Chemical Labeling Strategy and LC-MS/MS Analysis of Amine-containing Neurotoxin in Shark's Fins

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Introduction

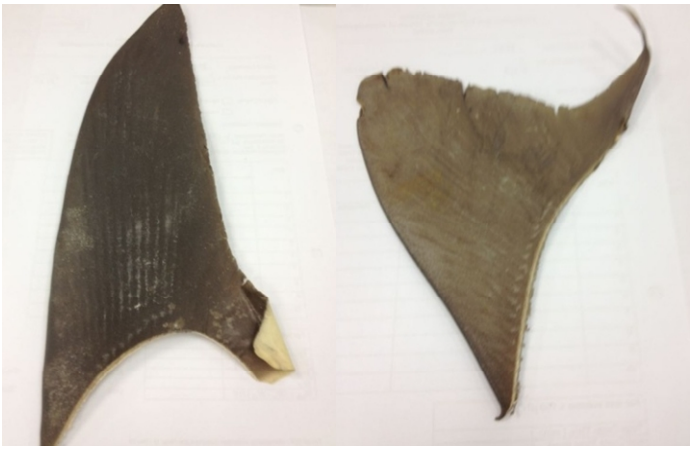
β-Methylamino-L-alanine (BMAA), is a neurotoxin[1] and a non-proteinogenic amino acid. BMAA has been identified and quantified in cycad seeds and cyanobacteria by Rosen[2] et al. using liquid chromatography tandem mass spectrometry without pre-derivatisation. Kushnir et al.[3] has also developed a quantitative analysis of BMAA with iTRAQ® reagent as derivative in biological samples and plant extracts. No BMAA was detected in human serum samples and cerebrospinal samples. However, 50 mg/kg (ppm) of BMAA was found in the extract of the cycad seed.

Recently, a high concentration ranging from 144 to 1836 ng/mg of BMAA was discovered in shark fins by a group of University of Miami scientists. [4] In Mondo et al.'s analysis, BMAA was pre-labeled with a fluorescent tag 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) before injecting into LC-MS/MS.

The main challenges of determining BMAA are that the neurotoxin is highly polar and it provides low sensitivity for MRM when analyzed in the free form. In this paper, a simple labeling of BMAA using mTRAQ® Δ0 reagent for detection with HPLC and AB SCIEX 4000 QTRAP® system is reported for shark's fin.

The mTRAQ® Δ0 is an amine labeling reagent useful for performing relative quantitation experiments of targeted proteins, peptides and post-translational modifications by LC-MS/MS.

Here, mTRAQ® was used for labeling of BMAA prior LC-MS/MS detection using Multiple Reaction Monitoring (MRM).



Experimental

Sample Preparation

The sample preparation protocol was adopted from the Mondo et al.[4] About 1 g of dried sample was weighed and digested with 6N HCl for 18 hours.

Sample Labeling

5 µL of the filtered sample was mixed with 15 µL dissolution buffer (pH 8.5, 0.5 M triethylammonium bicarbonate). 50 µL of isopropanol was added to the lyophilized mTRAQ® tube and vortexed. The mTRAQ® solution was then added to the 5 µL sample and vortexed. The mixture was incubated at room temperature for 1 hour and then injected into LC-MS/MS.

LC

A Shimadzu Prominence LC system with a Waters Atlantis HILIC column (100 x 2.1 mm, 3 µm) at 40°C with a gradient of eluent A: 20 mM ammonium acetate + 0.1% formic acid in water and eluent B: 0.1% formic acid in acetonitrile was used at a flow rate of 300 µL/min. The injection volume was set to 10 µL.

MS/MS

An AB SCIEX 4000 QTRAP® system with Turbo V™ source and electrospray ionization (ESI) probe was used. 2 MRM transitions were monitored for BMAA; 399/228 was selected as quantifier and 399/172 was selected as qualifier transition.

Results and Discussion

mTRAQ® reagent was used for labeling of BMAA. The proposed structure of the BMAA/mTRAQ® complex and the two monitored product ions are shown in Figure 1.

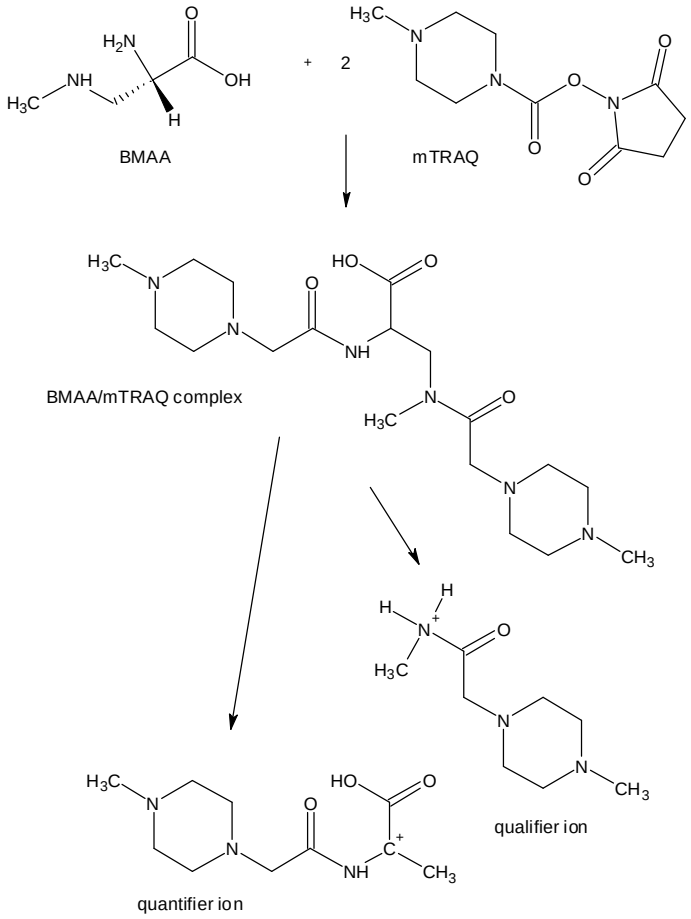


Figure 1. Proposed structure the of BMAA/mTRAQ® complex and the product ions monitored using MRM mode

An example chromatogram of a standard injection with a retention time of about 6.2 minutes is shown in Figure 2.

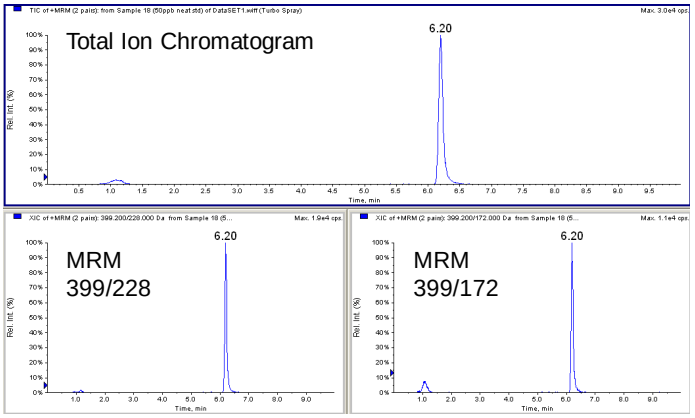


Figure 2. Detection of BMAA/mTRAQ® complex in neat standard of 50 ng/mL by LC-MS/MS

A 0.5, 1, 5, 10, 50, 100, 200, and 1000 mg/kg of BMAA was pre-spiked into the shark's fin, acid-digested, labeled with mTRAQ® and injected into the LC-MS/MS. A good linearity with r-value of greater 0.999 was observed (Figure 3). Repeatability was studied at 1, 5, 10, and 50 mg/kg over three consecutive days. The resulting coefficients of variation (%CV) are shown in Table 1.

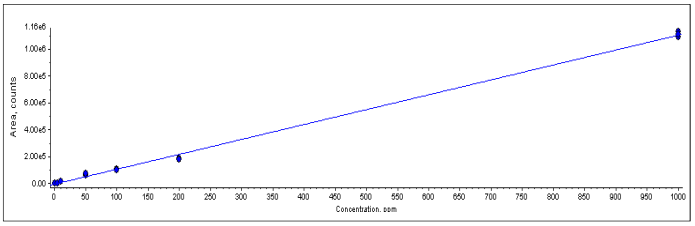


Figure 3. Calibration line from 0.5 to 1000 mg/kg BMAA spiked into shark's fin

Table 1. Repeatability of analysis on three consecutive days

%CV at	1 mg/kg	5 mg/kg	10 mg/kg	50 mg/kg
Day 1	19.7	14.5	3.2	9.5
Day 2	8.3	5.8	6.4	0.9
Day 3	10.9	7.8	6.4	1.2

The Limit of Quantitation (LOQ) was found to be 0.5 mg/kg BMAA in shark's fin with a signal to noise ratio of ~10 (Figure 4).

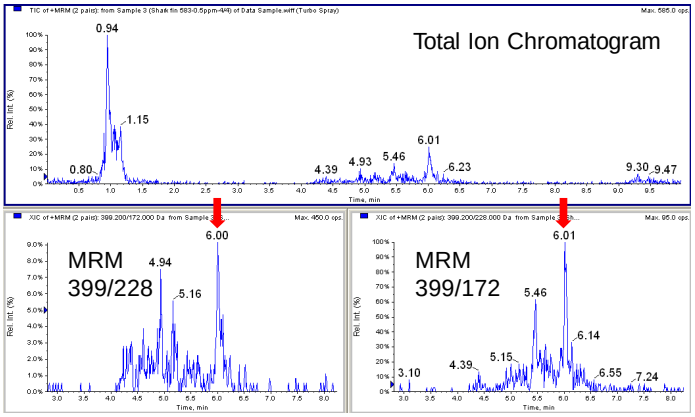


Figure 4. Detection of BMAA in 0.5 ppm spiked shark's fin after derivatization with mTRAQ® reagent

Summary

A fast, simple and reliable labeling method with mTRAQ® Δ0 reagent, for the detection of BMAA in the shark's fin matrix, was developed for quantitative study on the AB SCIEX 4000 QTRAP® system. Multiple Reaction Monitoring (MRM) was used because of its high selectivity and sensitivity. The Limit of Quantitation (LOQ) of the method was found to be 0.5 mg/kg (ppm).

Acknowledgements

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References

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