Rapid and robust PTM peptide mapping for biologics with the Evosep One and the ZenoTOF 7600

Moritz Heusel[1], Stephen Lock[2], Nick Morrice[2], Sibylle Heidelberger[2], Jakob Bunkenborg[1], Michael Barrett Andersen[1], Nicolai Bache[1]

[1]Evosep Biosystems, Odense, Denmark, [2]SCIEX Macclesfield, United Kingdom

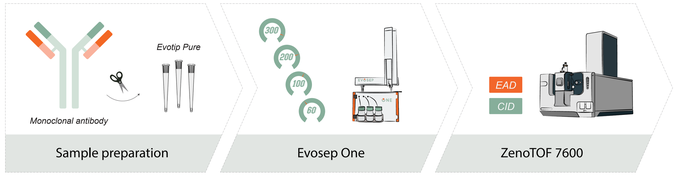
Highlights

- Implementation of rapid and robust LC-MS based workflow for antibody characterization.
- Deep sequence coverage of primary structure as well as process and product related modifications with high run-to-run retention time reproducibility.
- Achieved through standardized Evosep One methods in combination with ZenoTOF 7600 CID and EAD based fragmentation.

Evaluating Evosep One standard methods with ZenoTOF 7600

Standardized workflow for antibody peptide mapping

A commercial tryptic digest of NIST reference material (Waters, 186009126) was loaded on Evotip Pure with 50, 100 and 200 ng respectively. Four Evosep One standard methods, 300 SPD, 200 SPD, 100 SPD & 60 SPD were evaluated. The ZenoTOF 7600 (SCIEX) mass spectrometer was operated in information dependent acquisition (IDA) mode utilizing a Top15 method with either CID or EAD fragmentation by using the OptiFlow Turbo V ion source with the low microflow emitter.



Workflow for antibody peptide mapping.

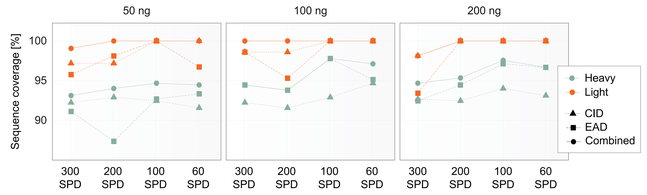
	CID	EAD			
	Collision energy	Beam current	Acceleration voltage	MS2 fill time	Dynamic exclusion
300 SPD	Dynamic CE	5000 nA	5 eV	35 ms	5 sec
200 SPD	Dynamic CE	5000 nA	5 eV	35 ms	3 sec
100 SPD	Dynamic CE	5000 nA	5 eV	30 ms	3 sec
60 SPD	Dynamic CE	5000 nA	5 eV	30 ms	3 sec

IDA parameters for CID and EAD fragmentation on the ZenoTOF 7600 for sample acquisition.

Evaluating sequence coverage

High heavy and light chain sequence coverage

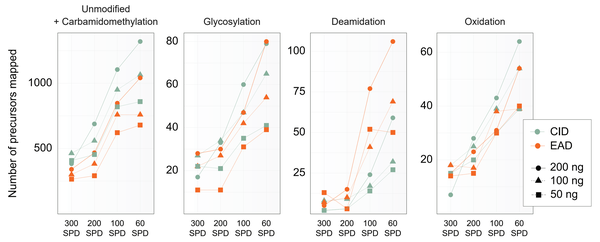
The antibody light chain was identified with a sequence coverage of 100% at 100 ng load when combining CID and EAD fragmentation independent of sample throughput. Additionally, we achieved 97.5% sequence coverage of the heavy chain at 100 ng load, using 100 SPD and EAD fragmentation.



Sequence coverage of reference antibody chains from tryptic peptide map.

Peptide identification depth

The depth of mAb sequence maps was explored using the number of mapped precursors as a metric which were grouped by modification class to monitor for potential site-specific biases. A 200 ng load in combination with 60SPD provided the deepest coverage for all modification classes. EAD fragmentation was particularly beneficial to increase the detection of deamidated precursors. Faster methods could be valuable in targeted workflows for monitoring specific peptides and modification sites.

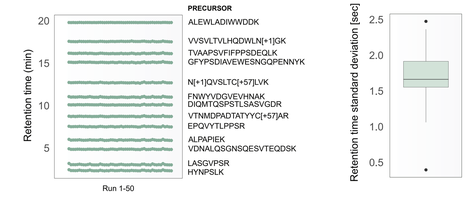


Number of precursors mapped as function of Evosep One method, sample load, fragmentation and modification class.

Robust and reproducible workflow

Stable retention times along runs

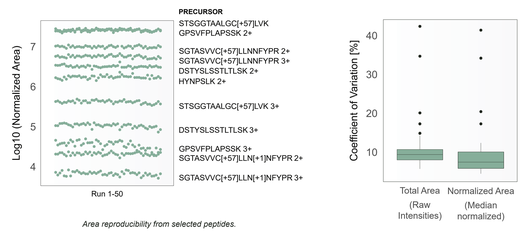
Fifty consecutive injections were performed using 60 SPD method. The retention time variability was less then 1% across all 33 monitored peptides irrespective of elution time.



Retention time stability from 50 injections.

Low variance in precursor XIC area

MS1 area under the curve observed across the 50 injections was robust with a median CV of 10% for raw intensities and 7.8% following a median normalization of raw intensities.

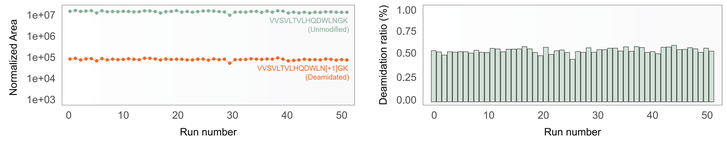


Area reproducibility from selected peptides.

Boxplot showing area reproducibility for all peptides with and without normalization.

Deamidation levels are stable on the Evotip

Samples are stored on moisturized Evotips at room temperature prior to analysis. We monitored the development of deamidation ratios over a time frame of ~22 hours and did not observe a change.



Deamidation ratio of VVSVLTVLHQDWLNGK