the selective verification of stearates using a two-criteria decision algorithm combined with the "Analogous Sample" software feature.

## Introduction

Stearates are widely used in the pharmaceutical industry as lubricants or flowing agents to prevent components from sticking to manufacturing equipment during tablet pressing or capsule formation. Magnesium stearate is the most common example of a flowing agent, while calcium or zinc stearates are also frequently used based on their lubricating properties. However, despite sharing common properties, the metal stearates are not interchangeable in the manufacturing process and must be differentiated at receipt. Metal stearates share a common anion of stearate, a long carbon chain polymeric material with a series of 16 $CH_2$ repeat units ($CH_3$-$[CH_2]_{16}$-$COO^-$), as shown in Figure 1. Identification (ID) testing of metal stearates by Raman spectroscopy can be a challenge, as differentiation or selectivity is based purely on the influence of the counter metal cation.

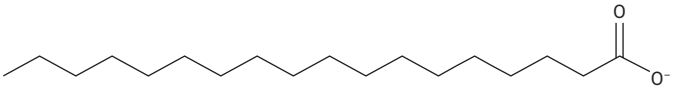



**Figure 1.** Structure of a stearate anion.

This application note demonstrates how the Agilent Vaya handheld Raman spectrometer uses Spatially Offset Raman Spectroscopy (SORS) to provide robust selectivity of stearate analogs. SORS enables the accurate verification of the compounds through transparent low-density polyethylene (LDPE) bags. Verification of stearates is also possible using the Agilent RapID, a mobile Raman instrument that also uses SORS technology.

### What is the Agilent Vaya Raman handheld system?

Vaya is a handheld Raman spectrometer that uses SORS for sample ID verification through containers, including common pharmaceutical raw material container types. SORS provides high-quality through-barrier performance for packaging materials such as plastic tubs and barrels, glass bottles, flexible intermediate bulk containers (FIBC), as well as plastic and paper sacks (Figure 2). The portable instrument is powered by rechargeable batteries that can be quickly exchanged to keep it operational. Vaya includes wizard-driven method development software that provides fully automated operation, including data acquisition, data processing, and pass/fail reporting.

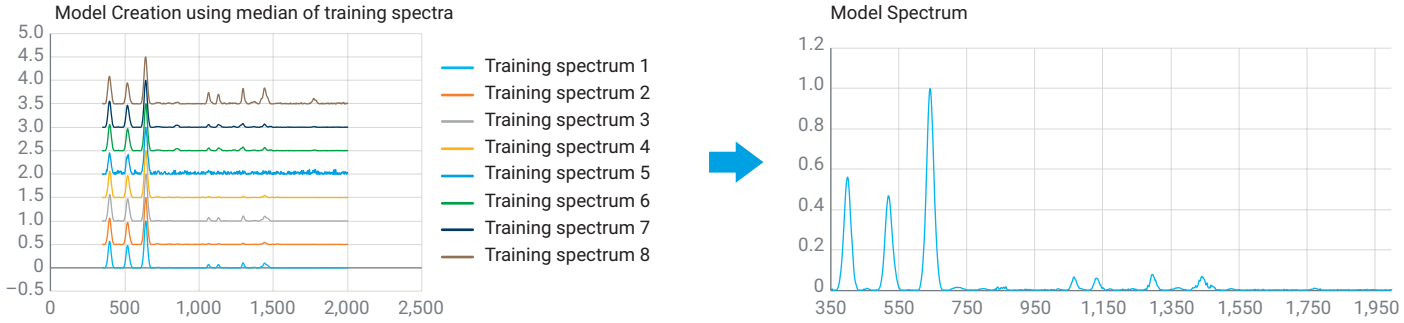
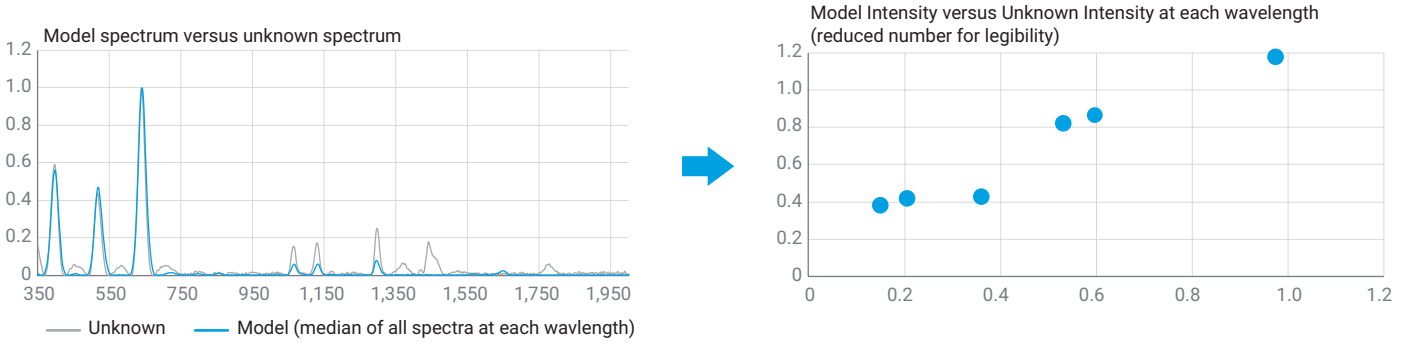
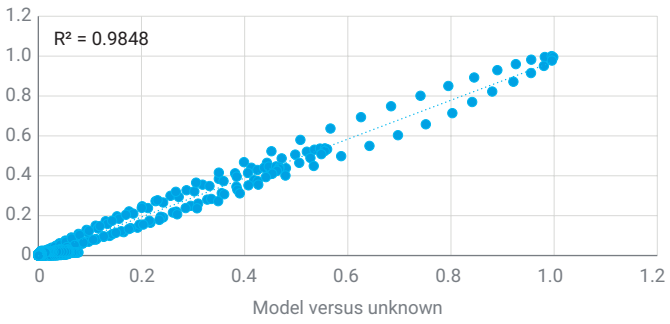
**Figure 2.** The Agilent Vaya Raman handheld spectrometer being used to identify materials inside paper sacks that typically comprise three layers of paper and a PE liner.

### Vaya method development: decision algorithm

To differentiate metal stearates and other similar materials and verify the ID of a material, Vaya employs a decision algorithm based on two criteria: the coefficient of determination ($R^2$) and the linear model coefficient (LMC). Details of the two criteria and how they are calculated are summarized in Table 1.

When a raw material is analyzed using Vaya, it will receive a "Pass" result, provided the scan of the raw material scores above the threshold limit for $R^2$ and LMC. The scoring system detailed in Table 1 provides a high degree of selectivity, enabling similar materials to be verified with a high degree of specificity.

**Table 1.** Criteria used by the Agilent Vaya software decision algorithm to differentiate materials with similar chemical structures.

<table>
<tr><th colspan="2">Vaya Two-Criteria Decision Algorithm</th></tr>
<tr><th>Coefficient of Determination ($R^2$)</th><th>Linear Model Coefficient (LMC)</th></tr>
<tr><td>– The coefficient of determination is calculated from the method's model Raman spectrum of a compound and the unknown Raman sample spectrum. It is defined in the conventional way, as the square of the correlation coefficient ($R^2$) between the two spectra.<br>– $R^2$ ranges between 0 and 1, where the closer the $R^2$ value is to 1, the higher the similarity between the unknown sample spectrum and the model spectrum.</td><td>– The linear model coefficient (LMC) is calculated as the slope of a first order polynomial fit using a first order linear regression model between the model Raman spectrum and the unknown sample Raman spectrum.<br>– LMC ranges between 0 and 1:<br>– 1 is the ideal value, indicating that the unknown sample spectrum is highly correlated to the model spectrum.<br>– 0.7 to 1: values in this range indicate a specificity that is considered optimal.<br>– < 0.7: LMC values below 0.7 indicate a poorer fit of the unknown spectrum to the model spectrum.</td></tr>
<tr><th colspan="2">How are the decision criteria calculated?</th></tr>
<tr><td colspan="2">Step 1. A model is generated using the median of multiple training Raman spectra of the material in container.<br> 
<br>Step 2. At each wavelength, the Raman intensity value for the model is compared to the one of the unknown. A pair (Intensity model, Intensity unknown) is obtained for each wavelength in the spectral range (350 to 2,000 $cm^{-1}$) of the detector.<br> 
</td></tr>
<tr><td>Step 3A. The $R^2$ of the pair (Intensity model, Intensity unknown) is calculated.<br> 
</td><td>Step 3B. The LMC (slope of the first order linear regression) of the pair (Intensity model, Intensity unknown) is calculated.<br> 
</td></tr>
</table>

### Vaya method development: analogous samples

The two-criteria decision algorithm works well for most applications. Occasionally, however, the Raman spectra of closely related materials have characteristics that are highly similar. In these cases, full selectivity or differentiation requires the use of the "Analogous Samples" feature of the Vaya software during method development, as shown in Figure 3.

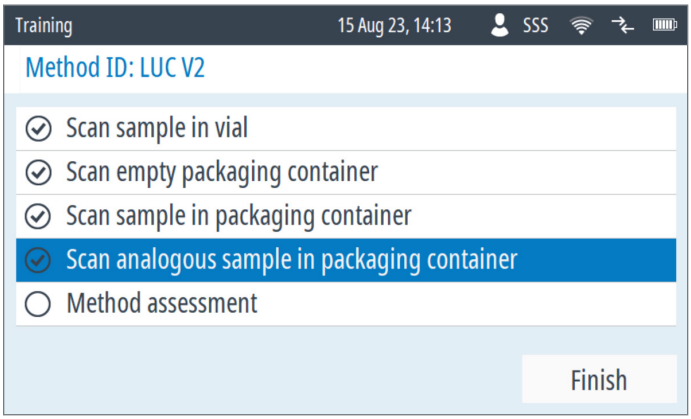



**Figure 3.** Screenshot of the Agilent Vaya handheld Raman spectrometer method development software.

Adding spectra of analogous samples to the method is a simple way to optimize the thresholds of the decision algorithm and achieve high selectivity between similar materials. Scans of analogous samples are added to the method quickly and easily during the "training phase" of method development. Once established, the method can then be assessed/tested before use. All data processing is performed automatically by the instrument software. A description of the automated data processing steps performed on Vaya when analogous sample scans are added to the method during method development is given in Table 2.

**Table 2.** Stepwise description of automated data processing steps performed on Vaya when Analogous Sample scans are added to the method during method development.

| Vaya method development: Data processing of analogous samples | |
|---|---|
| Step 1 | During method development, the user adds scans of the subject sample in its container and scans of samples with highly similar chemical structures (analogous samples). It is useful to add scans of samples that have been falsely identified as the subject material during the initial method validation stage. The Vaya software then creates a "model median" for the subject material and each of the analogous materials. |
| Step 2 | Multilinear regression is applied to the sample scan data against both the model medians for the subject and all of the analogous samples. When multiple analogous samples are added, the analogous median that generates the lowest LMC score against the unknown sample is removed from the method. This process is conducted iteratively until only one analogous model and the subject model remain. |
| Step 3 | From this linear regression, an LMC score is calculated that is equivalent to the slope of the regression plot, for each sample scan data with the model median. These scores are then used to calculate the method LMC threshold. |
| Step 4 | For unknown batch scans, LMC scores are calculated against the model median for the subject method and any analogous samples using the same approach as during method development Steps 2 and 3. If the LMC score is calculated to be higher than the method LMC threshold against the subject method, then the batch result is a PASS. If the LMC score is calculated to be lower than the threshold, then the batch result is delivered as a FAIL. A low LMC can arise due to a penalization of the LMC score because of a better fit of the unknown batch scan against the one remaining analogous sample than the model median, for example. |

## Experimental

### Samples

Technical grade calcium, magnesium, and zinc stearate were bought from Sigma Aldrich. Upon receipt, the metal stearate powders were placed into transparent LDPE bags as primary containers.

### Instrumentation and data acquisition

The Vaya handheld Raman spectrometer was used to create a method for the identification of each metal stearate through the LDPE layer that is representative of the liner used in multilayer paper sacks. Each metal stearate method (three in total) is based on a model built from 10 repeat scans of the corresponding metal stearate samples. Each of the methods was then validated to confirm specificity.

Following USP <1225> requirements, the method under validation was positively challenged five times using samples with the correct metal stearate. The software automatically processes the acquired data against the method and confirms the sample ID with a pass. Five negative challenges were also conducted. Each method was successfully validated against each of the remaining stearates, ensuring that the negative results were repeatable.

## Results and discussion

Figure 4 shows the spectra obtained for the three metal stearate samples through LDPE and the clear similarities between them. Using SORS, it was possible to acquire low noise spectra for each stearate in the plastic bags.

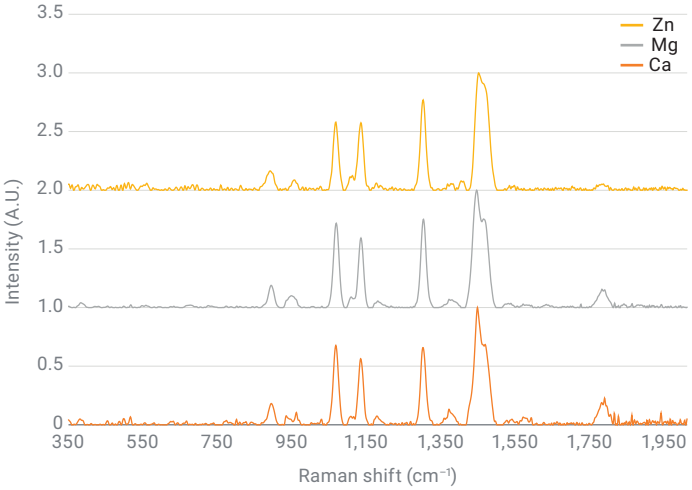



**Figure 4.** Raman spectra of the three metal stearate samples in clear LDPE bags obtained by Agilent Vaya handheld Raman spectrometer in SORS mode.

## Verification of the Vaya methods

The challenge matrix in Figure 5A shows that the two-criteria decision algorithm can separate calcium and magnesium stearate from zinc stearate through an LDPE liner, but it cannot separate calcium and magnesium stearate from each other.

Zinc stearate exhibits some significant spectral differences that explain why an analogous model for this material is not required to achieve differentiation with calcium and magnesium stearate. Calcium and magnesium stearate have similar spectra and therefore score similarly using the decision algorithm that is based on $R^2$ and LMC, resulting in false-positive results.

To improve the robustness of the magnesium stearate method, spectra of calcium stearate were added as analogous samples. Likewise, spectra of magnesium stearate were added to the calcium stearate method, resulting in excellent differentiation for both compounds, as shown in Figure 5B.

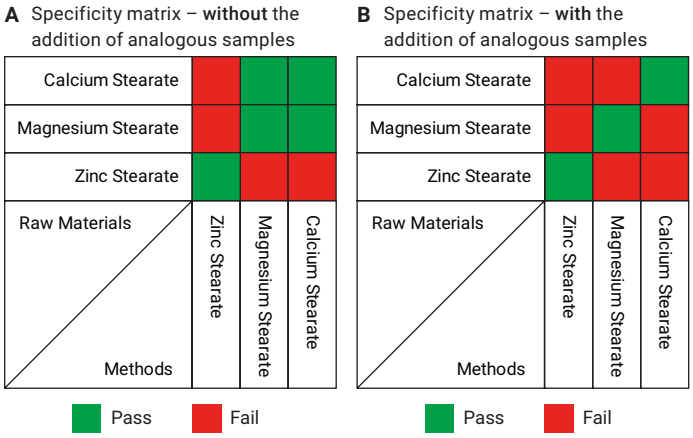



**Figure 5.** (A) Specificity matrix – without the addition of analogous samples. (B) Specificity matrix – with the addition of analogous samples. Green indicates a "Pass" result when scanning the raw material versus the method built using that raw material. Red indicates a "Fail", which should occur when scanning a different raw material versus the developed method.

## Conclusion

The study has shown that the Agilent Vaya with SORS can verify the identity of stearate analogs through LDPE liners without the use of offline chemometric software. The fast, highly selective method enables the quick movement of pharmaceutical raw materials from receipt into production.

Vaya was able to differentiate and correctly verify raw materials that share similar spectra due to a combination of a powerful decision engine and the addition of analogous samples during method development. Using this approach, Vaya provided the selectivity necessary to correctly verify calcium, magnesium, and zinc stearates using on-board software tools.

In a warehouse setting, the Vaya handheld Raman spectrometer will accelerate throughput of mandated quality testing of raw materials, without compromising testing integrity or increasing operational costs.

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