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The analysis of tetrodotoxins in fish and shellfish using UPLC-MS/MS

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Introduction

Tetrodotoxins (TTXs) are natural toxins produced by symbiotic bacteria, which can be present in marine animals such as gobies, newts, trumpet shells and puffer fish. Consumption of seafood containing these toxins can cause severe intoxications. TTX intoxications mostly occur in Japan and other Asian countries, where puffer fish (fugu) is served as a delicacy. The adverse effects observed after exposure to TTX include nausea, vomiting and paralysis of muscles resulting in breathlessness. In severe occasions death can occur. Notwithstanding the fact that these toxins are not that often found within Europe, methods for rapid testing are still needed in order to protect the consumers from exposure to this highly toxic substances.

Material and methods

Extraction procedure

Weigh 1 g of fish or shellfish homogenate and extract three times with 3 mL ACN/H_2O (50:50 v/v) containing 7.5 mM NH_4COOH at pH3. Centrifuge at 3500*g* and combine the supernatants in a volumetric flask of 10mL and make up till the mark. Dilute the extract 1:1 with ACN to precipitate proteins. Filter the extract through a 0.2 µm nylon filter prior to UPLC-MS/MS analysis.

UPLC separation

Column	Waters UPLC BEH Amide column 100x2.1 mm, 1.7 µm
Column temp	30°C
Injection volume	20 µL
Flowrate	0.4 ml/min
Mobile phase A	H_2O containing 7.5 mM NH_4COOH at pH3
Mobile phase B	ACN/H_2O (95:5 v/v) + 7.5mM NH_4COOH at pH3
UPLC gradient	0 - 1 min: isocratic at 15% A / 85% B 1 - 6 min: linear to 60% A / 40% B 6 - 7 min: isocratic at 70% A / 30% B 7 - 10 min: isocratic at 15% A / 85% B

MS/MS detection

Mass spec	Waters Quattro Ultima
Polarity	Positive electrospray ionization
Capillary voltage	3 kV

By infusion with a TTX standard solution the optimal fragmentation and multiple-reaction-monitoring (MRM) conditions were determined (Table 1, Figure 1).

Table 1. Optimal MS settings for TTX

Parent ion (m/z)	Fragment ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
320.1	302.1	20	20
320.1	162.1	20	35

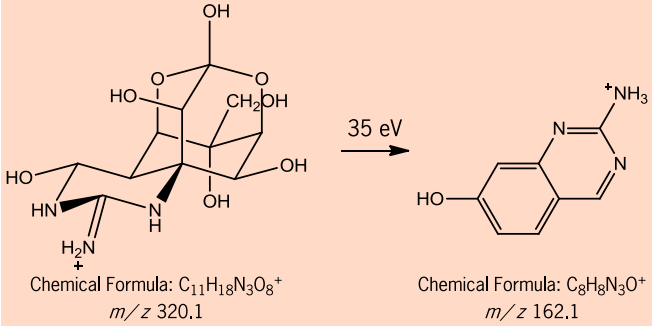

Figure 1. Specific fragmentation of TTX

Results

- Triplicate extraction from fish and shellfish resulted in an extraction efficiency of more than 90% for TTX.
- For quantification a matrix matched standard calibration curve in fish or shellfish extract was used depending on the type of sample to be analyzed.
- Good repeatability was obtained for fish (94.7 ± 6.0%, n = 8) and shellfish (99.8 ± 12.7%, n = 6) spiked with 800 µg/kg TTX
- Parent scanning on fragment *m/z* 162.1 of a naturally contaminated fish sample (*Lagocephalus lunaris*) revealed the presence of several TTX analogues (Figure 2).

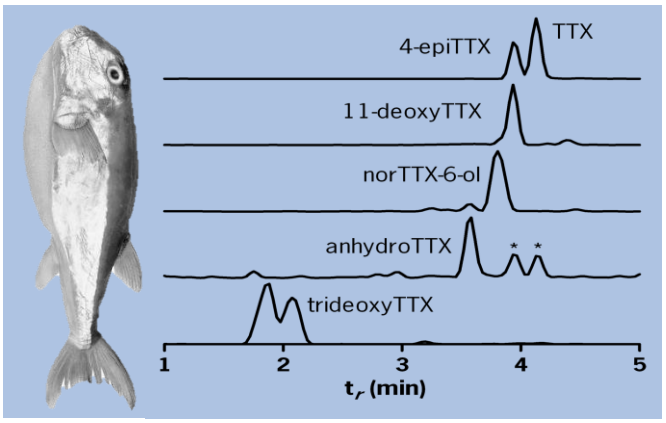

Figure 2. TTX profile in fish (L. lunaris)

Conclusions

- An UPLC-MS/MS method has been developed capable of analysing TTX and several analogues in a 10 min run.
- Matrix matched standard calibration is necessary for fish and shellfish to compensate for matrix effects.

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