Measuring Site Occupancy and Site-specific Drug Distribution of Cysteine-Conjugated ADCs by a LC-MS/MS Approach

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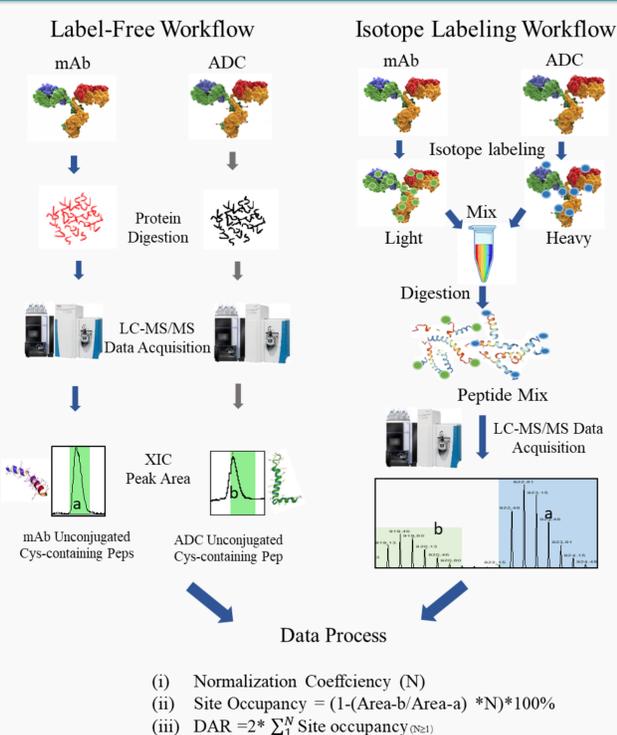
Introduction

- The cysteine-conjugated antibody-drug conjugates (ADCs) consist of subpopulations that differ in the number of drugs attached, as well as the location of the drug linkage.

- The drug distribution plays an important role in the pharmacokinetics of the ADC, and eventually impacting its safety and efficacy.

- Common analytical techniques such as hydrophobic interaction chromatography (HIC), reversed phase liquid chromatography (RPLC), and native mass spectrometry (MS) cannot generate site-specific information when used to measure drug distribution and drug-to-antibody ratios (DAR).

- Traditional peptide mapping typically does not take into account the MS response difference caused by payload-linkers, therefore it would not produce accurate conjugation site occupancy information.

- In this poster, we present our study to simultaneously obtain site occupancy and site-specific drug distribution information for cysteine-conjugated ADCs using a label-free or an isotope labeling method.

Analytical Workflow of the Methods

Label-Free Workflow

mAb → ADC

Protein Digestion

LC-MS/MS Data Acquisition

XIC Peak Area

mAb Unconjugated Cys-containing Peps / ADC Unconjugated Cys-containing Pep

Isotope Labeling Workflow

mAb → ADC

Isotope labeling

Light / Mix / Heavy

Digestion

Peptide Mix

LC-MS/MS Data Acquisition

Data Process

(i) Normalization Coefficiency (N)
(ii) Site Occupancy = (1-(Area-b/Area-a) *N)*100%
(iii) DAR = $2 * \sum_1^N$ Site occupancy (N≥1)

Methods & Results

Method 1: Label-free Method – Analysis Steps

1) Peptide digests from the equal amount (moles) of unconjugated (naked) antibody and an ADC derived from the same mAb were analyzed by LC-MS/MS in separate analytical runs;

2) The response of the peptides that do not contain cysteine residues in the two data sets were first compared and normalized to tender a normalization coefficient to compensate for any potential signal variation that may exist during the analysis;

3) The MS response values for the cysteine-containing peptides were corrected based on the normalization coefficient;

4) The MS response ratio of the cysteine-containing peptides from the two data sets was used to calculate the site occupancy of each cysteine residue in the ADC.
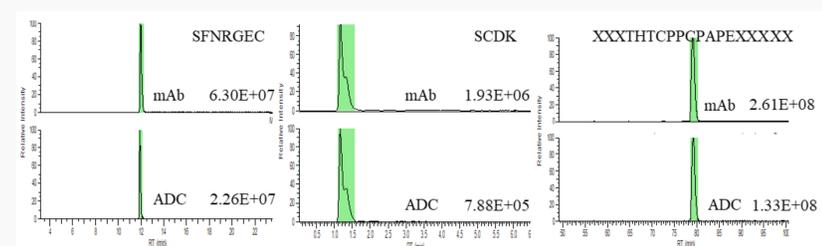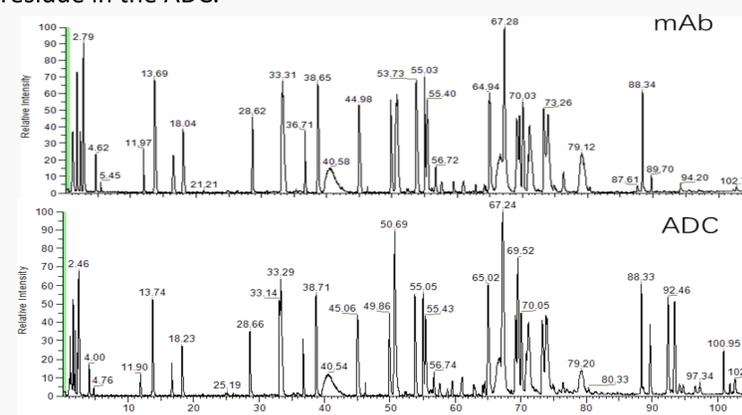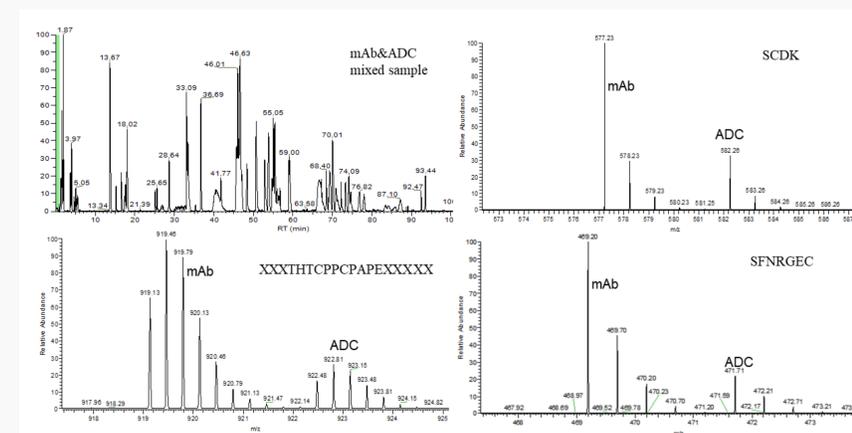
Peptide	RT（min）	MonoMass（Da）	Mass Error (ppm)	Response (B) ADC	Response (A) mAb	Site Occupancy	DAR
SFNRGEC	11.98	868.349	-1.1	2.26E+07	6.30E+07	0.61	
SCDK	1.17	508.195	-0.3	7.88E+05	1.93E+06	0.55	
XXXTHTCPPCPAPEXXXXX	79.09	2618.310	2.7	1.33E+08	2.61E+08	0.46	3.2
XXXTHTCPPCPAPEXXXXXXK	73.45	2843.459	3.0	7.40E+07	1.53E+08		

Method & Results

Method 2: Isotope Labelling Method – Analysis Steps

a) The unconjugated (naked) antibody and an ADC derived from the same mAb was alkylated, after a full reduction, by either light or heavy isotope labeled alkylating reagent separately;

b) An equal amount (moles) of alkylated antibody and ADC were mixed together;

c) The mixture was digested by Lys-C, and the peptide digest was analyzed together in a single run by LC-MS/MS;

d) The extracted ion chromatograms were generated for the cysteine-containing peptides that contain either light or heavy isotope labeled alkylating reagent. The peak area ratio of the cysteine-containing peptides from the same data sets was used to calculate the site occupancy of each cysteine residue in the ADC.

Peptide	RT（min）	MonoMass（Da）	Mass Error (ppm)	Response (B) ADC	Response (A) mAb	Site Occupancy	DAR
SFNRGEC	25.66	936.376	1.6	3.66E+08	1.14E+09	0.67	
	25.18	936.376	1.3				
SCDK	3.75	576.2214	-1.5	3.13E+08	1.15E+09	0.72	4.1
XXXTHTCPPCPAPEXXXXX	87.14	2754.355	1.5	1.39E+09	3.92E+09	0.64	

Conclusions

Two LC-MS/MS methods measuring site occupancy and drug distribution of cysteine-conjugated ADCs were developed. The methods were successfully applied to a model ADC molecule, anti-CD19-vc-MMAE, and delivered good results on occupancy values. The label-free method is cost-saving, while the isotope labeling method delivers more accurate measurement.