Application Note

Pharma & Biopharma

Verification of Raw Materials for Synthetic Peptide Production with the Agilent Vaya Raman System

Authors

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Abstract

Advancements in the manufacturing technology of peptide drug products have enabled large-scale synthetic peptide production, producing high-purity synthetic peptides in bulk. The widespread demand for glucagon-like peptide receptor agonists (GLP-1 RAs) has put pressure on synthetic peptide manufacturing capacity and revealed the need for efficient and scalable tools. The Agilent Vaya Raman raw material identity verification system enables uniquely efficient raw material verification due to its through-container identification capabilities. This application note showcases Vaya's successful through-container identification and differentiation of key building blocks for synthetic peptide manufacturing: fluorenylmethoxycarbonyl (Fmoc)-protected amino acids.

Introduction

Peptides are short chains of amino acids (< 40) that can be powerful therapeutics due to their specificity, selectivity, and potency. Additionally, peptides can be designed to be orally active, allowing for an improved patient experience compared to parenterally administered drugs. Peptides that regulate metabolism, such as GLP-1 RAs, have escalated rapidly in popularity due to their efficacy in treating diabetes and weight management. This growing family of medicine includes dipeptidyl peptidase-4 inhibitor (DPP4) degradation-resistant peptides such as semaglutide, liraglutide, tirzepatide, and exenatide.

Peptides can be manufactured using recombinant DNA technology or by synthetic means. For manufacture by use of recombinant DNA, raw materials would have similarities to those outlined in a previous application note.[1] Production using synthetic means requires a significantly different set of raw materials, involving specialized amino acids, solvents, and reagents.

Synthetic peptide production

The manufacturing of synthetic peptides typically follows two methods: solid-phase peptide synthesis (SPPS) or liquid-phase peptide synthesis (LPPS). SPPS is commonly used for longer or more complex amino acid sequences, such as GLP-1 RAs, involving the sequential assembly of amino acids on a solid support resin.

Raw materials for synthetic peptide synthesis

From a raw material perspective, the crucial starting materials in peptide synthesis are Fmoc-protected amino acids. Fmoc protection is necessary to support the synthesis process and amino acid coupling to produce the specific peptide. In SPPS manufacturing, it is critical that the building blocks and reagents have their identity verified prior to use to ensure a high-quality finished product. Regulatory bodies also globally enforce the verification of raw materials for drug manufacturing to ensure product safety.

Traditionally, Raman handheld devices using conventional backscattering require the container to be opened and sampled, potentially introducing contamination and shortening the shelf life of raw materials. Fmoc-protected amino acids are sensitive to light and air, so identification without opening the container is ideal to help ensure the integrity of the raw material before use in production.

The Vaya Raman is a handheld device that uses spatially offset Raman spectroscopy (SORS). SORS enables the verification of raw materials through transparent or opaque containers such as amber bottles, thick plastic, and paper sacks, eliminating the need to open them. In this application note, the through-container identity verification ability of the Vaya was tested to identify Fmoc-protected amino acids.

Experimental

Samples of Fmoc-protected amino acids were supplied by Sigma-Aldrich in white high-density polyethylene (HDPE) or amber bottles. Table 1 outlines the structure and nomenclature of each raw material used in this application note, as well as a picture of the raw materials in their original containers.

Methods were developed on the Vaya for four Fmoc-protected amino acids without opening their respective containers. The guided on-device software provides steps to create methods and ensure models are differentiable. During method development, the software prompts the user to select Container Type for optimal container subtraction. In this case, Glass and Thick Plastic were chosen for amber bottles and white HDPE bottles, respectively.

Once methods were created and validated, Fmoc-protected amino acids could be identified and differentiated through amber bottles or white HDPE in a scan time of less than 40 seconds, delivering a clear and simple Pass/Fail result. A visual representation of analysis using the Vaya is shown in Figure 1.

Table 1. Outline of raw materials.

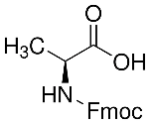
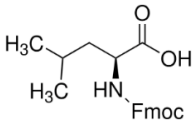
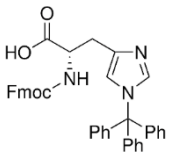
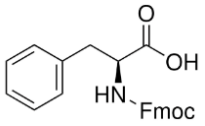
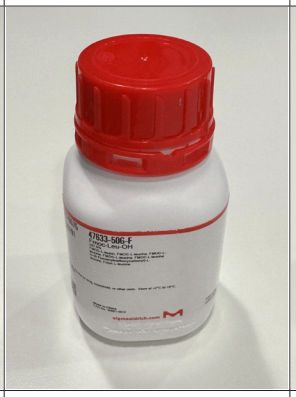
Structure of Raw Material				
Name of Raw Material	Fmoc alanine hydroxide (Fmoc-Ala-OH)	Fmoc leucine hydroxide (Fmoc-Leu-OH)	Fmoc histidine TRT hydroxide (Fmoc-HIS(Trt)-OH)	Fmoc phenylalanine hydroxide (Fmoc-Phe-OH)
Picture of Raw Material in Native Container				
Container Type Selected on the Device for Method Development	Glass	Thick plastic	Thick plastic	Thick plastic

Figure 1. The Agilent Vaya Raman raw material identity verification system being used to verify Fmoc-protected amino acids.

Results and discussion

Fmoc-protected amino acids show differentiable Raman spectra through both amber bottles and white HDPE. Despite their chemical similarities, Vaya SORS quickly distinguishes and verifies the identity of Fmoc-protected amino acids through the container, preserving the integrity of the raw materials.

In Figure 2, the features at 1,481cm^{-1} are attributed to the Fmoc-protecting group, and are consistent across the raw material group. The protecting group feature is unique to Fmoc-protected amino acids versus natural amino acids. Additionally, there are features corresponding to the aromatic groups at 1,003, 1,026, 1,582 cm^{-1}, and carbonyl groups can be identified by features at 1,675 and 1,687 cm^{-1}.

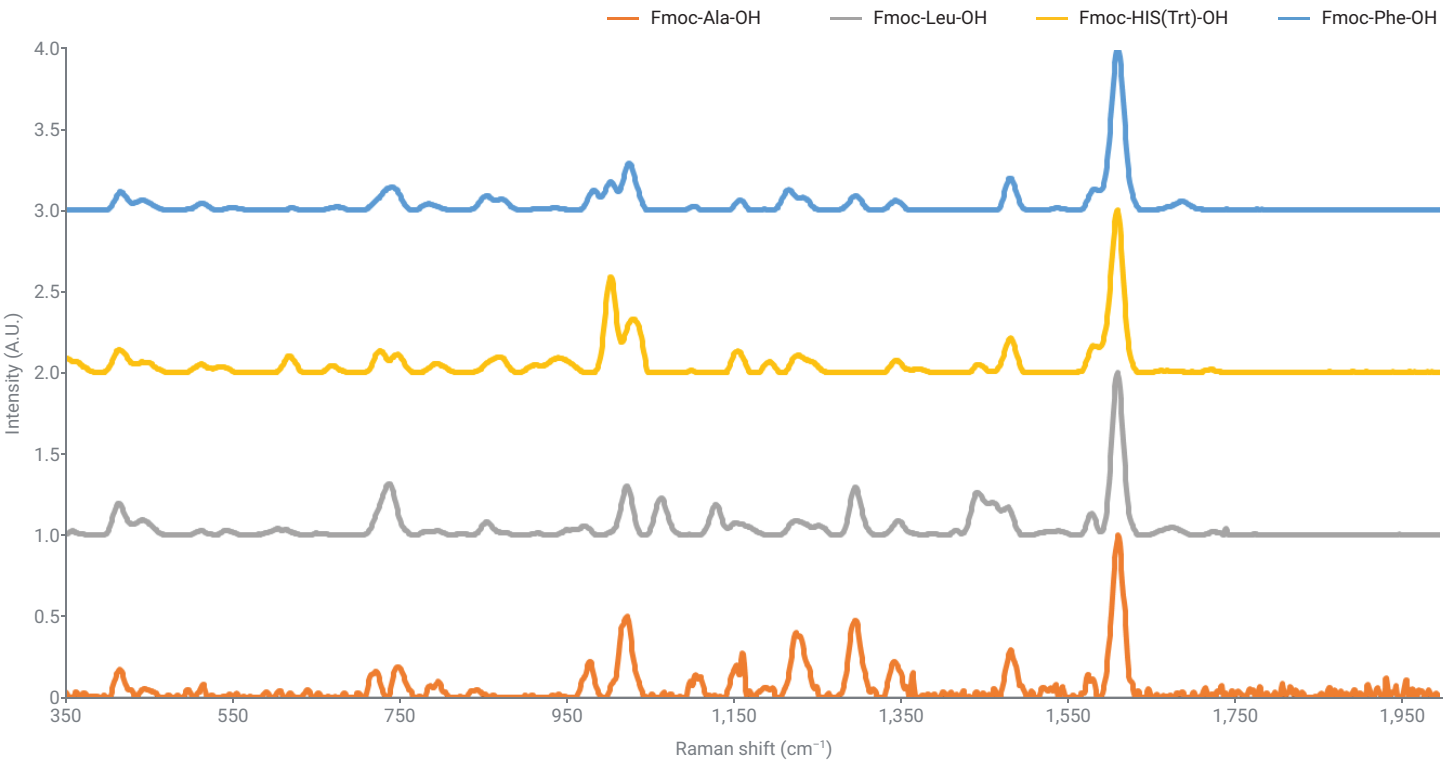


Figure 2. Spectra of Fmoc-protected amino acids.

Conclusion

The increasing demand for peptide biologics, such as GLP-1 receptor agonists, is expected to continue growing and outpacing current manufacturing capacity. To effectively meet demand, scalable solutions like the Agilent Vaya Raman raw material identity verification system introduce efficiencies to avoid bottlenecks that could be faced in raw material identification and regulatory-enforced verification. Additionally, the Vaya's ability to perform nondestructive, noninvasive identification ensures that raw materials are transferred expediently to production while preserving their integrity.

Reference

1. Prullière, F.; Welsby, C. Differentiating Biopharmaceutical Raw Materials Using Spatially Offset Raman Spectroscopy, *Agilent Technologies application note*, publication number 5994-3534EN, **2021**.

Additional resources

Vaya Handheld Raman Spectrometer

Rapid Testing of Biopharmaceutical Solvents

Differentiating Biopharmaceutical Raw Materials

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