Analytical Approaches for Assessing Immunoconjugate Integrity and Characterization prior to radiolabeling

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Introduction

Immunoconjugates, which have highly specific targeting towards certain antigen, are molecules that are built from three parts: an antibody, a therapeutic payload and a linker between them.
Based on the mechanism of the therapeutic agent, they are divided into defined groups: antibody-drug conjugates (therapeutic payload), radioimmunoconjugates (radioisotope) or immunotoxins (catalytic, protein toxins).

The major interest of this research is focused on immunoconjugates ready to be radiolabeled, and the analytical techniques used for assessing their integrity and characterization.
Testing of the immunoconjugates generally can be as quality control testing in a qualified good manufacturing practices laboratories or characterization using research and development methods.

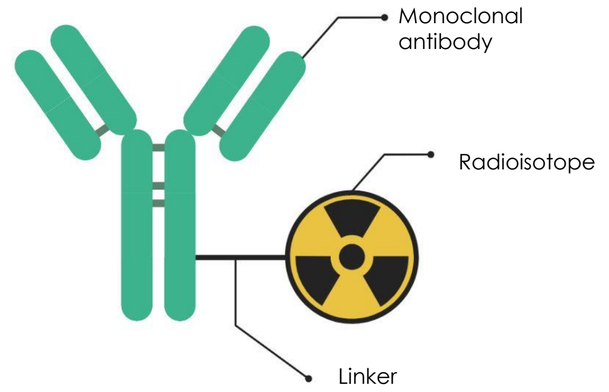


Figure 1. Simple graph of radioimmunoconjugates

Characterization parameters

Physicochemical properties — Structural characterization

- Molecular weight or size
- Extinction coefficient
- Electrophoretic patterns (identity, homogeneity and purity)
- Liquid chromatography patterns (identity, homogeneity and purity)

Biological activity — To complete characterization profile

- Animal-based biological assays
- Cell culture-based biological assays
- Biochemical assays
- Binding assays

Immunochemical properties — For Ab and Ab-based products

- Binding assays

Purity, Impurities and contaminants

- Appropriate test accordingly to the product

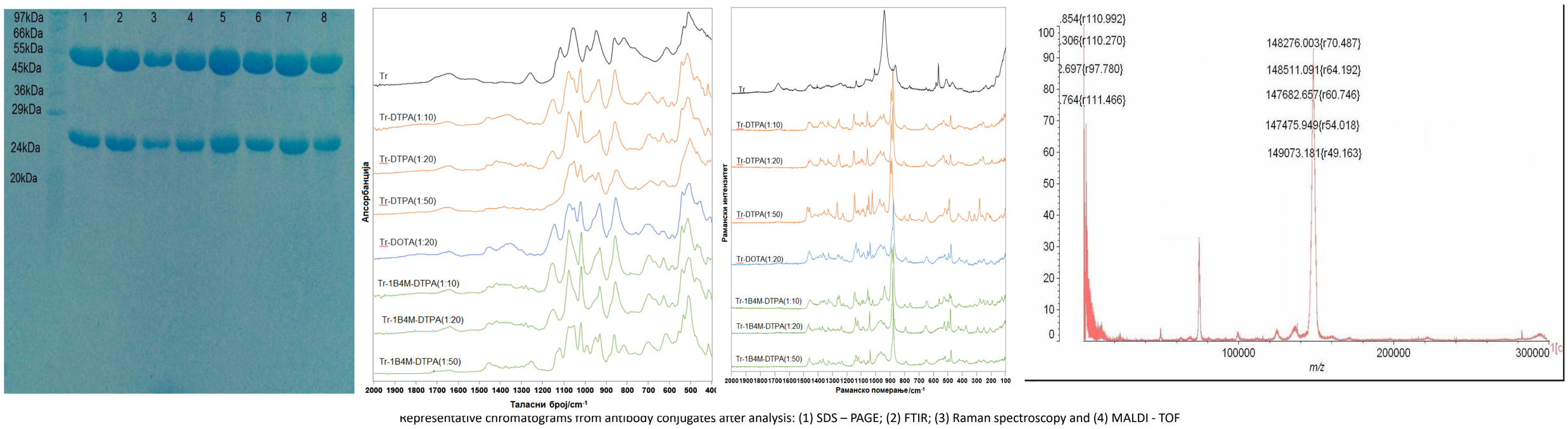
Potency and Quantity

*According to ICH Q6B

Size	Aggregation /Fragmentation	- Size Exclusion Chromatography - Electrophoresis - RP - HPLC
	Total mass	- Mass spectroscopy
Structural integrity of the immunoconjugate		- FTIR - RAMAN
Number of chelates per antibody		- MALDI – TOF
Stability of the immunoconjugate		- SEC

*For research and development purposes

Methods and Results



Representative chromatograms from antibody conjugates after analysis: (1) SDS – PAGE; (2) FTIR; (3) Raman spectroscopy and (4) MALDI - TOF

The used techniques, electrophoresis (reducing conditions), Infrared spectroscopy and Raman spectroscopy, give information about the integrity, primary structure and the number of chelators attached to the mAb with MALDI – TOF analysis. This can be as primary analysis for integrity and characterization of immunoconjugates prior radiolabeling.

Conclusion

The success of a radiolabeling process, as well as the distribution of immunoconjugate after labeling to tumor cells, depends primarily on antibody characteristics, the appropriate chelator, and the method of their conjugation.
The choice of methods used for quality control of the formed immunoconjugates is crucial to correctly assess and perform categorization prior to radiolabeling process.