The characterization and quantification of the cyclic peptide Oxytocin using Zeno CID and Zeno EAD

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ABSTRACT

The characterization and quantification of cyclic peptides using LC-MS/MS with CID represents a large challenge. Due to the nature of these molecules the number of fragment ions can be significantly higher than equivalent linear peptides, and often unselective for quantification in complex matrices.

One of the challenges with quantification is finding selective fragment ions which will meet the analytical concentrations demanded with accuracy and precision. In CID typically large m/z fragments above the multiply charged precursor are chosen as they typically offer greatest selectivity in matrix.

In this study we examine the potential of electron activated dissociation (EAD) for the quantification of the cyclic peptide Oxytocin.

A unique workflow that fully leverages a Zeno trap (Figure 1) coupled to a novel QTOF system in which >90% duty cycle is achieved in MS/MS mode is demonstrated [1] and when coupled with MRMHR in either CID or EAD for quantification.

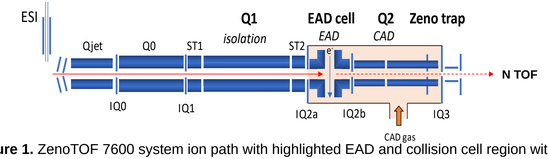

Figure 1. ZenoTOF 7600 system ion path with highlighted EAD and collision cell region with the Zeno trap

MATERIALS AND METHODS

Sample preparation:
Oxytocin was prepared in protein precipitated rat plasma with 2:1 CH_3CN: plasma and diluted 10-fold in water. Serial dilution was performed from 100nM to 0.1nM.

HPLC conditions:
An ExionLC AD system with a Phenomenex Kinetex XB-C18, 50x2.1mm, 1.8 µm column at 40˚ C. Gradient separation using 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used at a flow rate of 400 µL/min. Separation was performed over 2.15 minutes from 5% B to 99% B with a total runtime of 4 minutes. The injection volume was set to 10 µL.

MS/MS conditions:
A SCIEX ZenoTOF 7600 system with Turbo V ion source and electrospray ionization (ESI) was used.

The samples were analyzed in using Zeno CID MRM^{HR} and Zeno EAD MRM^{HR}. The TOF MS scan was scanned between *m/z* 100-1500, the CID and EAD MS/MS covering *m/z* 100 to 1400. EAD kinetic energy of 1eV and beam current of 5500nA.

RESULTS

The acquisition method for analyzing Oxytocin was setup to simultaneously monitor the TOF MS, EAD MS/MS and CID MS/MS. Figure 2 shows the 3 collision energy settings acquired for Oxytocin. Acquiring a low, mid and higher collision energy the challenge of cyclic peptides is present. These molecules shift from low fragmentation efficiency to highly fragmented in a short energy range. In addition, the fragmentation is unselective and multiple pathways not just classic b- and y- ions are observed.

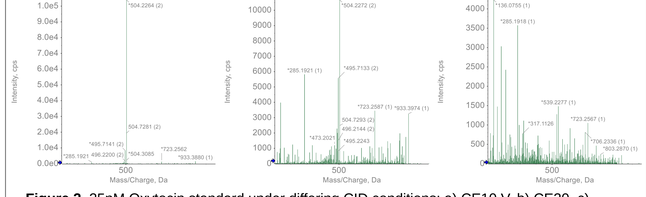

Figure 2. 25nM Oxytocin standard under differing CID conditions; a) CE10 V, b) CE20, c) CE30V

For the later quantification method, the collision energy 20 was chosen and the most abundant fragment ion above the precursor, *m/z* 933.3880. Figure 3 shows the EAD MS/MS spectrum acquired for Oxytocin also at 25 nM. The conditions employed an electron kinetic energy of 1 eV. Under these conditions the fragmentation is very different to CID.

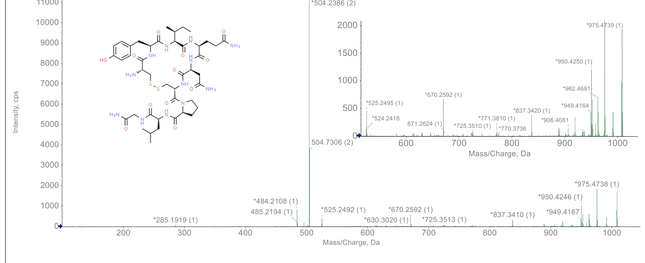

Figure 3. 25nM Oxytocin standard under differing EAD condition with an electron kinetic energy of 1eV. Inset zoom region of EAD fragments generated above the precursor up to the charge reduced precursor

First classic charge reduced species of the 2+ precursor is observed, confirming the capture of an electron. The fragmentation opens the disulfide bond and the observed fragment ions are predominantly the c- and z- ions as if fragmenting a linear peptide using electrons. For the quantification using EAD the most abundant high mass fragment was chosen, *m/z* 975.4738.

In assessing the quantification performance of EAD, selectivity, limit of quantification, dynamic range, accuracy and precision were all compared to CID. Here the EAD extracted chromatograms (XICs) are shown for the blank, lower limit of quantification and next two concentrations, Figure 4.

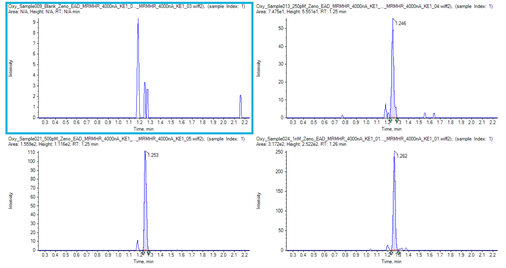

Figure 4. a) Blank, b) 0.25 nM, c) 0.5 nM, d) 1 nM EAD extracted ion chromatograms

At the LLOQ the peak is greater than five times the blank noise and with greater than 10:1 signal to noise.

To assess linear dynamic range, accuracy and precision a dilution series from 0.25nM to 50nM was analyzed with 6 replicates per concentration. The calibration curves for both CID and EAD are shown in Figure 5 and the statistics in Table 1.

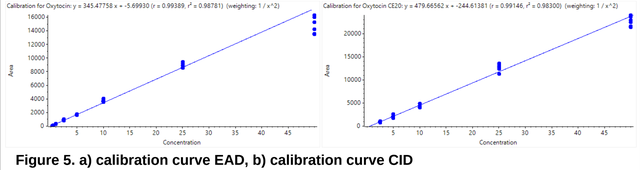

Figure 5. a) calibration curve EAD, b) calibration curve CID

The key difference between the CID and EAD calibration curves is the LLOQ achievable. With CID as shown in Table 1 the LLOQ is limited by the precision of the low concentrations.

For 0.5 and 1 nM the percent CV is over 30 % which is outside of regular bioanalytical criteria. Therefore, the LLOQ is limited to 2.5nM.

Table 1. Accuracy and precision measurements for n=6 replicates.

Concentration	Mean Conc. EAD	Std Dev. EAD	Percent CV EAD	Accuracy EAD	Mean Conc. CID	Std Dev. CID	Percent CV CID	Accuracy CID
0.25 nM	0.256	0.0184	7.20%	102.5%	N/A	N/A	N/A	N/A
0.5 nM	0.47	0.0236	5.02%	94.2%	0.50	0.154	30.8%	100.1%
1 nM	0.98	0.0594	6.07%	98.0%	1.06	0.341	32.29%	105.8%
2.5 nM	2.68	0.2229	8.31%	107.4%	2.17	0.246	11.38%	86.7%
5 nM	4.94	0.1870	3.78%	98.9%	4.71	0.634	13.47%	94.3%
10 nM	10.91	0.5338	4.89%	109.1%	9.89	0.902	9.12%	98.9%
25 nM	25.91	0.9729	3.75%	103.6%	28.29	1.757	6.21%	113.2%
50 nM	42.85	3.554	8.29%	85.7%	50.50	2.364	4.68%	101.0%

For EAD the accuracy and precision is maintained down to 0.25 nM and linear to 50 nM. Percent CVs maintain good precision less than 9% and accuracy within 15%.

CONCLUSIONS

This work highlights the potential new avenue for both the characterization and quantification of cyclic or poorly fragmenting compounds. Electron activated dissociation (EAD) shows promise as shown in this data where poor reproducibility impacts the limit of quantification achievable.

Further work will continue to explore across a wider range of cyclic molecules to understand how EAD can improve the analytical methodology.

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