Enhanced Structural Elucidation of Microcystins by Electron Activated Dissociation (EAD)

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Introduction

- The structural diversity of microcystins (MCs) stems from variable amino acid substitutions within the cyclic peptide structures of MC and nodularin (NOD).
- Conventional immunoassay-based methods lack the unambiguously identify individual specificity to congeners
- CID-based MS/MS produces non-selective fragments at a common side chair
- Unique and alternative dissociation pathways are critical for identifying and differentiating known and new MCs
- EAD fragmentation in the ZenoTOF 7600 system enhances structural elucidation through production of unique fragments









Figure 2. Kinetic energy ramping from -10 to 25 eV using EAD fragmentation mode for the MC-YR congener.







Tunable kinetic energy (KE) in EAD reveals 80% more unique and alternative dissociation pathways not observed with CID



Figure 4. Fragmentation sites in the MC-YR congener (top) and closer examination of various unique fragments only observed in EAD MS/MS spectra of MC-YR (bottom). EAD-specific fragments corresponded to different modifications of the amino acid side chains and internal cleavages of the amide peptide bonds within the cyclic ring structure of MC-YR.



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	MC-RR	$\mathbf{MC} - \mathbf{RR}$		$\frac{MC-YR}{AA2} = \frac{AA4}{AA4} = $		
Predicted formula EAD C	D Predicted formula	a EAD CID	Predicted formula	EAD	CID	
C ₄₉ H ₇₅ N ₁₀ O ₁₂ + ✓	✓ C ₄₉ H ₇₆ N ₁₃ O ₁₂ +	\checkmark	$C_{52}H_{73}N_{10}O_{13}^+$	\checkmark		
$C_{48}H_{75}N_{10}O_{11}^{+}$	$\sim C_{48}H_{74}N_{13}O_{12}^{+}$	\checkmark	$C_{51}H_{71}N_{10}O_{13}^+$	\checkmark		
$C_{48}H_{74}N_{10}O_{10}^{+}$	✓ C ₄₉ H ₇₄ N ₁₃ O ₁₁ +	\checkmark	$C_{52}H_{71}N_9O_{13}^+$	\checkmark		
C ₄₇ H ₇₃ N ₁₀ O ₈ ⁺ ✓	C ₄₈ H ₇₇ N ₁₃ O ₁₁ +	\checkmark	$C_{51}H_{74}N_{10}O_{12}^+$	\checkmark		
C ₄₇ H ₇₁ N ₁₀ O ₉ ⁺ ✓	$C_{48}H_{74}N_{13}O_{11}^{+}$	\checkmark	$C_{51}H_{71}N_{10}O_{12}^+$	\checkmark		
$C_{40}H_{63}N_{10}O_{11}^{+}$	$\sim C_{48}H_{76}N_{13}O_{10}^+$	\checkmark	$C_{51}H_{73}N_{10}O_{11}^{+}$	\checkmark		
C ₃₉ H ₆₃ N ₁₀ O ₉ ⁺ ✓	$C_{48}H_{74}N_{13}O_{9}^{+}$	\checkmark	$C_{50}H_{71}N_{10}O_{11}^{+}$	\checkmark		
	$C_{47}H_{74}N_{13}O_{9}^{+}$	\checkmark	$C_{49}H_{70}N_{10}O_{12}^+$	\checkmark		
	$C_{47}H_{72}N_{10}O_{10}^{+}$	\checkmark	$C_{49}H_{70}N_9O_{11}^+$	\checkmark		
	$C_{47}H_{70}N_{11}O_{10}^+$	✓	$C_{48}H_{68}N_{10}O_{11}^+$	\checkmark		
	C ₄₆ H ₇₀ N ₁₃ O ₁₁ +	✓	$C_{45}H_{67}N_{10}O_{13}^+$	✓		
	$C_{45}H_{71}N_{13}O_{10}^{+}$	✓	$C_{45}H_{67}N_{10}O_{12}^{+}$	✓		
	$C_{40}H_{66}N_{13}O_{11}^+$	✓ ✓	$C_{43}H_{63}N_{10}O_{12}^+$	✓	✓	
	$C_{40}H_{65}N_{10}O_{12}^{+}$	√	$C_{42}H_{63}N_{10}O_{10}^{+}$	× 		
	С Н N O +	• •	$C_{40} \square_{56} \square_7 O_8^{-1}$	•		
	$C_{39} I_{64} I_{13} O_{9}$	· · · · · · · · · · · · · · · · · · ·	V30 ¹ 46 ¹ ³ 5 ^V 5	•		
	C20H50N0O0+	\checkmark				
C ₉ H ₁₁ O⁺ ✓	✓ C₀H₁₁O+	 ✓ 	C ₉ H ₁₁ O ⁺	\checkmark	✓	
$C_5H_{10}N_3^+$	$\sim C_5 H_{10} N_3^+$	 ✓ 	$C_5 H_{10} N_3^+$	\checkmark	\checkmark	
C ₈ H ₇ + ✓	\checkmark C ₈ H ₇ +	 ✓ 	C ₈ H ₇ +	\checkmark	\checkmark	
$C_4H_{10}N_3^+$	C ₄ H ₁₀ N ₃ +	\checkmark	$C_4H_{10}N_3^+$	✓		

Table 1. Comparison of diagnostic fragments produced by EAD and CID for structural elucidation of different MC congeners. Molecular formulas in bold represent the parent ions.

Summary

- EAD fragmentation on the ZenoTOF 7600 provides complementary MS/MS data for the structural elucidation of MCs
- KE ramping in EAD induces different dissociation pathways to produce unique fragmentation profiles in the ECD, hot ECD and EIEIO regions
- EAD yields unique product ions that reveal additional fragmentation pathways, such as sidechain modifications and ring-opening bond breakages within the cyclic peptide structures

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