Improved sensitivity using liquid chromatography mass spectrometry (LC-MS) for detection of propyl chloroformate derivatised β-*N*-methylamino-L-alanine (BMAA) in cyanobacteria

M Esterhuizen-Londt, S Downing and TG Downing*

Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa

Abstract

β-*N*-methylamino-L-alanine (BMAA) is a difficult molecule to detect, primarily due to its presence in low concentrations in complex matrices. This has resulted in contradictory reports on the presence of BMAA in cyanobacteria. We report improved sensitivity of detection using propyl chloroformate derivatisation, liquid chromatographic (LC) separation, and single quadrupole mass spectrometry (MS) detection. Triple quadrupole mass spectrometry (MS/MS) was used to confirm the identity of BMAA in cyanobacteria based on product ions. We show a 10-fold increase in sensitivity with the LC-MS method compared to the previously published gas chromatography mass spectrometry (GC-MS) method with pre-column derivatised BMAA using a commercially available amino acid derivatisation kit. Clear chromatographic separation of BMAA from 2,4-diaminobutyric acid (DAB), as well as the 20 standard amino acids, was achieved. The analytical method was validated by multiple derivatisation of samples, multiple users, and multiple injections, as well as in various matrices. The quantifier ion used was $[M + H]^+ = 333$ *m/z*. The MS/MS product ions 273 *m/z* and 245 *m/z* were used in identification and peak confirmation. Additionally, we confirm the presence of BMAA in cyanobacteria previously screened with GC-MS as well as the presence of BMAA in newly isolated cultures.

Keywords: β-*N*-methylamino-L-alanine, BMAA, LC-MS, LC-MS/MS