

Comparative Approaches for the Analysis of a Site-Specific ADC Produced by AJICAP™ Technology

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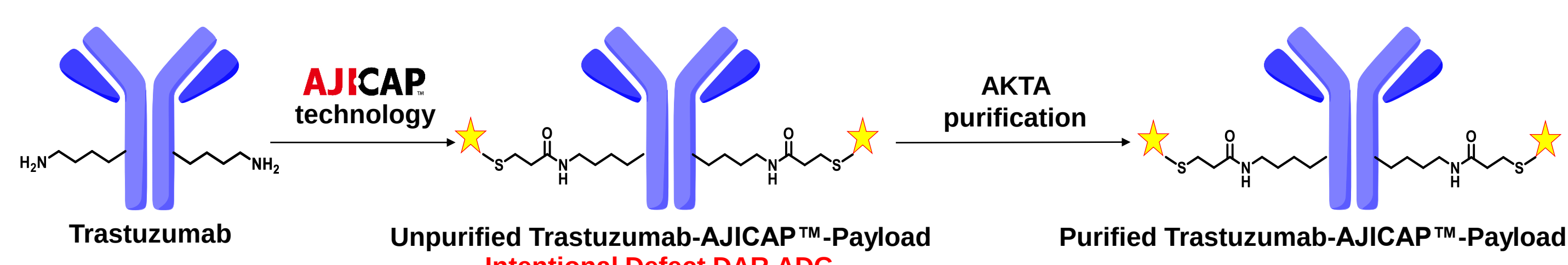
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Introduction

AJICAP™ technology is a novel platform for the site-specific conjugation of antibodies through the use of a class of Fc-affinity compounds to install payload-compatible linkers to well-defined amino acid residue(s). In this poster, we present six comparative analytical methods for drug-antibody ratio (DAR) determination to apply to site-specific ADCs, using an AJICAP™ ADC. We are also able to confirm the site-specificity of the AJICAP™-ADCs by MS/MS peptide mapping.

Analytical Comparison in DAR Characterization



DAR characterization by 6 different methods

- RP-HPLC
- RPLC-QTOFMS
- “Denaturing” SEC-QTOF-MS
- HIC-HPLC
- Ellman’s assay
- “Native” SEC-QTOF-MS

Analytical Summary of “Unpurified” AJICAP™-ADC

	PR-HPLC	HIC	RPLC-QTOFMS	Denaturing QTOFMS	Native QTOFMS	Ellman's Assay
Column	Reverse Phase Column	HIC-Column	Reverse Phase Column	SEC-Column	SEC-Column	-
Mobile Phase	MPA: Organic Solvent with TFA MPB: Aqueous Solvent with TFA	MPA: Ammonium derivatives MPB: Aqueous Solvent with IPA	MPA: Organic Solvent with TFA MPB: Aqueous Solvent with TFA	MPA: Organic Solvent with TFA MPB: Aqueous Solvent with TFA	NH ₄ ⁺ buffer	-
DAR	DAR = 1.6	DAR = 1.6	DAR = 1.8	DAR = 1.8	DAR = 1.6	DAR = 1.6*

* Calculation from SHimAb ratio

Slightly different results were obtained

Analytical Summary of “Purified” AJICAP™-ADC

	PR-HPLC	HIC	RPLC-QTOFMS	Denaturing QTOFMS	Native QTOFMS	Ellman's Assay
Column	Reverse Phase Column	HIC-Column	Reverse Phase Column	SEC-Column	SEC-Column	-
Mobile Phase	MPA: Organic Solvent with TFA MPB: Aqueous Solvent with TFA	MPA: Ammonium derivatives MPB: Aqueous Solvent with IPA	MPA: Organic Solvent with TFA MPB: Aqueous Solvent with TFA	MPA: Organic Solvent with TFA MPB: Aqueous Solvent with TFA	NH ₄ ⁺ buffer	-
DAR	DAR = 2.0	DAR = 2.0	DAR = 2.0	DAR = 2.0	DAR = 2.0	DAR = 2.0*

* Calculation from SHimAb ratio

Importance of Analytical Strategy

	Reaction Screening	Reaction Familiarization	Process Development
Reaction Scale	µg	mg	g
Data Accuracy	Semi-quantitative	Monitoring: Semi-quantitative Final Product: Quantitative	Monitoring: Semi-quantitative Final Product: Quantitative
What is the goal?	“Green light” or “Red light”	Fine tuning/optimization	Establishment of scalable process

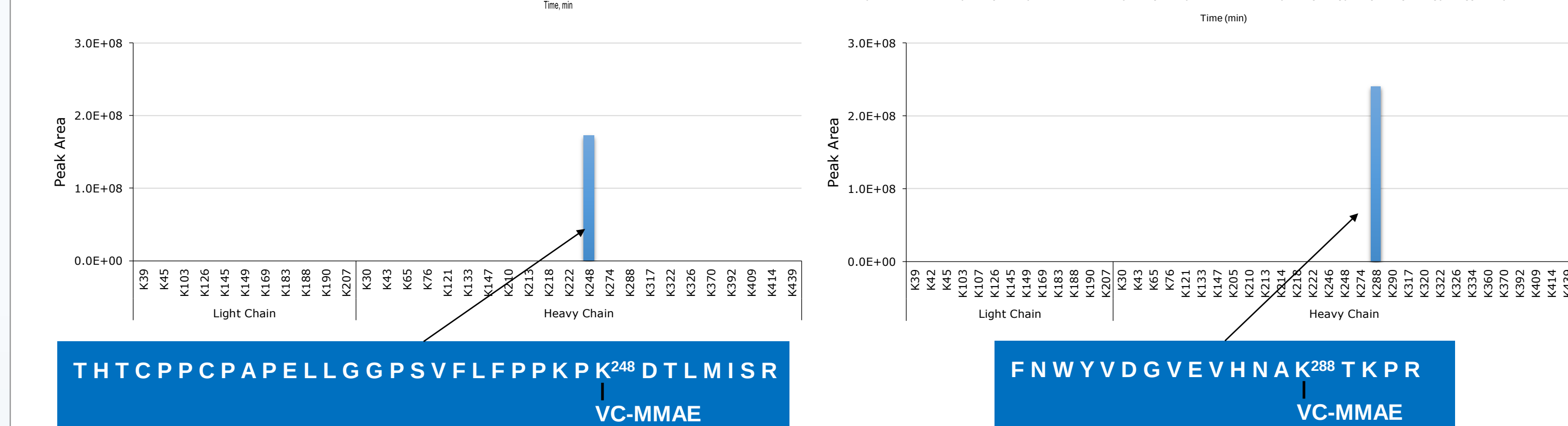
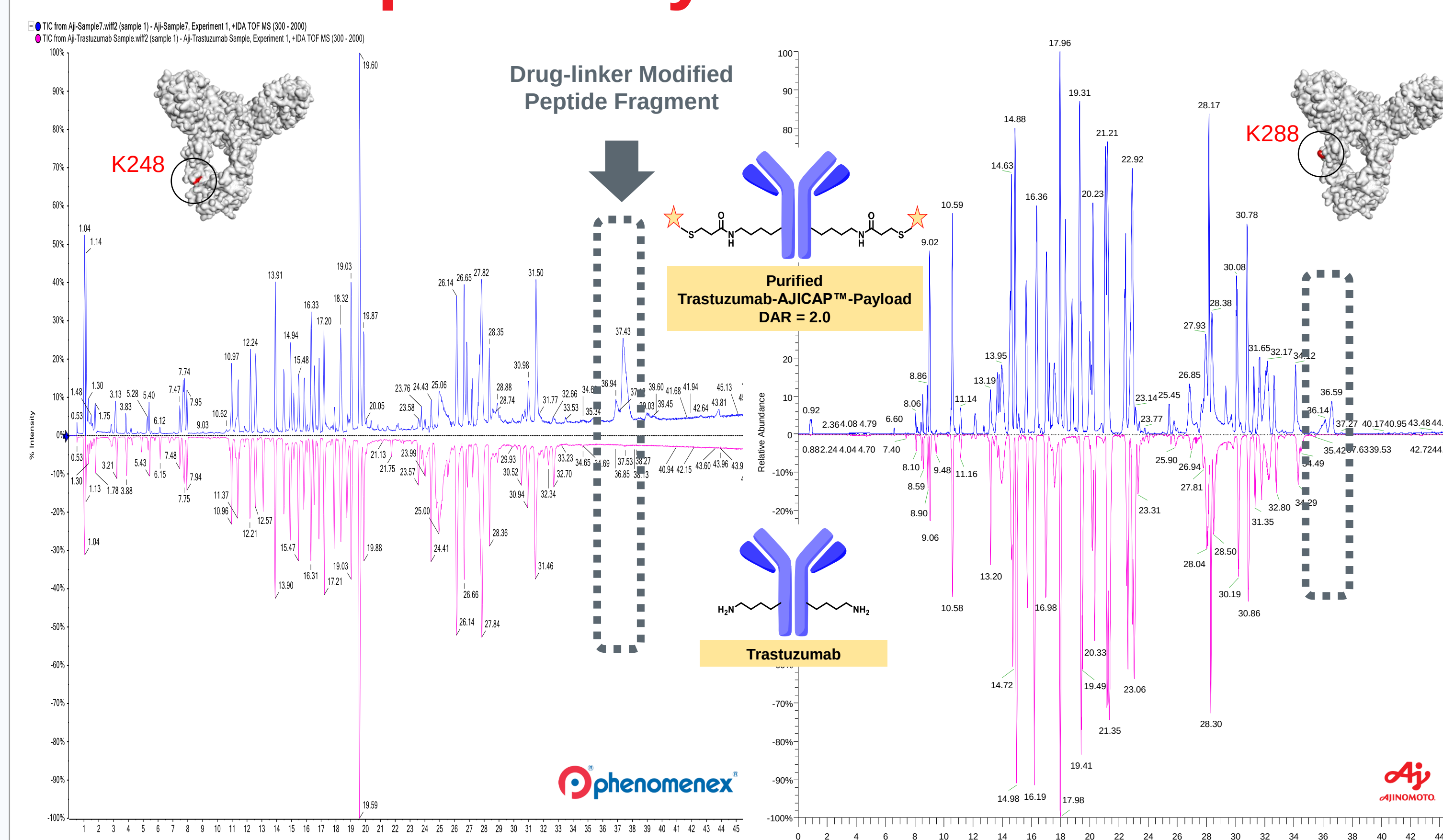
Project Evolution

Characteristics of Three Different Q-TOF-MS Analyses

	RPLC-Q-TOF-MS	Denaturing SEC-Q-TOF-MS	Native SEC-Q-TOF-MS
Column	Reverse Phase Column	SEC-Column	SEC-Column
Mobile Phases	MeCN/Water	MeCN/Water	NH ₄ ⁺ buffer
Required ADC amount	5 µg	20 µg	20 µg
Accuracy	Semi-quantitative	Semi-quantitative	Quantitative
Pretreatment	Necessary	Not Necessary	Necessary
Recommended Purpose	Reaction Screening	Reaction Monitoring	Accurate DAR Determination

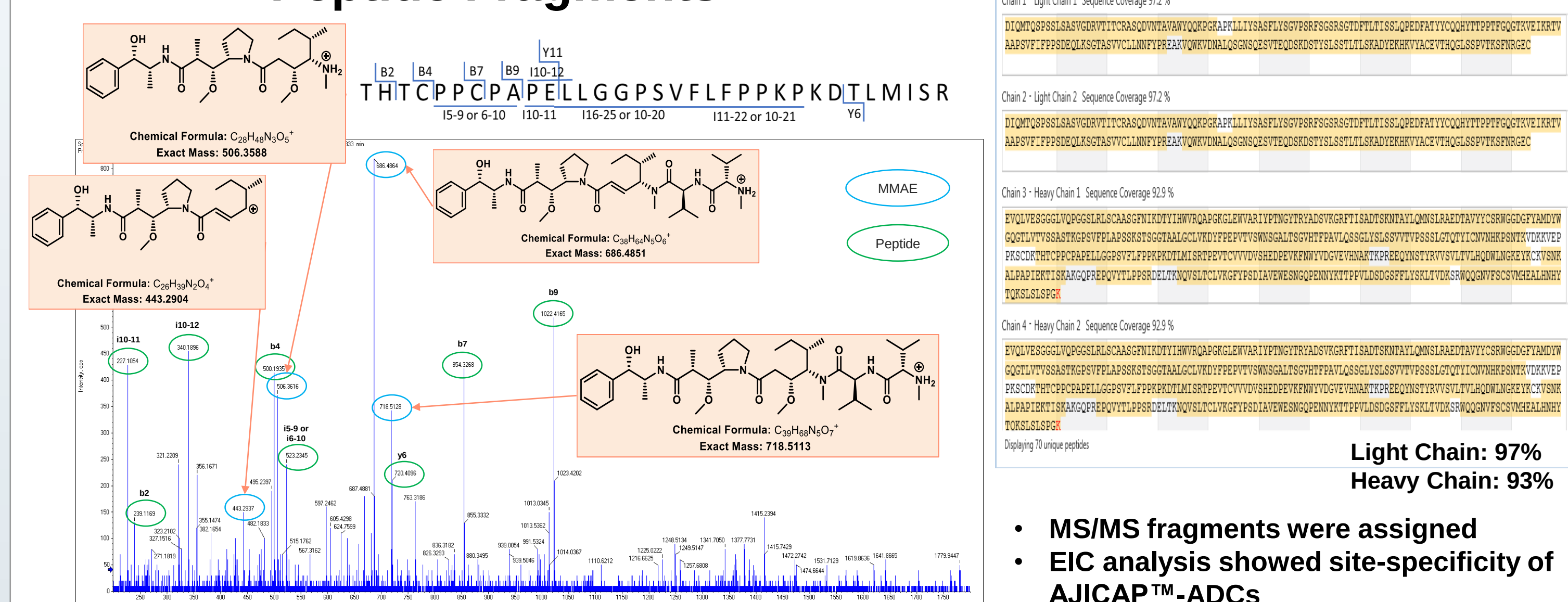
We recommend that all of these methods be used, at different stages, to increase the efficiency given specific analytical objectives

Site-Specificity of AJICAP™-ADC



MS/MS Results of MMAE Modified Peptide Fragments

Sequence Coverage



Site-specificity of AJICAP™ technology was confirmed

Conclusion

- Six analytical methods were utilized for DAR determination of a single batch of an unoptimized site-specific ADC (intentional defect DAR ADC) to allow for a direct comparison and evaluation of their use at different stages
- For a final ADC sample which underwent chromatographic purification, all analytical techniques gave the same results
- Site-specificity of AJICAP™ technology was confirmed by peptide mapping

References

- 1) Matsuda, Y.; Robles, V.; Malinao, M-C.; James, S.; Mendelsohn, B. A. *Anal. Chem.*, **2019**, *91*, 12724–12732.
- 2) Matsuda, Y.; Malinao, M-C.; Robles, V.; James, S.; Yamada, K.; Mendelsohn, B. A. *J. Chromatogr. B*, **2020**, 121981.

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