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The biosynthesis of  $^{15}\text{N}$ -labeled microcystins and the comparative MS/MS fragmentation of natural abundance and their  $^{15}\text{N}$ -labeled congeners using LC-MS/MS

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1 The biosynthesis of <sup>15</sup>N-labeled microcystins and the comparative MS/MS  
2 fragmentation of natural abundance and their <sup>15</sup>N-labeled congeners using LC-  
3 MS/MS

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10 **Abstract**

11 The global need for accurate and sensitive quantitation of microcystins (MCs) persists as incidents of  
12 cyanobacterial harmful algal blooms continue to rise and recent research reveals an underestimation of  
13 the human health implications of these toxins. An optimal approach for their accurate quantitation  
14 relies on the availability of stable isotope-labeled MC standards for use in stable isotope dilution analysis  
15 (SIDA) strategies involving liquid chromatography tandem mass spectrometry (LC-MS/MS). Due to the  
16 dearth of isotopically labeled MCs, ten different <sup>15</sup>N-enriched MCs were biosynthesized from producing  
17 cultures and fully characterized. This involved the comparative MS/MS fragmentation of natural  
18 abundance or unlabeled metabolites with their <sup>15</sup>N-labeled congeners for improved confidence in  
19 product ion annotation. These results revealed a series of incorrect annotations described previously in  
20 the literature. In this manuscript, the biosynthesis of labeled microcystin is detailed, and their complete  
21 analytical characterization for prospective use in targeted SIDA applications, such as routine water  
22 testing is described.

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25 **Keywords:** microcystin, cyanotoxin, hepatotoxin, isotope-labeled, mass spectrometry

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