Characterization of Interchain Cysteine Linked Antibody Drug Conjugates in Mouse Plasma by LC/MS

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INTRODUCTION

Antibody drug conjugates (ADCs) has become promising therapy for the treatment of cancers. Among all the ADCs under developing, 2/3 of them are interchain cysteine linked ADCs. The ADCs are manufactured by partially reduce the 4 pairs of interchain disulfide bond followed by conjugate cytotoxic payloads to the thiols, as a consequence, the antibodies are linked with 0, 2, 4, 6, 8 drugs. The drug to antibody ratio (DAR) and the drug linking position are important parameters that affect the therapeutic effects and need to be well characterized.

Sample preparation:

The ADCs in the mouse plasma were purified by affinity capture with anti-human IgG beads followed wash with PBS supplied with 0.1% tween-20. The ADC was eluted with 0.1% TFA and neutralized with 1M tris (PH=8).

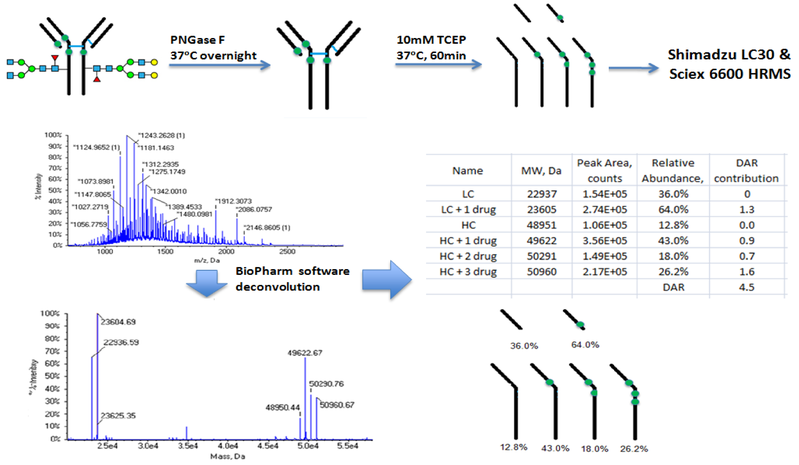
Challenges:

1. Heavy chain positional isomers separation and Identification.
2. Heavy chain positional isomers abundance determination.

Methods:

1. Reduced ADC HRMS analysis. The Purified ADC was deglycosylated and reduced and then analyzed with HRMS.
2. Bottom up analysis. Purified ADC was treated with IdeS to remove Fc part followed by denaturation, reduction, alkylation, chymotrpsin digestion and then subject to LC/MS analysis.
3. LC-MS/MS analysis. Isomer fraction were collected and digested with trypsin, the digest was analyzed by LC/MS/MS for drug linking position identification.

RESULTS



Name	MW, Da	Peak Area, counts	Relative Abundance,	DAR contribution
LC	22937	1.54E+05	36.0%	0
LC + 1 drug	23605	2.74E+05	64.0%	1.3
HC	48951	1.06E+05	12.8%	0.0
HC + 1 drug	49622	3.56E+05	43.0%	0.9
HC + 2 drug	50291	1.49E+05	18.0%	0.7
HC + 3 drug	50960	2.17E+05	26.2%	1.6
			DAR	4.5

Figure 1. Reduced ADC analysis

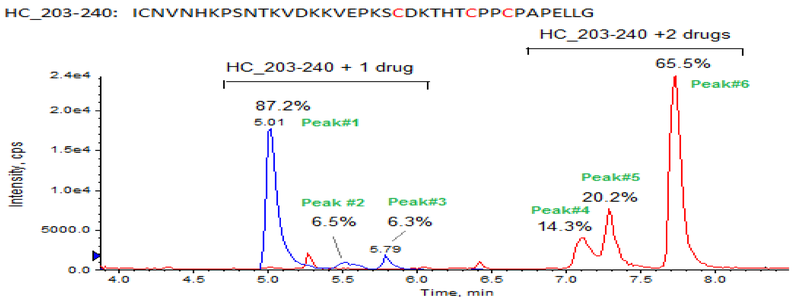


Figure 2. Heavy chain positional isomers separation

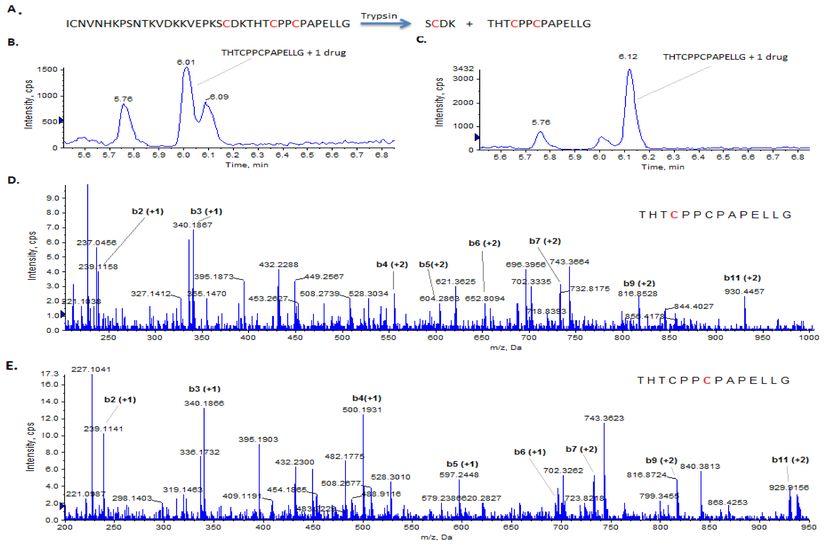


Figure 3. Drug linking position Identification. A) Trypsin digestion of isomer peptide, B) Chromatogram of Peak #2 &4 digest, C)Chromatogram of Peak#3 & 5 digest, D) MS/MS fragmentation of drug linking peptide at RT=6.01min, E) MS/MS fragmentation of peptide at RT=6.12min.

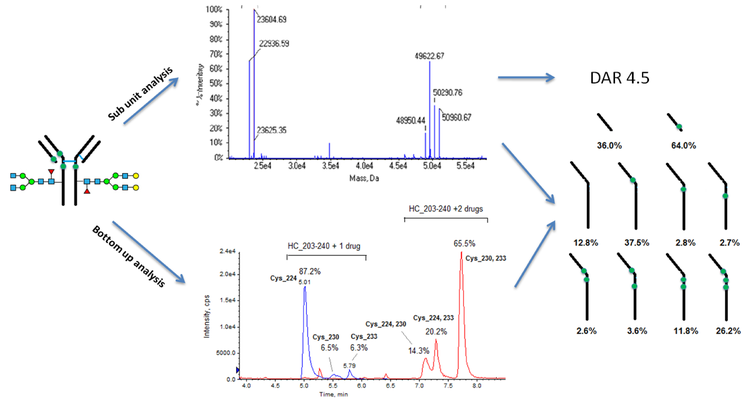


Figure 4. Data analysis combine reduced ADC analysis and bottom up analysis.

CONCLUSIONS

A method for the characterization of interchain cysteine linked ADC in biological matrix has been successfully developed. The DAR and drug distribution on each chain were calculated in reduced ADC HRMS analysis. The HC isomers, including the hinge region isomers, are separated and their relative abundance were determined. The following LC/MS/MS analysis of each isomer fraction provide solid information for the identification of drug linking positions on each isomer. The reduced ADC analysis, bottom up analysis and following LC/MS/MS analysis provide comprehensive information regarding the DAR and the drug linking position information.

REFERENCES

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