

Analytical Strategy for LC-MS Quantification of Enfortumab and Free MMAE in Plasma from Patients with Advanced Urothelial Carcinoma Treated with Enfortumab Vedotin

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01. Clinical Context & Challenges

Antibody-drug conjugates (ADCs) combine the specificity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs, delivered directly to tumor cells.



Efficacy

ADCs aim to improve therapeutic efficacy while limiting systemic toxicity.



Toxicity

Off-target release of the cytotoxic payload remains a key concern and may contribute to adverse effects.

Main objective:

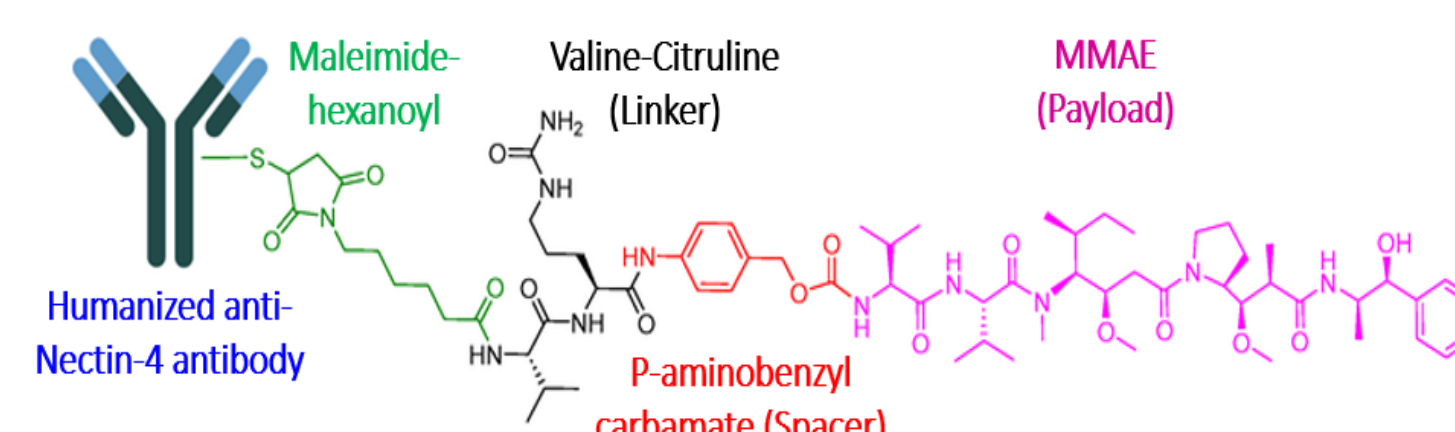
To develop a quantitative LC-MS/MS assay for measuring both the total antibody and the released payload, with the aim of providing a tool to improve patient safety and predict treatment-related toxicities.

ADC selected to achieve the main objective:

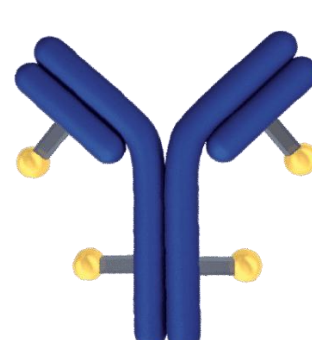
Enfortumab vedotin (EV) has been chosen. EV is an ADC composed of a monoclonal antibody targeting Nectin-4 conjugated to monomethyl auristatin E (MMAE), a potent microtubule-disrupting agent.

02. Enfortumab Vedotin (EV)

EV is approved for the treatment of patients with advanced or metastatic urothelial carcinoma, particularly after progression following platinum-based chemotherapy and immune checkpoint inhibitors. Despite its clinical benefits, treatment-related toxicity, potentially associated with circulating free MMAE, remains a limitation.



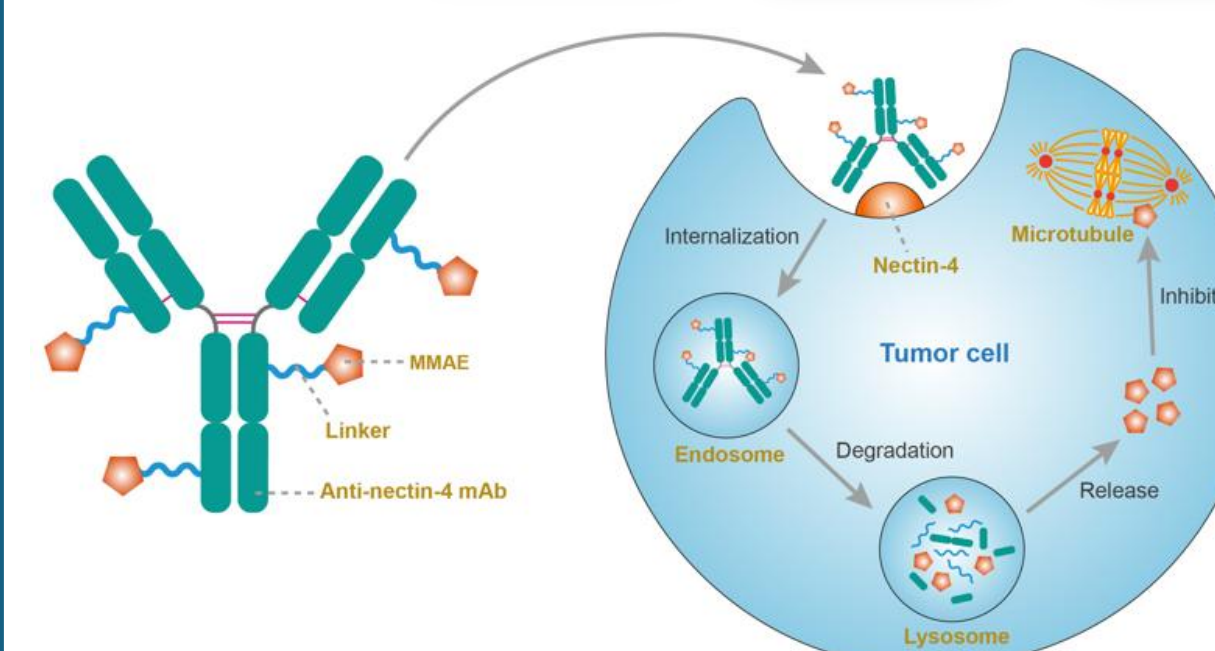
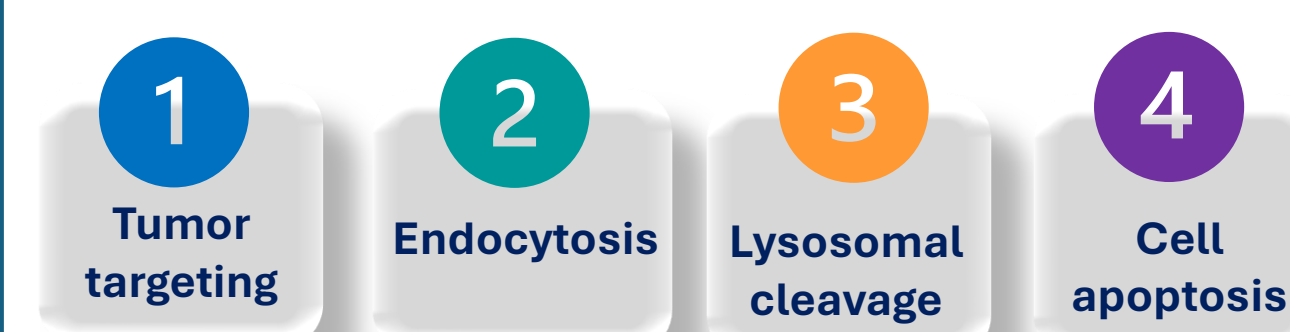
Enfortumab Vedotin (payload: MMAE (Monomethyl Auristatin E))



EV is composed of a humanized monoclonal antibody directed against the protein Nectin-4, linked to the cytotoxic monomethyl Auristatin E. Each mAb molecule binds in average 4 molecules of cytotoxic. The mean Drug-Antibody-Ratio (DAR) is thus 4.

Mechanism of action of EV:

Enfortumab vedotin (EV) binds to Nectin-4 expressed on the surface of tumor cells and is subsequently internalized via endocytosis. Lysosomal degradation of the ADC results in the release of its cytotoxic payload. The released payload disrupts microtubule dynamics and induces mitotic arrest, ultimately leading to tumor-cell apoptosis.



Source: see Ref1

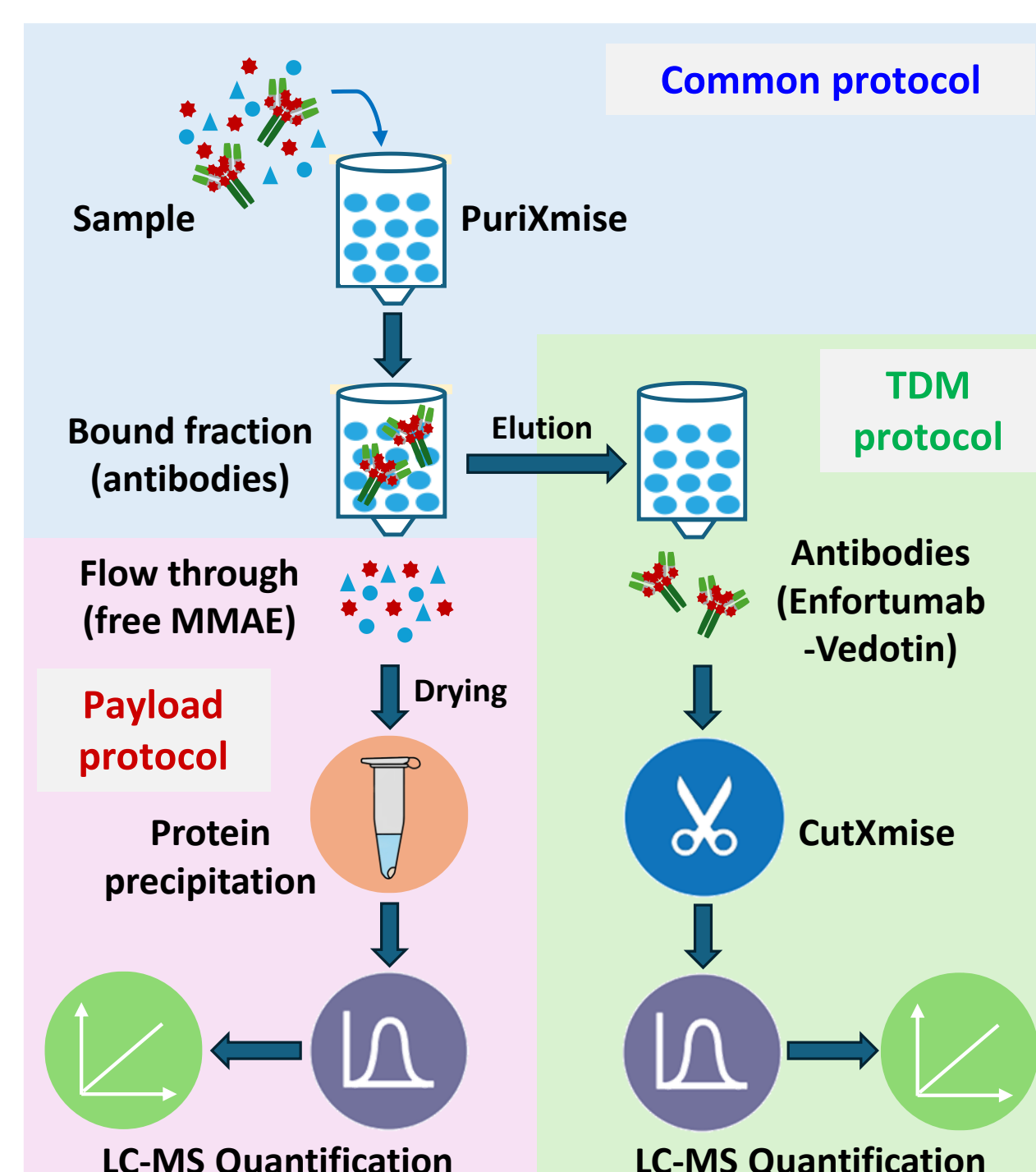
03. Therapeutic Drug Monitoring (TDM)

Therapeutic drug monitoring (TDM) is the clinical practice of measuring specific drug concentrations in a patient's blood at set times to keep the amount within a safe and effective target range.

For patients treated with EV, TDM is therefore of high interest to optimize patient management by enabling individualized dosing, improving efficacy, and minimizing toxicity. In this context, simultaneous monitoring of both the antibody component (Enfortumab) and free MMAE in circulation is particularly relevant.

04. Analytical strategy

A LC-MS/MS-based analytical strategy was developed for the quantification of Enfortumab and free MMAE in plasma or serum from patients treated with Enfortumab-Vedotin. The workflow relies on a single initial sample preparation (from the same aliquot), followed by two parallel processing procedures and two distinct LC-MS/MS methods dedicated to each analyte.



05. Performances, Validation & Clinical data

Initial sample volume required: 5 μ L

Target	Internal Standard (IS)	Measuring range	LLOQ	Analytical performance	Type of sample
Enfortumab	Full length stable isotope labelled enfortumab	1-50 μ g/mL	1 μ g/mL	Linearity Accuracy Precision Interference Matrix Effect Stability study	Plasma collected on Citrate, EDTA, Heparin tubes, Serum
MMAE	MMAE-d8	0.25-25 nM (0.18-17.9 ng/mL)	0.25 nM (0.18 ng/mL)		

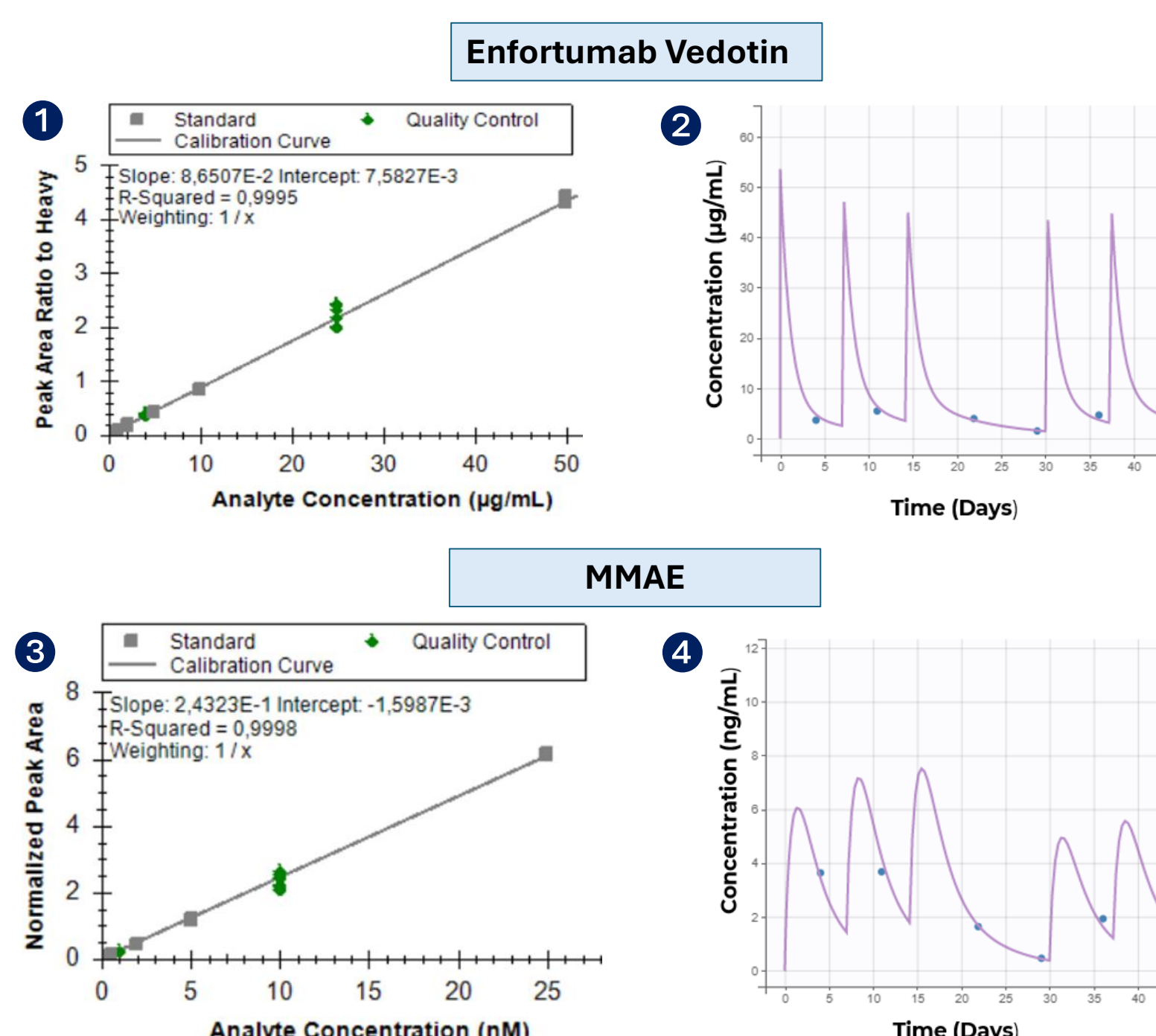


Fig. 1: Calibration curves performed in triplicates for total ADC quantification (Enfortumab vedotin), using SIL-Enfortumab as IS.

Fig. 2: Total ADC (EV) concentrations measured at different time points for one patient (blue points) and correlation with the published PK model (purple curve).

Fig. 3: Calibration curves performed in triplicates for circulating MMAE, using deuterated MMAE as IS.

Fig. 4: MMAE concentrations measured at different time points for one patient (blue points) and correlation with the published PK model (purple curve).

Enfortumab-Vedotin and MMAE were successfully quantified in a total of 94 samples, corresponding to different collection time points from 12 patients.

Enfortumab vedotin
patient concentrations

[0.36 – 29.45] μ g/mL

MMAE
patient concentrations

[0.18 – 12.72] nM
[0.13 – 9.13] ng/mL

Calibration curves performed in triplicates for the quantification of Enfortumab-Vedotin (1) and circulating MMAE (3)

For 1 patient with 6 time points measured, overlay of measured concentrations for total EV (2) and MMAE (4) with the concentration-time profile estimated from the published PK model (Ref2).

Conclusion

This LC-MS/MS analytical strategy provides a robust and practical tool for the simultaneous assessment of Enfortumab and free MMAE concentrations from a single patient sample. Its application in TDM could enhance patient monitoring, support personalized treatment adjustments, and contribute to reducing toxicity while maintaining therapeutic efficacy. A commercial kit-based approach has been developed by the company Promise Proteomics.