A Novel Diagnostic Method for Detection and Quantitation of Paralytic Shellfish Poisoning in Humans

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OVERVIEW

• 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

INTRODUCTION

Paralytic shellfish toxins (PSTs) are the most potent neurotoxins and include Gonyautoxins (GTX) 12,3, and 4. Exposure to PST occurs upon consumption of contaminated shellfish that have bioaccumulated the toxins through filter feeding of toxic algae. Humans consuming contaminated shellfish can be exposed to these bioaccumulated PSTs causing paralytic shellfish poisoning(Figure 1). PSTs block voltage-gated sodium channels in muscle and nerve cell membranes thereby stopping the nerve impulse propagation(Figure 2). This can result in respiratory paralysis and death when exposed to high amounts of the toxins.

METHOD

GTxs were extracted from plasma using mixed mode strong cationic exchange cartridges. Sample pH was adjusted to 4.0 with 10mM acetate buffer. Cartridges were conditioned with methanol and acetate buffer and toxins were eluted with 5% ammonium hydroxide in methanol. Five calibrants and two quality controls were prepared by spiking the toxin into 100µl of pooled human plasma. The final calibrant concentration ranges were: GTX1 8.13-317.66 ng/mL, GTX2 6.98-473.25 ng/mL, GTX3 2.96-200.68 ng/mL and GTX4 2.56-162.91 ng/mL. Quality controls were prepared at a concentration of 44 and 124 ng/mL for GTX1, 40 and 113 ng/mL for GTX2, 17 and 71 ng/mL for GTX3, 14 and 39 ng/mL for GTX4. Splayed samples were then extracted as described above, dried and reconstituted. Extracted samples were injected onto HPLC-MS/MS for analysis (Figure 3).

RESULTS

GTxs were successfully extracted from plasma by solid phase extraction and the developed method was further validated according to FDA guidance for bioanalytical method validation. The method was analyzed by validating six calibration curves for each GTX. Each curve has 5 calibrants, 2 quality controls and blank sample. 8 plasma specimens were analyzed with each curve. A typical chromatogram for GTxs extracted from plasma is shown in Figure 4. The calibration curves were linear with the coefficients of determination value (R-squared value) greater than 0.99 for all toxins (Graph 1). The percent accuracies of all the analytes were within the range of 80-120% indicating that the method can accurately detect the analytes within the range selected (Tables 1 and 2). The developed method was validated using specimen plasma samples with good recovery making it more specific (Table 3). LODs were calculated by Taylor method and were shown in Table 4. Blank samples were analysed to characterize the selectivity of the method and there were no peaks at the retention times of our analytes indicating minimal interference with endogenous matrix components (Figure 5).

CONCLUSION

We have successfully developed a solid phase extraction method for extracting GTxs 1,2,3,4 from human plasma. The developed method has been characterized and validated for diagnosing GTX-based PSP using clinical specimens. This method has all the validation parameters within the limits specified by FDA. This is a fast and simple diagnostic method which is easily accessible to common clinical laboratories and can be applied to clinical specimens.