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*Short communication*

# Integrated vibrational spectroscopic and chemometric strategies for advanced quality control of therapeutic peptides

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## Introduction

In recent years, peptide-based medicines have gained considerable importance and represent one of the fastest-growing classes of pharmaceuticals, with applications in metabolic, endocrine, oncological, and rare diseases (Xiao et al., 2025). However, their structural complexity, sensitivity to environmental conditions, and formulation diversity pose significant analytical challenges. The growing occurrence of falsified and substandard peptide medicines highlights the need for rapid and reliable screening methods (Janvier et al., 2018). Conventional analytical techniques, including chromatography, mass spectrometry, electrophoretic methods, and biological assays, remain indispensable for the analysis of peptide-based pharmaceuticals. However, they are often labor-intensive and require extensive sample preparation, limiting their suitability for rapid screening (Fekete et al., 2015).

Vibrational spectroscopy (VS), including attenuated total reflectance Fourier-transform infrared (ATR-FTIR), near-infrared (NIR), and Raman spectroscopy, offers a rapid and non-destructive alternative by providing molecular fingerprints with little or no sample preparation. Combined with chemometric approaches such as principal component analysis (PCA), hierarchical cluster analysis (HCA), partial least squares discriminant analysis (PLS-DA), and partial least squares (PLS) regression, these techniques can support qualitative identification, provide insights into structural features, and enable quantitative assessment of peptide pharmaceuticals (Godzo et al., 2023; Godzo et al., 2024). Therefore, integrated VS with chemometric analysis represents a promising complementary approach for the quality assessment of therapeutic peptides.

## Quality attributes addressed by vibrational spectroscopy and chemometrics

*Verification of active pharmaceutical ingredient identity*

One of the most important applications of VS in the analysis of peptide therapeutics is the identification of the active pharmaceutical ingredient (API). The presence of an incorrect, undeclared, or absent API represents a critical quality issue frequently associated with falsified medicines. Vibrational spectral fingerprints combined with

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chemometric analysis can potentially discriminate between products containing different peptide APIs, enabling rapid screening and preliminary identification. In this context, ATR-FTIR spectroscopy has been proposed as a screening approach for the differentiation of biopharmaceutical preparations (Godzo et al., 2023). A combination of exploratory and supervised chemometric approaches is often advantageous, where PCA and HCA can provide insight into natural clustering patterns, while PLS-DA can enable classification of unknown samples into predefined API classes.

*Quantitative assessment*

Another quality-related issue is the mismatch between the declared amount of API and the actual composition of a product. This can be due to manufacturing deviations, degradation, or intentional falsification. VS combined with multivariate analysis, particularly PLS regression, can provide rapid insight into API content and support the detection of quantitative deviations. The applicability of this approach was demonstrated for quantitative determination of insulin aspart using Raman spectroscopy coupled with PLS regression (Godzo et al., 2024).

*Structural characterization*

Given the sensitivity of peptide therapeutics to environmental conditions, which can induce conformational changes and degradation, structural integrity represents a critical quality attribute that affects their efficacy and safety. VS can provide insight into structural alterations through spectral features associated with the peptide backbone, particularly the Amide I and Amide II bands, which are sensitive to secondary structure (Tintor et al., 2024). These bands reflect secondary structure elements, including α-helices, β-sheets and β-turns (Waeytens et al., 2021). In combination with chemometric analysis, PCA score and loading plots can reveal clustering patterns and identify spectral regions associated with structural variability. Consequently, these techniques can potentially detect structural alterations caused by improper manufacturing, storage, transportation, or handling.

## Conclusion

Integrated VS with chemometric strategies offers considerable potential for advanced quality control of therapeutic peptides across multiple contexts. Although these approaches are not intended to replace conventional techniques, they provide rapid, non-destructive, and cost-effective preliminary screening and quality assessment of peptide pharmaceuticals, facilitating identification of potential quality deviations and prioritization of samples for confirmatory analysis. Consequently, VS and chemometrics represent promising complementary tools for the analysis of peptide-based pharmaceuticals.

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