

A novel clean-up method based on cyclodextrin polymers for the detection of tetrodotoxins in natural samples with a cell-based assay

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Tetrodotoxins (TTXs) are a group of potent neurotoxins produced by bacteria, usually found in pufferfish. These toxins may reach consumers and cause them an intoxication with symptoms such as muscle weakness, respiratory failure, and ultimately death. Therefore, the detection of TTXs is of great importance in seafood safety. The cell-based assay (CBA) is commonly used to detect TTXs because of its high sensitivity and because it provides an estimation of the composite toxicity of a sample. However, when analysing natural seafood samples, matrix effects may interfere in the CBA. In this work, five insoluble cyclodextrin polymers (β CDPi-1.4SO₃ (DS2), β CDPi-1.3SO₃ (DS2), β CDPi-1.3SO₃ (DS4), β CDPi-1.4SO₃ (DS4) and β CDPi-NCH₃ (DS5)) have been investigated as novel clean-up materials for natural extracts containing TTXs. The clean-up method involved an incubation of the cyclodextrin polymers with TTX standard (at different concentrations), followed by an extraction of the TTXs captured by the cyclodextrins allowing it to bind with TTX. The purified TTXs were then detected using a CBA, since these toxins act on voltage-gated sodium channels. The best recoveries were achieved with β CDPi-1.4SO₃ (DS2) (~80%). This cyclodextrin polymer had the capability of capturing as much as ~20 ng TTX/mg cyclodextrin polymer. Therefore, the developed clean-up method combined with CBA is a promising strategy for the detection of low TTXs contents in pufferfish and shellfish extracts, which could be implemented in research and monitoring programs for seafood safety.

Keywords: Cyclodextrin polymers, cell-based assay, tetrodotoxins