Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 563 - 564 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.271
Short communication

Raman spectroscopy combined with PLS-DA for discrimination of insulin types and sample forms

Evgenija Dobreska[1*], Aleksandra Petrovska[1], Sanja Bobevska[1], Hrisanta Godzo[1], Olga Gigopulu[1], Nikola Geskovski[2], Katerina Brezovska[1]

[1]*Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, North Macedonia*
[2]*Institute of Pharmaceutical Technology, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, North Macedonia*

Introduction

Therapeutic peptides are an important class of biopharmaceuticals in modern pharmacotherapy. Their structural complexity and formulation variability pose analytical challenges and highlight the need for rapid and simple approaches for quality assessment (Fekete et al., 2015).

Raman spectroscopy is widely applied for the analysis of biological materials due to its high chemical and structural specificity, minimal sample preparation requirements, and relatively low interference from water. The technique provides detailed information about protein structure, including secondary and tertiary conformational features, and is suitable for the analysis of samples in different physical states (Shashilov et al., 2010). Insulin and its analogues have previously been analyzed using Raman spectroscopy coupled with chemometric methods for pharmaceutical applications (Godzo et al., 2024). Due to the complexity of Raman spectral data, multivariate statistical methods such as Partial Least Squares Discriminant Analysis (PLS-DA), are frequently used for data analysis and sample classification (Lasalvia et al., 2022).

The aim of this study was to evaluate the applicability of Raman spectroscopy combined with PLS-DA for the differentiation and classification of different insulin types and sample forms, including pure standard substances and formulated samples.

Materials and methods

The study included insulin aspart secondary reference material (SRM) (602 nmol/mL; 100 IU/mL), insulin aspart standard substances from three different batches (purities of 95.4, 95.8% and 95.8%), insulin detemir standard substances (potencies of 154 and 155 nmol/mg), human insulin SRM (98.8 IU/mL), and insulin detemir SRM (596 nmol/mL), all obtained from the manufacturer. Highly purified water obtained using a TKA-LAB purification system was used throughout the study.

Insulin aspart SRM, human insulin SRM, and insulin detemir SRM were freeze-dried prior to analysis using a freeze-dryer operated at -50 °C and 7 Pa for 24 h (FreeZone 2.5 Liter Benchtop Freeze Dry System; Labconco Corp., Kansas City, MO, USA) to obtain solid formulation samples for Raman spectroscopic measurements.

*evgenijadobreska@yahoo.com

Raman Spectroscopy

Raman spectra were recorded using a portable ATR 3000 DH Raman spectrometer (Optosky, China) equipped with a 1064 nm excitation laser. Spectra were acquired in the spectral range from 2600 to 190 cm^{-1} with a spectral resolution of 5 cm^{-1} and laser power of 400 mW. Measurements were performed directly on the samples by focusing the probe on the sample surface. Six replicate spectra were recorded for each sample under identical experimental conditions.

Multivariate Statistical Analysis

Spectral preprocessing, including spectral smoothing was performed using GRAMS/32 software (Thermo Fisher Scientific, MA, USA). Baseline correction, standard normal variate (SNV) transformation, and chemometric analysis were carried out using SIMCA® 14 software (Umetrics, Sweden). PLS-DA was used for the discrimination and classification of different insulin types and formulations based on Raman spectral data.

Results and discussion

A PLS-DA model was developed using 47 SNV-transformed spectra in the 200-1800 cm^{-1} Raman fingerprint region. Internal cross-validation resulted in the following model statistics: $R^2X = 0.743$, $R^2Y = 0.941$, and $Q^2 = 0.904$, indicating good model fit and internal predictive ability. The obtained Raman spectra showed characteristic spectral differences between the analyzed samples. Application of the PLS-DA approach enabled successful classification and clear clustering of samples according to insulin type and sample form. The first component, t[1], mainly separates the different insulin types and distinguishes pure standards from formulated samples, whereas the second component, t[2], enabled additional differentiation between the lyophilized formulated samples. However, no independent external test set was available for validation. Therefore, the results should be considered exploratory and require confirmation using external validation samples.

Evaluation of the VIP and coefficient plots identified characteristic Raman spectral regions contributing to sample discrimination, which were consistent with literature-reported Raman bands of peptides (Godzo et al., 2024). The most significant variables were associated with the Amide I (~1650 cm^{-1}) and Amide II (~1550 cm^{-1}) regions, corresponding to characteristic vibrations of the peptide backbone. In addition, spectral features within the 1000-1100 cm^{-1} region, which are likely associated with excipient-related vibrations, contributed to the differentiation between pure standards and formulated samples (De Veij et al., 2009). These findings demonstrate the potential of Raman spectroscopy combined with PLS-DA for rapid discrimination of insulin types and formulations.

Conclusion

Raman spectroscopy combined with PLS-DA demonstrated potential as a rapid and non-destructive approach for the classification of insulin types and discrimination between pure standards and formulated samples. These results highlight the applicability of this methodology for the characterization and quality assessment of therapeutic peptides and support its further exploration as a screening tool in pharmaceutical analysis, following validation with independent external samples.

References

De Veij, M. et al., 2009. Reference database of Raman spectra of pharmaceutical excipients. J. Raman Spectrosc. 40, 297–307. https://doi.org/10.1002/jrs.2125

Fekete, S. et al., 2015. Chromatographic, electrophoretic, and mass spectrometric methods for the analytical characterization of protein biopharmaceuticals. Anal. Chem. 88, 480-507. https://doi.org/10.1021/acs.analchem.5b04561

Godzo, H. et al., 2024. PLS based quantitative determination of insulin aspart in solution using Raman spectroscopy. Arh. Farm. 74, 206-215. https://doi.org/10.5937/arhfarm74-49901

Lasalvia, M. et al., 2022. A comparison of PCA-LDA and PLS-DA techniques for classification of vibrational spectra. Appl. Sci. 12, 5345. https://doi.org/10.3390/app12115345

Shashilov, V.A. et al., 2010. Quantitative methods for structural characterization of proteins based on deep UV resonance Raman spectroscopy. Methods 52, 23-37. https://doi.org/10.1016/j.ymeth.2010.05.004