Application Note
Pharmaceuticals

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Rapid Identification of Raw Materials Inside Packaging

Using through-container spatially offset Raman spectroscopy

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Introduction

Glycerin is a color-less odorless, viscous liquid with a sweet taste and is widely used in liquid pharmaceutical, cosmetic, and food products. Diethylene glycol (DEG) is also a colorless, viscous liquid with a sweet test. The analogy stops here. DEG is a glycol with acute toxicity when ingested. In 1995/96, more than 60 children in Haiti died after ingesting a cough syrup containing glycerin tainted with DEG. Following this tragedy, the US FDA published extra guidance on the testing of glycerin for DEG (*1*). The extra guidance stated that deaths were caused by the ingestion of DEG-tainted glycerin due to the absence of a full identity test on the glycerin raw material. The FDA also noted that the pharmaceutical manufacturers solely relied on the Certificates of Analysis (CoA) provided by the supplier. The obtained COA was often a copy on the letterhead of a distributor and not the original glycerin manufacturer CoA. The chain of custody of the document could not be demonstrated because the glycerin raw material changed hands multiple times.

In this guidance, the FDA highlights the need for pharmaceutical manufacturers to use specific identification test to distinguish raw materials from closely related analogs.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) topic Q2(R1) "Validation of Analytical Procedures" sets requirements around analytical techniques used for identity verification and their validation. In particular, identification tests are intended to ensure the identity of an analyte in a sample. The tests must be able to select between compounds of closely related structure that are likely to be present. This ability should be confirmed by obtaining positive results from samples containing the analyte coupled with negative results from samples that do not contain the analyte.

Effective raw material identification verification equipment must therefore be selective and dependable. Being able to test materials without removing samples from packaging is also valuable to pharmaceutical companies, saving time and money, and reducing the risk of contamination.

The Agilent Vaya Raman system is a SORS (Spatially Offset Raman Spectroscopy) handheld spectrometer. The instrument can identify raw materials through transparent and opaque containers to simplify and accelerate the receipt of raw materials in cGMP facilities.

This study demonstrates the use of an Agilent Vaya Raman system to differentiate closely related materials. The analytical procedure guidelines for identity testing, as defined in ICH Q2 (R1) were followed.

Agilent Vaya Raman System for identification testing

Figure 1. The Agilent Vaya Raman handheld spectrometer being used to identify materials inside sacks in a warehouse.

The Agilent Vaya Raman handheld spectrometer (see Figure 1) simplifies the identity verification of raw materials by quickly analyzing materials at receipt or in quarantine.

Using Spatially Offset Raman Spectroscopy (SORS), Vaya performs the ID test directly through both transparent and opaque containers. A single operator can easily receive and release large numbers of containers in a matter of hours, rather than days. This method eliminates the movement of containers, sampling booth clean up, sampling consumables, and PPE for testing personnel.

Spatially offset Raman spectroscopy

SORS uses the property of light propagation through diffusely scattering materials, combined with Raman spectroscopy to achieve through-barrier analysis.

Unlike a conventional Raman back-scattering setup, SORS uses a physical offset between the region of the sample being excited by the monochromatic light (a laser) and the region of the sample the detector is collecting information from. This offset geometry collects Raman photons in the detection area originating mostly from beneath the sample surface. This geometry yields a spectrum rich in subsurface "information". In contrast, the spectrum with no or "zero" physical offset yields a spectrum rich in the top layer "information". When measuring a raw material in a container, the container-rich "zero offset" spectrum is subtracted from the raw material-rich "offset" spectrum. The resultant spectrum is a container-free raw material spectrum that can be used for ID verification purposes.

Unlike conventional Raman back-scattering spectroscopy, SORS can perform identification tests through many different containers. Tests can be done through transparent and opaque containers such as amber bottles, multilayer paper sacks, colored and transparent plastic liners, and opaque polyethylene containers.

Pharmacopeia requirements for identification tests

The SORS technique is subject to the same rules as those for Raman spectroscopy in the various pharmacopeia chapters (*2*) and identification test methods. In addition to spectrometer requirements, guidance was established for the identification test to ensure deployment readiness and effectiveness in operation.

The International Conference on Harmonization (ICH) Harmonized Tripartite Guideline, "Validation of Analytical Procedures: Text and Methodology" (*3*) specifies the validation process that an identification method must undergo before being deployed.

As a category IV assay, an identification test need only be able to unequivocally assess the identity of an analyte.

You must test the specificity of the method: the ability of the method to correctly identify compounds with similar chemical structures. The accepted validation methodology consists of positively and negatively challenging the method under operational conditions. The positive challenge verifies the identity of well-characterized samples of different batches[1] of the analyte. The negative challenge must confirm that the method correctly rejects analogs under the same conditions.

Experimental

To demonstrate the specificity of the Vaya instrument, a series of methods for the identification of raw materials was developed. Each method was generated using reagent grade products (from Sigma-Aldrich UK or Colorcon) to verify the identity of each raw material listed in Table 1. The methods varied with the primary packaging (multilayer paper sack, polyethylene (PE) transparent bag, white polyethylene (HDPE) bottle, FIBC bag, and amber bottle) and with the raw material.

Three Vaya instruments were used to develop a total of 39 methods. 10 scans were acquired for each model. The methods were grouped into four analog sets, based on raw material similarity:

- Sugars
- Glycols/Diols
- Long chains
- Coating agents

Each method was subjected to a series of positive and negative challenges using multiple analogs for validation following USP<1225> (equivalent to protocol ICHQ2 (R1)).

For each analog set, a challenge matrix was developed. Each matrix included the pass rate of each raw material in the class against each identification method in that class. Each identification test was repeated 10 times and an average of the pass score was reported. The analytical conditions used to acquire each scan (for method training) or for identification tests were automatically set by the instrument with no intervention from the operator.

Table 1. List of raw materials with their respective containers

Chemical groups	Chemicals with associated container
Sugars	Anhydrous Dextrose in paper sack, Anhydrous Dextrose in white HDPE, Dextrose monohydrate in 3-layer paper sack, Dextrose monohydrate in white HDPE, Galactose in white HDPE
Glycols, diols	Propylene glycol in clear PE, Diethylene glycol in amber glass, 1,2-Butanediol in amber glass, 2,3-Butanediol in amber glass, Triethylene glycol in clear PE, 1-3-Butanediol in amber glass, Glycerin in clear PE, 1,2-Pentanediol in amber glass, 1,4-Pentanediol in amber glass, 1,3-Propanediol in clear PE, 1,4-Butanediol in clear PE, 1,5-Pentanediol in clear PE, 2,4-Pentanediol in amber glass, Dipropylene glycol in clear PE, Tripropylene glycol in amber glass, 1,2,4-Butanetriol in amber glass, Tetraethylene glycol in amber glass, Ethylene glycol in amber glass, Polyethylene glycol (MW 8000) in amber glass, Polyethylene glycol (MW 4000) in amber glass
Long Chains	Adipic acid in white HDPE, Cetyl alcohol in white HDPE, Decanoic acid in amber glass, Isopropyl myristate in amber glass, Octanoic acid in glass, Palmitic acid in glass, Paraffin wax in white HDPE, Sodium dodecyl sulfate in white HDPE, Sorbitan monopalmitate in amber glass, Stearic acid in white HDPE, Magnesium stearate in glass, Lauric acid in white HDPE
Coating agents	orange coating agent in clear vial, green coating agent in clear vial, yellow coating agent in clear vial, white coating agent in clear vial

Results and discussion

Figure 2 A shows that SORS is an effective technique to verify ID through non-transparent containers. It correctly subtracts the container contribution of non-transparent containers to yield container-independent analyte spectra. The results show the similarity of the SORS Raman spectra acquired for anhydrous dextrose through multilayer paper sack, white HDPE container, and its reference spectrum acquired through clear glass. For example, the bands for HDPE are not visible on the anhydrous dextrose SORS spectrum acquired through the white HDPE container (i.e. 1065 cm^{-1}, 1129 cm^{-1}, 1295 cm^{-1} and 1440 cm^{-1}). Figure 2B demonstrates that SORS is adaptable to other materials. The technique retains minor spectral features, as shown by the small differences in the different SORS spectra obtained for the 'sugars class' anhydrous dextrose, dextrose monohydrate and galactose in paper sacks, white HDPE plastic containers, and clear glass.

1. Samples of batches not used during method development

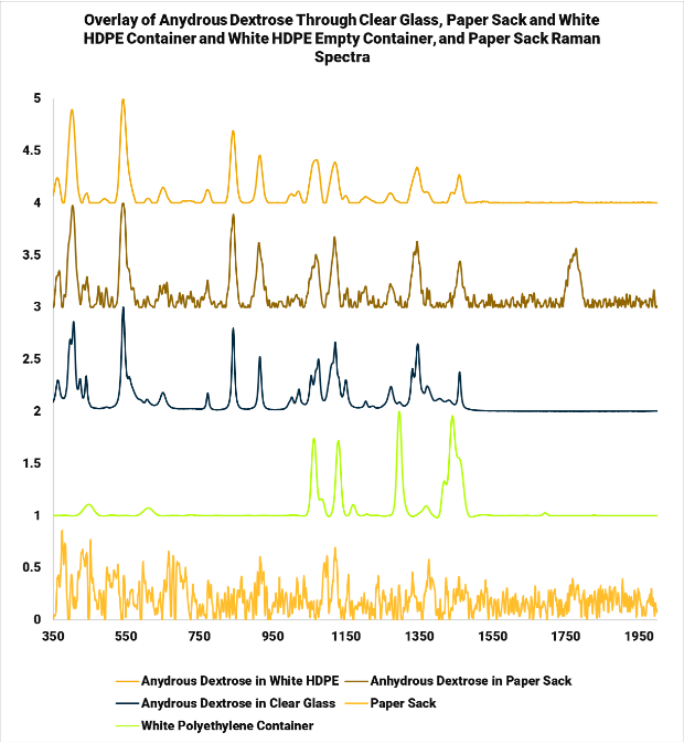

Figure 2A. Overlay of spectra for anhydrous dextrose acquired through clear glass, white HDPE, multilayer paper sack, and spectra of white HDPE container and paper sack

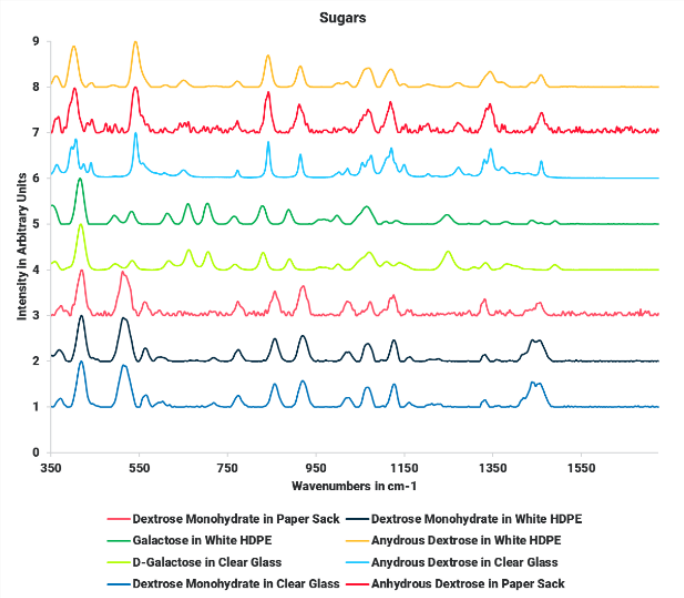

Figure 2B. Overlay of spectra for a set of similar sugars acquired through multilayer paper sack, white HDPE containers, and clear glass

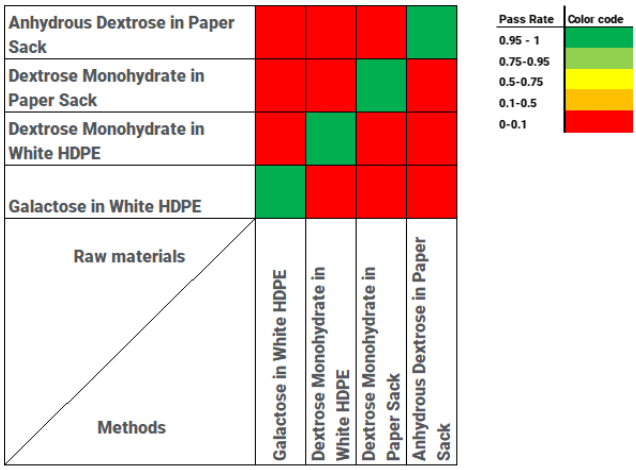

Figure 3. Challenge matrix for a set of analogous sugars stored in multilayer paper sacks and white HDPE containers.

Figure 3 shows a challenge matrix that graphically represents how the Vaya instrument differentiates and correctly verifies the identity of closely related structures. In a challenge matrix, an ID test is conducted for each analyte 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 above 0.95 along the matrix diagonal indicating that the method recognizes its corresponding material perfectly. Off the diagonal, the ideal matrix should display only pass scores below 0.1. A score of 0.1 indicates that the method correctly rejects incorrect analytes (i.e. dextrose anhydrous in paper sack tested with method galactose in white HDPE). An ideal matrix demonstrates that a group of methods has a zero level of false positives and can be deployed in a warehouse environment. Figure 3 shows the "Sugar class" challenge matrix and demonstrates that the Vaya instrument can easily differentiate between all the similar sugars in different containers.

It is to be noted that dextrose monohydrate and anhydrous dextrose were tested in both 3-layer paper sacks and white HDPE containers. The method for dextrose monohydrate and the method for anhydrous dextrose in paper sacks rejected the same sugars when they were contained in white HDPE containers. A Vaya method is developed to get the best specificity and sensitivity for a particular combination of analyte-container. A Vaya method will reject the correct material in a different container. The measurement conditions are refined for each analyte-container combination. For example, the optimum position or optimum threshold depend on the signal-to-noise ratio of that specific container.

Vaya enables an operator to verify that the raw material is received in an appropriate container, preventing the introduction of unsuitable containers for chemical storage in a warehouse.

Figures 4A and 4B demonstrate that the Vaya instrument is an effective solution for the verification of raw materials in different transparent containers. Raman spectra were easily acquired for all the diols and glycols in the "Diols/ Glycols class" stored in amber glass bottles[2] or PE bags[3] . Despite weaker Raman cross sections for some materials, all the spectra show little signal noise. The spectra also have no container contribution, as shown by the glycerin in a transparent PE liner spectrum in Figure 4C. PE Raman features, in particular the prominent 1295 cm^{-1} band, are not visible in the spectra. Figures 4A and B also show that SORS retains Raman features in the SORS Raman spectra of all small (C3-C6) analogous molecules measured through containers. This includes PE liners and containers made with light blocking materials like amber glass[4].

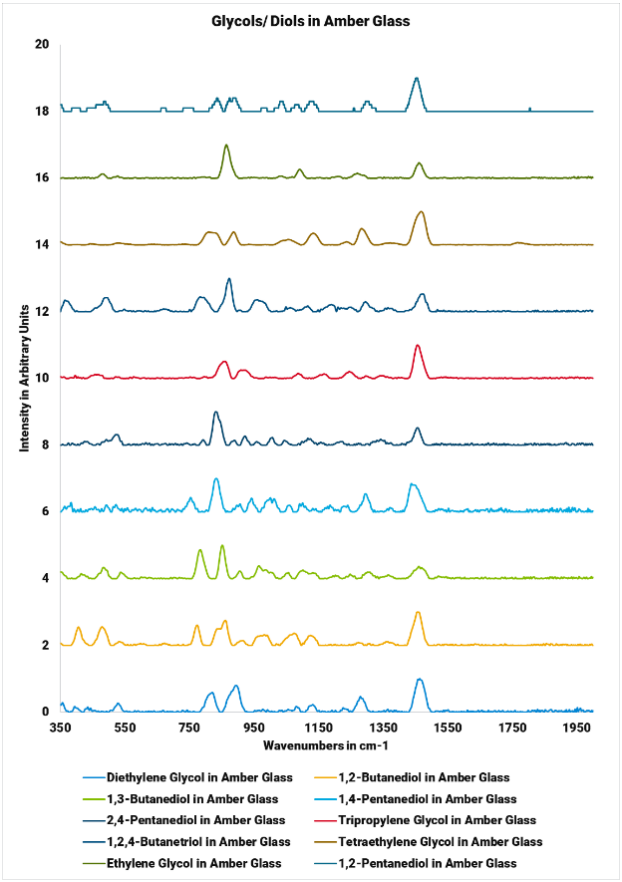

Figure 4A. Overlay of spectra for a set of similar glycols and diols acquired in amber glass containers

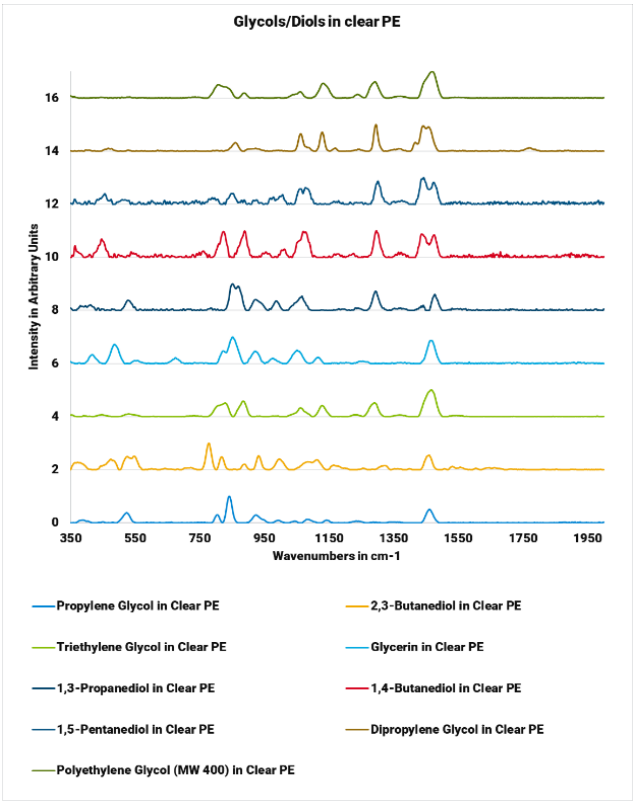

Figure 4B. Overlay of spectra for a set of similar glycols and diols acquired in clear PE liner

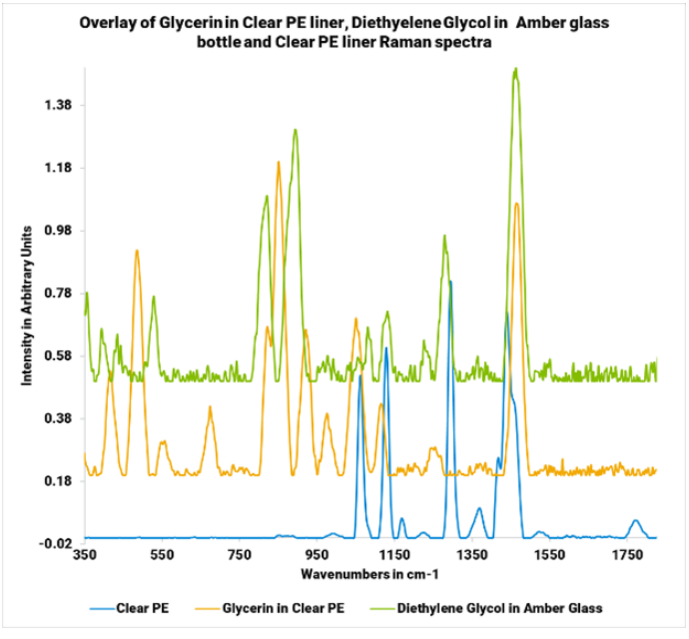

Figure 4C. Overlay of the Raman spectra of glycerin acquired in a clear PE liner, DEG acquired in an amber bottle and a clear PE liner Raman

2. Glass has typically a weak Raman cross section. Silicate and borosilicate glasses typically give three broad peaks centered on 80, 500 and 1000 cm^{-1}. A small peak is also visible around 800 cm^{-1}. Some glass may exhibit some photoluminescence that is easily eliminated by SORS by background subtraction.
3. A polymer commonly used as plastic liner in the pharmaceutical industry
4. Through amber glass analysis is often more difficult to perform given the extinction coefficient of this material. Amber glass often requires sensitive detectors for the acquisition of the Raman spectrum for weak Raman scatterers like small polar organic molecules.

Figure 4A and B show that the Vaya spectrometer can differentiate small hydrocarbon-based molecules (C3 through C6) with a varying number/position of hydroxy moieties and/or oxy substitutions. The Vaya instrument prevents DEG being substituted for glycerin due to the spectral differences in the 750 cm^{-1} to 1150 cm^{-1} region (*4*), see Figure 4C. It is also possible to differentiate polymeric versions of the small hydrocarbon-based units. Polyethylene glycols of different molecular weight (MW) can be separated as Raman bands are sensitive to change in MW (*5*).

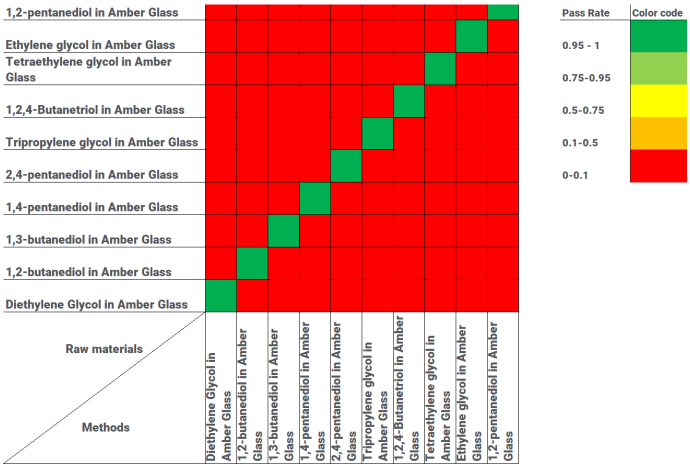

Figure 5A. Challenge matrix for a set of analogous diols/glycols stored in amber glass.

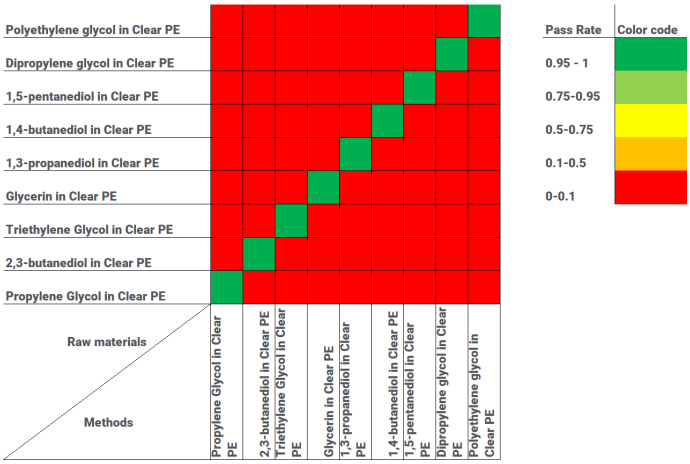

Figure 5B. Challenge matrix for a set of analogous diols/glycols stored in PE.

Figures 5A and 5B demonstrate the specificity power of the Vaya instrument. The challenge matrix results for the "glycols/diols" class in both types of transparent containers (amber glass and PE liners) are perfect.

Figures 6A and 6B show that the technique is also an effective solution for the acquisition and identification of long chain hydrocarbons through non-transparent containers. Overlaid spectra for long chain analogs like lauric acid, adipic acid and stearic acid show that the white HDPE container[5] spectrum was subtracted from the spectra. Minute spectral differences of each long chain analog were retained. Despite overlap with the raw materials' Raman bands, the bands for PE are correctly removed from the raw material spectra. Figure 6C shows myristic acid overlaid with a white HDPE container. The PE bands at 1440 cm^{-1}, 1295 cm^{-1}, 610 cm^{-1}, and 448 cm^{-1} are not observable in myristic acid when the spectrum is acquired through the white HDPE container.

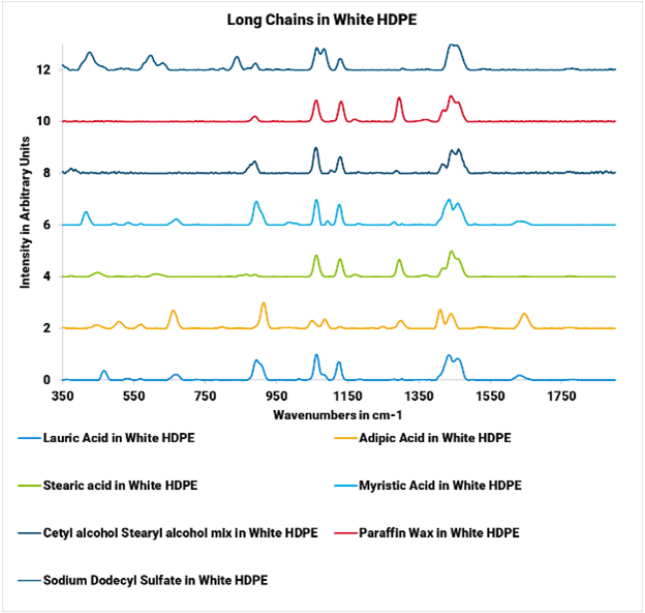

Figure 6A. Overlay of spectra for a set of similar long chain hydrocarbons acquired in white HDPE containers.

5. White HDPE bottles are typically made with HDPE (High Density Polyethylene) with a TiO_2 filler for white color.

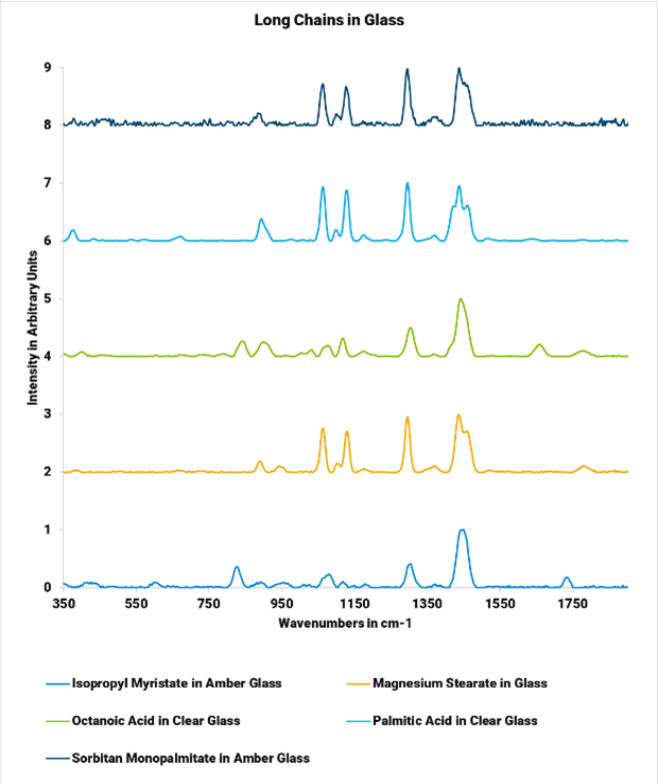

Figure 6B. Overlay of spectra for a set of similar long chain hydrocarbons acquired in amber and clear transparent glass (vials).

Figures 7A and B demonstrate the effectiveness of the Vaya instrument for selective identification of long chain hydrocarbons when analyzed through nontransparent and transparent containers.

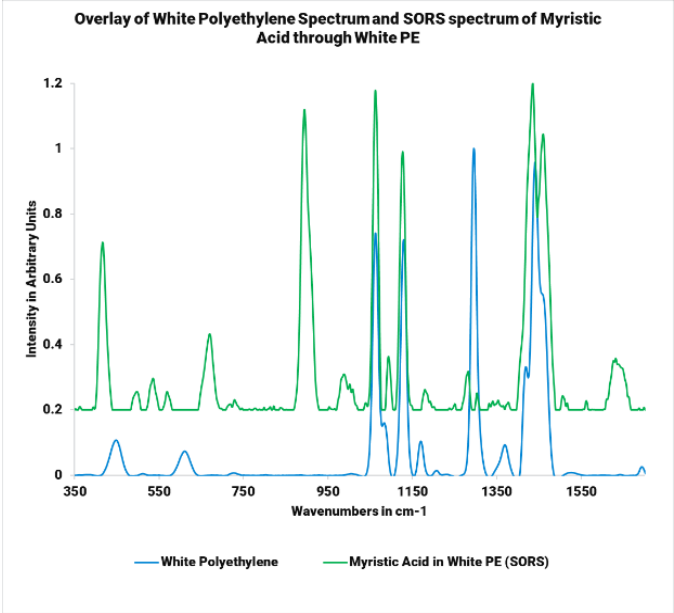

Figure 6C. Overlay of White HDPE (empty container) and SORS spectrum for myristic acid acquired through white HDPE.

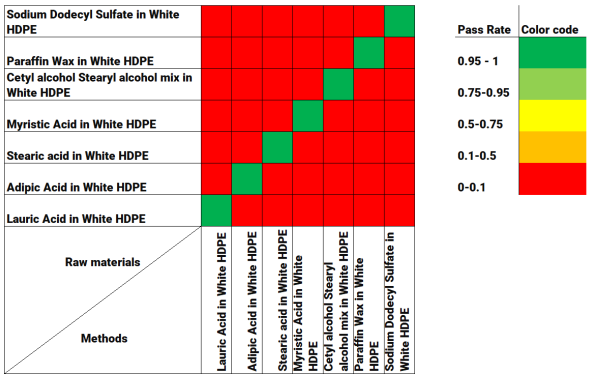

Figure 7A. Challenge matrix for a set of analogous long chains stored in PE.

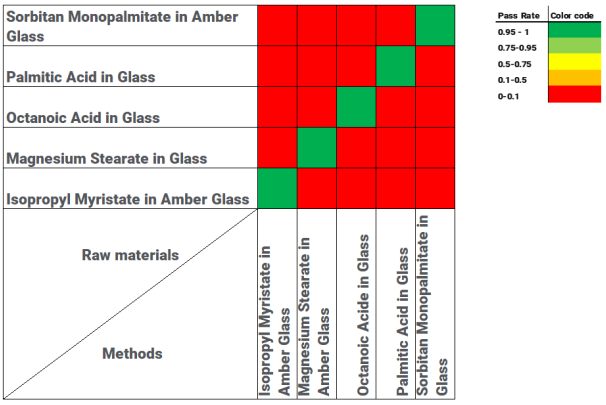

Figure 7B. Challenge matrix for a set of analogous long chains stored in PE.

Figures 8 and 9 demonstrate the technique's specificity for a blend of raw materials like coating agents of various color[6]. The coating agents can be sorted by the Vaya despite their similar composition and the dominance of the three bands (397 cm^{-1}, 515 cm^{-1} and 640 cm^{-1}) attributed to TiO_2 in the Raman spectra.

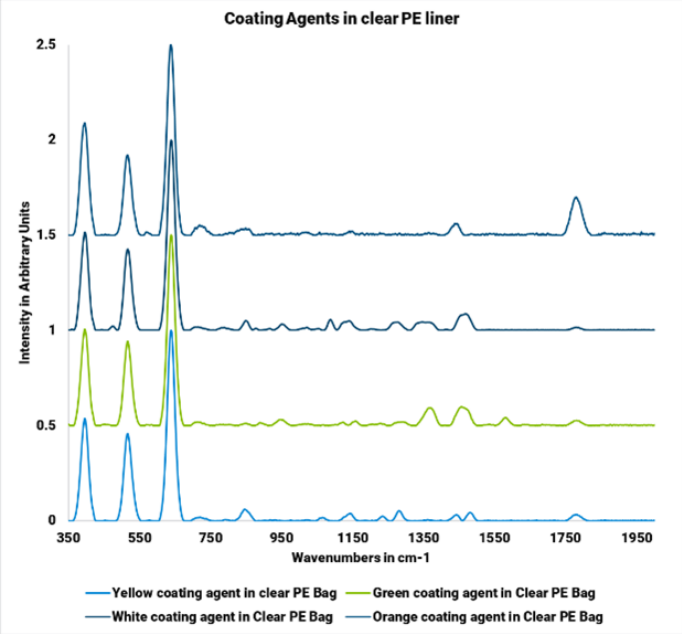

Figure 8. Overlay of Raman spectra of coating agents of different colors acquired in clear PE liner.

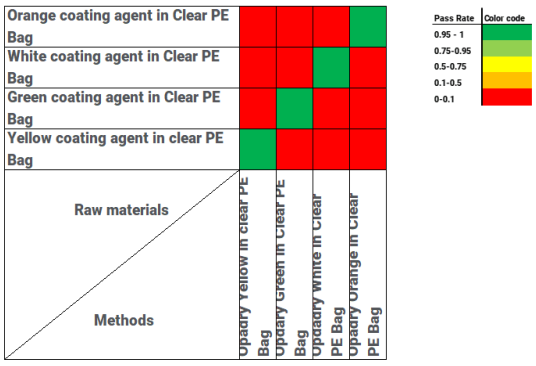

Figure 9. Coating agents Challenge Matrix with analogous raw materials added to the respective methods. Opadry® is manufactured by Colorcon, Dartford Kent, UK (www.colorcon.com/products-formulation/all-products/film-coatings/immediate-release/opadry). Opadry is typically made with Hypromellose, a plasticizer (Macrogol/Polyethylene Glycol) and appropriate mixture of dyes with Titanium Oxide

How does Vaya achieve selectivity?

The Vaya instrument identifies different chemicals in the same class by using an advantageous 2-score decision engine based on a coefficient correlation (R^2) and a linear model coefficient (LMC). R^2 is a quantitative metric used in spectroscopy to determine how similar the unknown sample spectrum is to the model spectrum in the method. The LMC provides an extra check to enhance the decision engine selectivity. It is based on the incorporation of more spectra into the model, such as a challenge material (i.e. analyte spectrum) and observing its influence over the LMC score.

When developing a Vaya method, it is possible to teach an ID method for a raw material to correctly reject the raw material's analogs (or similarly structured materials) by adding spectra of these analogs to the method. The extra spectra enhance method selectivity for a given raw material. Following the addition of analogs to the method, the decision engine automatically adjusts the R^2 and LMC PASS/FAIL thresholds to adapt to the raw material's new environment.

In the coating agents selectivity analysis, the use of additional analogous spectra produced a perfect selectivity matrix for all the different colors of coating agents tested. Although each coating agent is visually different, the chemical composition difference is not as dramatic and requires the model for each coating agent of color to be optimized for selectivity. Figure 10 shows the challenge matrix for coating agents yellow, green, white, and orange before the addition of the 3 respective analogous colors (green, white, orange and/or yellow).

The matrix shows that without the addition of analogs, the different coating agents' models cannot correctly differentiate agents. For example, the yellow coating agent is consistently misidentified by all models. The addition of analogs, as demonstrated in Figure 9, completely eliminates this issue, correctly identifying of all the coating agents.

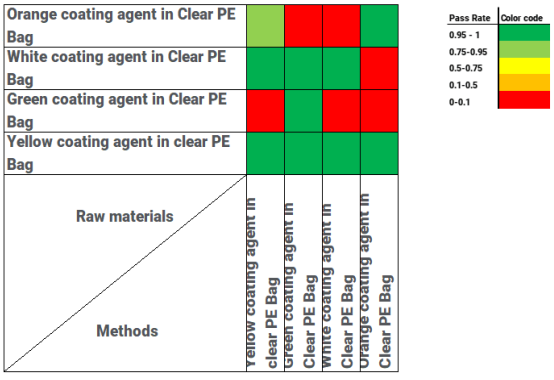

Figure 10. Coating agents Challenge Matrix without analogous added to the respective methods.

How does Vaya support method validation?

Post development, Vaya supports method validation through a dedicated validation platform. In this module, a method under development can be cross checked against other existing methods in production to verify the absence of identification conflict and the possibility of false positive. In addition, the method's robustness and validity can be tested through positive and negative challenges by the method developer.

- different production lots of a compound,
- different sampling locations of a raw material of confirmed identity in a container for positive testing
- closely related chemicals that can find their way into the warehouse for negative challenges.

For cGMP purposes, the validation module includes automated reporting and audit trail traceability.

Conclusion

The Agilent Vaya SORS Raman spectrometer was able to confirm the identity of raw materials inside their packaging container. The technique can quickly identify raw materials and detect shipment errors or willful substitutions, even with closely related, lower cost analogs.

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