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Rapid Identification Of Conjugation Sites In Antibody Drug Conjugates Using Microchip Capillary Electrophoresis Coupled with Mass Spectrometry

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Introduction

Antibody drug conjugates (ADC) are small molecule conjugated mAbs and a major class of oncology biotherapeutics. ADCs rely on combining the target specificity of mAbs with the potency of small molecule drugs. The localization of drug conjugation sites must be characterized. Peptide mapping is commonly used for this purpose. Conventional HPLC-MS uses long gradients to ensure sufficient separation for identification and quantification. ZipChip is a microfluidic device integrating capillary electrophoresis (CE) with mass spectrometry. Microchip CE-MS combined with the high duty cycle of PASEF shows the potential to enable rapid separation and identification of peptide mixtures from protein digests. In this study, peptide mapping results acquired with LC-MS and CE-MS for NISTmAb are evaluated and a 10min CE-MS peptide mapping method for a commercial ADC is developed.

Methods

Reverse phase LC separation was performed on an Elute HPLC (Bruker) using a CSH C18 1.7 um 2.1 x 150 mm column (Waters) with 0.2 ml/min flow rate and column oven 40 °C. 2 ug of digested ADC was injected onto the column. CE separation was performed on a ZipChip system (908 Devices). Digested peptides of the ADC trastuzumab-DM1 and NISTmAb were separated using a High Resolution (HR) chip and peptide background electrolyte with a field strength of 500 V/cm and a 10 min runtime.

8 nL of 0.5 mg/mL digested protein solutions were injected. A timsTOF Pro 2/HT mass spectrometer (Bruker) using PASEF was used for the MS detection and data analysis was performed using Compass DataAnalysis 6.0 and Byos 4.4 (Protein Metrics).

Results

NISTmAb digest is used as a reference control to develop the peptide mapping methods for LC-MS and CE-MS. 80 min LC runtime is used for the LC-MS reference data and 10 min total runtime is used for CE-MS. The PASEF total duty cycle is optimized to 0.3s (50ms frames and 5 MS/MS frames per cycle) to adapt to the narrow peak widths observed with CE. This ensures sufficient data points in extracted ion chromatograms and fragmentation of all the most intense precursor ions. In the LC-MS experiment, oxidized peptide DTLMISR has a ~ 3% relative quantitation level (data not shown) compared to wild type peptide. Similar 3% relative oxidation level was found on the peptide DTLMISR in the CE-MS experiment (Fig.1). The peak width for peptide DTLMISR is around 3s and the whole CE separation is finished before 7min.

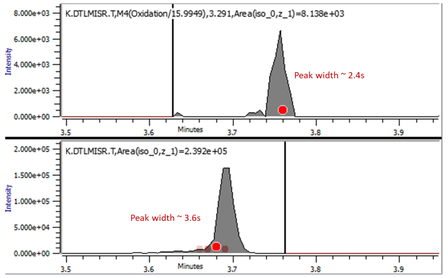

Fig. 1 Extracted ion chromatogram of oxidized peptide DTLMISR (top) and wild type peptide DTLMISR (bottom).

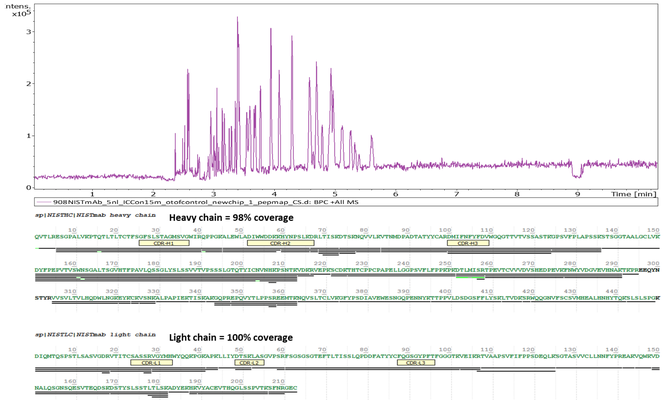

Fig. 2 Base peak chromatogram and sequence mapping results of the NISTmAb digest with the ZipChip coupled with Bruker timsTOF Pro 2 mass spectrometer. The sequence coverage for the light and heavy chains are 100% and 98%, respectively. All identified peptides eluted between 2 and 7min.

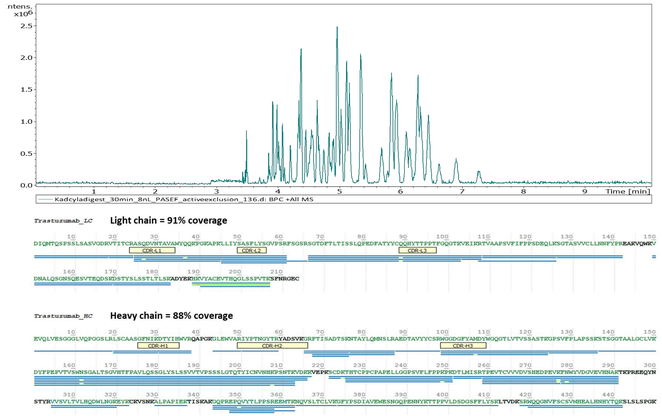

Fig 3. Base peak chromatogram and sequence mapping results of the ADC trastuzumab-DM1 digest with the ZipChip coupled to the timsTOF HT mass spectrometer. The sequence coverage for the light and heavy chains are 91% and 88%, respectively. All identified peptides eluted between 3 and 8min.

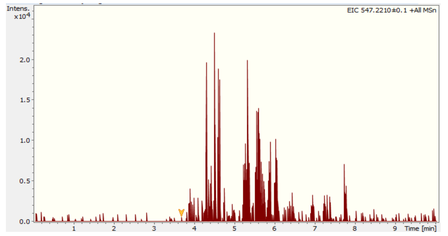

Fig. 4 Extracted ion chromatogram of signature MS/MS fragment m/z 547.221. MS/MS spectra of the conjugated peptides show a series of signature fragments with m/z of 547.221, 485.224 and 453.199. 【1】

The sequence coverage for the NISTmAb light and heavy chains are 100% and 98%, respectively with an elution window from 2 to 7min (Fig. 2). Good MS/MS sequence coverage is achieved for the ADC trastuzumab-DM1 using the optimized method (Fig.3) . Six drug conjugated peptides were identified by database search. Several other MS2 spectra containing fragments typical of DM1 containing peptides were acquired (Fig. 4). Fewer conjugated peptides were identified by CE-MS than LC-MS, possibly due to recovery issues. Further investigation to optimize the elution conditions and improve CE-MS identification rate of large hydrophobic conjugated peptides will be conducted.

- CE/MS method is optimized with NISTmAb digest as a reference control. Good MS/MS coverage of NISTmAb is obtained with narrow peak width and fast separation.
- With the developed CE/MS peptide mapping method the ADC under investigation yielded good sequence coverage.
- Further optimization is needed to enhance the conjugated peptides identification rate.

[1] Chen L, Wang L, Shion H, Yu C, Yu YQ, Zhu L, Li M, Chen W, Gao K. In-depth structural characterization of Kadcyla® (ado-trastuzumab emtansine) and its biosimilar candidate. MAbs. 2016 Oct;8(7):1210-1223.