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Development of a solid phase extraction method for measuring gonyautoxins 1-4 in human urine

Mani Deepika Vakkalanka, Padmanabhan Eangoor, Amruta Indapurkar and **Jennifer S Knaack**
Mercer University, Atlanta, USA

Purpose: Consumption of shellfish contaminated with gonyautoxins (GTX 1, 2, 3 or 4) can result in paralytic shellfish poisoning (PSP) which can be lethal. Identification and accurate determination of each GTX is important in assessing the severity of poisoning. Here, we describe the development of a solid phase extraction method to extract GTXs from urine followed by quantification using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS).

Methods: GTXs were extracted from urine using Oasis MCX mixed mode strong cationic exchange cartridges. Sample pH was adjusted with 10 mM acetate buffer and melamine was added to samples as an internal standard. Cartridges were conditioned with methanol and acetate buffer and toxins were eluted with 5% ammonium hydroxide in methanol. Experiments were conducted using pooled urine spiked with 50 ng/mL each of GTX 1, 4 and GTX 2, 3. Additional GTX spikes of 5.0, 10, 15 and 20 ng were added to urine samples and samples were extracted as described above. The percent recoveries were calculated by comparing the peak areas of extracted samples with those of reference standards. The amount of toxin in samples was calculated based on the line equation from the standard addition curve.

Results: Calculated recoveries for GTXs 1, 2, 3 and 4 from urine, based on reference standards, were 67.3±3.40, 44.1±5.79, 62.6±5.48 and 75.8±5.57%, respectively (Image 1). The coefficient of determination for GTXs extracted from pooled urine was above 0.98. The accuracies for GTX 1, 2, 3 and 4 in standard addition experiments were 70%, 86%, 89% and 90%, respectively.

Conclusion: GTXs can be extracted from urine using this SPE method. A standard addition method can be used to measure exposure to GTXs, but modifications are needed to increase sensitivity and accuracy of GTX 1. A successful method will be validated and used to test clinical specimens for GTXs.

Biography

Mani Deepika Vakkalanka is currently pursuing her PhD in an Analytical Toxicology Lab at Mercer University, Atlanta. Her research interests include developing diagnostic methods for toxin exposure. She is experienced in high performance liquid chromatography, liquid chromatography-mass spectrometry, ELISA and solid phase extraction..

Manideepika.vakkalanka@live.mercer.edu

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