In depth-characterization of a mAb combined with a routine multiple-attribute methodology (MAM) using a novel fragmentation type

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ABSTRACT

A complete qualitative and quantitative assessment is fundamental not only during quality control of any biotherapeutic product, but also during drug development. A streamlined approach is needed to obtain sequence information as well as type and localization of modifications. This qualitative analysis needs to be combined with a reproducible and accurate relative quantification of the quality attributes. Previous approaches were mainly based on peptide mapping with collision-induced dissociation (CID) for ID and a subsequent MAM leveraging the same data or MS only. Here, a novel fragmentation type based on ExD was investigated to combine information-richer fragmentation spectra and ID with an accurate MAM in one single injection.

INTRODUCTION

- Extensive qualitative and quantitative knowledge of a biotherapeutic product is fundamental, not only during quality control, but also during drug development for ensuring a safe and effective drug.
- As numbers of protein drug candidates are steadily rising, there is a need for streamlined approaches to obtain in-depth characterization information and reproducible quantification of defined product/critical quality attributes (PQA/CQA).
- Previous LC-MS/MS technologies suffer from either information gaps or lack of quantitative power for the same data set.

Key features of the ZenoTOF 7600 system from SCIEX

- **Higher levels of structural information:** electron activated dissociation (EAD) allows for localization of labile modifications[1] and differentiation of isomers such as Leu/Ile[2] and Asp/iso Asp.[3]
- **New depths of peptide mapping analysis:** novel alternative fragmentation enables in depth analysis[1-3] of next generation protein therapeutics and standard mAbs in a routine manner. Sequence coverages are highly reproducible and comparable to well established CID[4].
- **Higher MS/MS sensitivity for more confident identification:** 5-to-10-fold increase in sensitivity for detection of MS/MS fragments using the Zeno trap.
- **Streamlined and easy to use:** SCIEX OS software for streamlined data acquisition and quantification of PQA and CQA.

MATERIALS AND METHODS

Sample preparation:
The trastuzumab sample was denatured, reduced, and alkylated prior to enzymatic digestion using trypsin/LysC.

LC-MS:
Peptides were separated using an ExionLC system (SCIEX). The LC-MS data were acquired with data-dependent acquisition (DDA) using a ZenoTOF 7600 system (SCIEX).

Data analysis:
Peptide ID was performed with Byos software (Protein Metrics Inc.).
SCIEX OS software was used for fast and reliable quantification of selected PQAs.

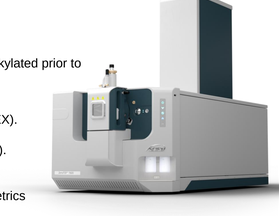

Figure 1. ZenoTOF 7600 system.

RESULTS

Table 1. Percentage sequence coverage of trastuzumab (*n*=5)

	Light chain (LC)		Heavy chain (HC)	
	Sequence coverage	Fragment coverage	Sequence coverage	Fragment coverage
CID	*96.0 ±0.9*	*93.7 ±1.1*	*93.6 ±0.0*	*86.9 ±0.4*
EAD	*96.0 ±0.9*	*96.0 ±0.9*	*93.2 ±0.7*	*90.5 ±1.3*

Consistently high sequence coverages of trastuzumab were obtained using either CID or EAD, with improved fragment coverage in the case of EAD. This proves the reliability of EAD for obtaining standard information in a routine manner.

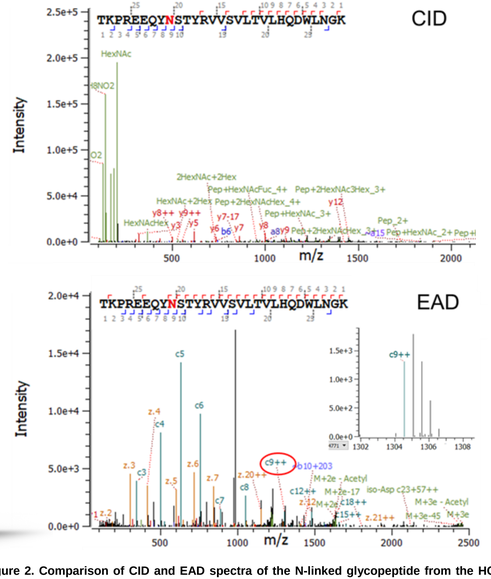

Figure 2. Comparison of CID and EAD spectra of the N-linked glycopeptide from the HC. In addition to confident peptide identification, EAD enables unambiguous localization of the N-linked glycan, whereas CID cannot provide fragments with the dissociation-labile modification still being attached to the fragments.

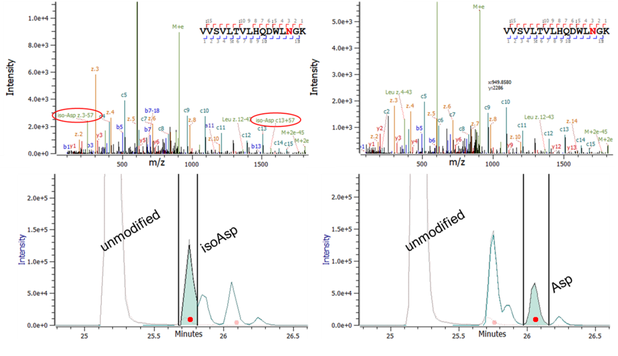

Figure 3. Differentiation of asparagine deamidation to iso-aspartic acid (isoAsp) or aspartic acid (Asp). The diagnostic ions (red circles) of isoAsp derived from alternative fragmentation with EAD allow for confident identification of this quality attribute and differentiation from Asp.

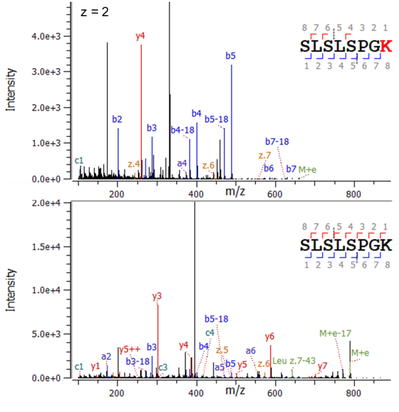

Figure 4. EAD MS/MS spectrum for the C-terminal peptide SLSLSPGK of the HC. Top: peptide with C-terminal lysine loss. Bottom: unmodified peptide.

Beside allowing improved fragmentation of challenging peptides, such as large peptides, peptides with dissociation-fragile modifications, and differentiation of amino acid isomers, EAD can also efficiently fragment low charged species (singly and double charged peptides), needed for a full characterization of biotherapeutic protein products.

Excellent reproducibility (%CVs </= 5%) was obtained for all quality attributes monitored using CID or EAD.

*Sample	*HC-T21 M Oxidation	*HC-T26 Dea-isoAsp	*HC-T26 Dea-Asp	*HC-T24-26 G0F	*HC-T24-26 G1F	*HC-T24-26 G2F	*N-t Glu->PyroGlu	*C-t Lys loss
CID #1	3.79	6.59	2.95	57.75	36.84	4.58	0.77	98.66
CID #2	3.70	6.75	3.09	57.64	36.78	4.68	0.73	98.58
CID #3	3.47	6.45	2.96	57.19	37.41	4.53	0.74	98.69
CID #4	3.73	6.76	3.01	57.83	36.89	4.39	0.74	98.71
CID #5	3.48	6.58	3.04	57.10	37.35	4.67	0.73	98.79
EAD #1	4.66	6.67	3.47	56.31	37.90	4.73	0.79	98.73
EAD #2	4.59	6.59	3.49	57.45	36.80	4.71	0.83	98.76
EAD #3	4.34	6.53	3.47	56.87	37.44	4.77	0.83	98.66
EAD #4	4.10	6.98	3.63	56.11	38.17	4.72	0.81	98.74
EAD #5	4.70	6.56	3.38	57.01	37.61	4.45	0.81	98.58

Figure 5. Comparison of CID and EAD data for relative quantification of selected peptides of trastuzumab for replicate injections. Several modifications were chosen for quantitative analysis in SCIEX OS software. Shown here are the relative %modifications for each attribute for five replicates of CID and EAD data.

CONCLUSIONS

- EAD produced comparable sequence coverage of trastuzumab to traditional CID while offering increased fragment coverage for more confident peptide identification.
- EAD enabled in depth structural analysis (accurate localization of labile modifications and differentiation of isomers in a single DDA method).
- Reproducible quantification of quality attributes with low %CVs (<5%) was obtained with EAD, demonstrating its capability for a MAM assay.
- SCIEX OS software provides an intuitive environment for data acquisition and processing including customizable MAM functions.

REFERENCES

1. A new electron activated dissociation (EAD) approach for comprehensive glycopeptide analysis of therapeutic proteins. SCIEX technical note RUO-MKT-02-12980-A.
2. Differentiation of leucine and isoleucine using electron activated dissociation (EAD). SCIEX technical note RUO-MKT-02-12605-B.
3. Differentiation of aspartic and isoaspartic acid using electron activated dissociation (EAD). SCIEX technical note RUO-MKT-02-12550-B.
4. Evaluation of biotherapeutic sequence coverage using electron activated dissociation (EAD). SCIEX technical note RUO-MKT-02-12920-B.

TRADEMARKS/LICENSING