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Short communication

Application of ATR-FTIR as a screening method for analysis of biopharmaceutical preparations containing trastuzumab

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Introduction

In recent years, the number of therapeutic proteins introduced to the biopharmaceutical market has increased significantly, as medicine research and development have focused on protein-based therapies. Along with the introduction of biosimilar and biobetter medicines, the competitiveness of the biopharmaceutical market has grown considerably (Torres-Obreque et al., 2022). As a result, there is a global emergence of falsified biopharmaceuticals, especially monoclonal antibodies (mAbs), including trastuzumab, a humanized IgG1 mAb used as HER2-targeted therapy for the treatment of breast cancer (Maadi et al., 2021). In most of the seized falsified samples of medicines declared to contain mAbs (trastuzumab, rituximab, bevacizumab), no active pharmaceutical ingredient (API) was detected (Janvier et al, 2018). Therefore, suitable testing strategy and workflow should be defined in cases where falsification is suspected. Taking into account that mAbs are predominantly glycoproteins of high molecular weight (~ 150 kDa) with complex higher-order structure, their characterization can be rather challenging. Most of the analytical methods used for protein identification and characterization of mAbs (chromatography, electrophoresis, mass spectrometry and nuclear magnetic resonance) are time-consuming, expensive and require extensive sample preparation (Alhazmi et al., 2023). Therefore, implementation of fast, simple, and direct screening tools is of vast importance for the analytical quality control of these medicines.

The use of vibrational spectroscopic techniques, particularly infrared (Hamla et al., 2022) for characterization of mAbs is drawing more attention since these are rapid and non-destructive methods. In this study, we demonstrate the potential use of Fourier transform infrared (FTIR) spectrophotometry, with attenuated total reflectance (ATR) for identification of trastuzumab in biopharmaceutical preparations.

Materials and methods

IR spectra were recorded on Cary 630 FTIR spectrometer fitted with a diamond attenuated total reflection (ATR) module, in the 4000-650 cm^{-1} region with the spectral resolution adjusted to 4 cm^{-1}. MicroLabPC software was used for data acquisition. GRAMS Spectroscopy Software was used for background subtraction and for obtaining the second order derivative spectra (Savitzky Golay). Trastuzumab powder for concentrate for solution for infusion, was used as a testing sample. Measurements were made directly on the non-reconstituted finished product and after reconstitution in water (HPLC grade), according to the instructions for product reconstitution. Additionally, four dilutions of the non-reconstituted finished product in the main excipient of the formulation, trehalose (Merck, USA), were prepared (33.3%, 50%, 66.7%, 83.3%) and analysed.

Results and discussion

A typical infrared (IR) spectrum of a protein consists of several amide bands, deriving from both the protein backbone and the amino acid side chain vibrations. The most significant bands are the Amide I band (which results from the C=O stretching vibration, located around 1650 cm^{-1}) and the Amide II band (linked to N-H bending vibrations, located around 1550 cm^{-1}) (Barth, 2007).

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Averaged spectra from sixteen scans were analyzed and peaks at around 1636 cm^{-1} and 1558 cm^{-1}, corresponding to Amide I and Amide II, were obtained, confirming the protein nature in all samples. The signal corresponding to the Amide I band, showed the strongest absorption (Fig.1). The spectra were comparable in all tested samples: non-reconstituted product, reconstituted product and product diluted in trehalose. Spectra of the reconstituted product showed interfering bands of the water molecules, due to frequency overlap of the amide I band with the H–O–H bending vibrational mode of H_2O (Rutherford et al., 2023). This interference was removed in the second order derivative spectra after subtraction of the background using GRAMS Spectroscopy Software (Fig.2).

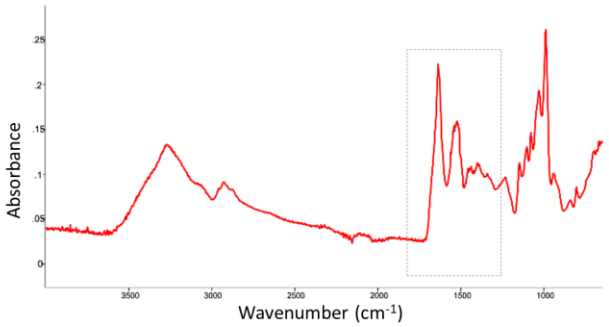

Fig. 1. ATR-FTIR spectrum of trastuzumab powder for concentrate for solution for infusion

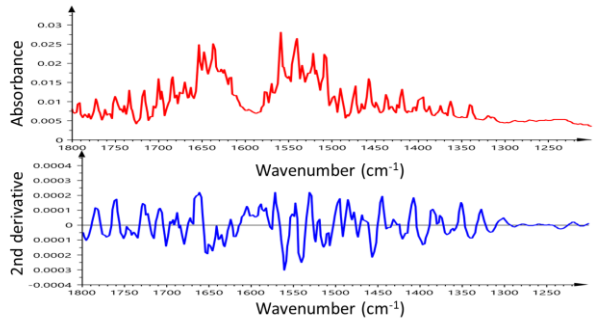

Fig. 2. ATR-FTIR spectrum of trastuzumab reconstituted solution: a) blank subtracted spectrum, b) second derivative spectrum.

Taking into account that Amide I and Amide II bands largely depend on the secondary structure of the protein (Barth, 2007), the similarity of the spectra, both first and second order, confirmed presence of trastuzumab in all tested samples.

Combination of ATR-FTIR with multivariate data analysis can be used as a powerful tool for specific identification, providing an insight into stability of the active ingredient with protein nature, by monitoring of aggregation, degradation, and post-translational modifications (i.e., glycosylation). Compared to electrophoretic and chromatographic methods, which are destructive and the sample cannot be reused after analysis, ATR-FTIR could help avoid unnecessary time-consuming analysis. This is of great importance since analysis of suspected falsified products should be simple and rapid.

Conclusion

The obtained results indicate that ATR-FTIR spectroscopy in combination with multivariate data analysis (partial least square regression) can be used as a screening technique for qualitative and quantitative characterization of trastuzumab, as well as other mAbs, in biopharmaceutical preparations. The non-destructive manner of ATR-FTIR is of particular significance, considering the high price of these products.

The recognition of critical quality attributes of biopharmaceuticals, by ATR-FTIR spectroscopy can help in confirming authenticity of mAbs and enable identification of suspected falsified products.

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