

Mussels Based Food Supplements: Evaluation of Emerging Marine Toxins is a Necessary Evil

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Abstract: Food supplements containing mussel extracts are becoming popular in human diet, providing high levels of proteins, omega-3 polyunsaturated fatty acid (PUFAs), iodine and carbohydrates. Besides the beneficial effects and bioactives that mussel may yield, it is vital to consider the potential harmful phycotoxins that can be present in mussel extracts and marine dietary supplements. Recently, we have detected for the first time the marine toxin 13-desmethyl spirolide C in food supplements containing green lipped mussels of *Perna canaliculus* at levels up to 98 µg/kg. In this work, we provide new data about the presence of pinnatoxin-G (trace amounts) in the dietary supplements intended for human consumption after the analysis of the green lipped mussel powder by UPLC-MS/MS. Moreover, the status of microalgae phycotoxin contaminants is also assessed in these products and in animal dietary supplements which contained 13-desmethyl spirolide C at levels up to 39 µg/kg. The mechanism of action of spirolides and pinnatoxins is associated with the blockage of the muscarinic and nicotinic receptors (mAChR and nAChR) on the nervous system. Despite the fact that human intoxications have not been reported, it is important to identify the impact of such toxins on public health since dietary products constitute an important part of the global market.

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1. Introduction

Marine food supplements constitute an important part of the worldwide market. They are produced from different matrix, providing a large number of bioactive molecules like proteins, omega-3 fatty acids, vitamins, minerals, carotenoids and polysaccharides [1–3]. Especially, green lipped mussels' powder is sold for their benefits in the treatment of osteoarthritis [4]. This product has high anti-inflammatory activity due to the polyunsaturated fatty acids profile [5]. Despite they are announced as beneficial for health, food supplements could also lead to potential toxic risks related to the contamination by toxins [6].

In this study, we investigate the presence of lipophilic marine toxins in dietary supplements of *Perna canaliculus* from New Zealand but commercialized in Europe. Due to the increase of emerging toxin detection worldwide, it is convenient to study the contamination level of marine food supplements. These products are legislated as foods and therefore, it is necessary to guarantee the safety derived from their consumption [7]. Moreover, food supplements are not subjected to pharmacovigilance as is the case with drugs, so the homogeneity among batches is not guaranteed [8].

2. Material and Methods

2.1. Samples Acquisition

Green lipped mussels' powder (*P. canaliculus*) supplements were obtained by 3 different European Union (EU) distributors at random. Once in the laboratory, food supplement capsules were opened, and the powder content was passed to a 45mL empty tube. Each sample was a homogenate of 40 capsules content (20 g approx.). 6 samples were sold for human consumption and 3 of them were labelled as dietary supplements for dogs (Table 1). Each capsule has 500 mg of concentrated green mussel.

Table 1. Dietary samples for human and animal consumption.

Food Product	No. of samples
Dietary supplements (Green lipped mussels) for human	6
Dietary supplements (Green lipped mussels) for animals (dogs)	3

2.2. Toxin Extraction

Food supplements were extracted adding methanol after their rehydration. First an amount of 1.64 mL of water was added to 0.36 g of power food supplement in order to have a humid mussel extract with the same water content as the fresh mollusc (82 ± 0.02 g H₂O/100 g). After that, it was extracted according to the standard operation procedure (SOP) for the determination of lipophilic toxins in shellfish. The amount of 2 grams of food supplement homogenate were extracted with 9 mL of methanol. The extract was homogenized via vortex and then, the samples were centrifuged (3700 rpm × 10 min). The content was transferred to 20 mL volumetric flask. The extraction of the residual tissue pellet was repeated with another 9 mL of methanol. After centrifugation (3700 rpm × 10 min), the supernatants were combined into a final volume of 20 mL with methanol. The volume of 15 mL was concentrated to 5 mL to improve the sensitivity of the analyses. One aliquot of 500 µL was filtered through 0.22 µm filter and then analysed by UPLC-MS/MS.

2.3. UPLC-MS Analysis

Analyses were performed by a 1290 Infinity ultra-high-performance liquid chromatography system coupled to an Agilent G6460C Triple Quadrupole mass spectrometer equipped with an Agilent Jet Stream ESI source (Agilent Technologies, Waldbronn, Germany). The toxins were separated using a column AQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 µm, Waters) at 40 °C. Mobile phase A was 100% water and B acetonitrile-water (95:5), both containing 50 mM formic acid and 2 mM ammonium formate. The gradient program with a flow rate of 0.4 mL/min was started with 30% B and then a linear gradient to 70% B in 3 min. After an isocratic hold time linear of 1.5 min at 70% B and return to the starting conditions of 30% B in 0.1 min. Finally, 30% B was kept for 1.99 min before the next injection. The samples in the autosampler were cooled to 4 °C and the injection volume was 5 µL. Source conditions were: 350 °C of drying gas temperature with 8 L/min flow, nebulizer gas pressure of 45 psi (Nitrocrafter NCLC/MS from Air Liquid, Madrid, Spain), sheath gas temperature of 400 °C and a flow of 11 L/min. The capillary voltage was set to 4000 V in negative mode with a nozzle voltage of 0 V and 3500 V in positive mode with a nozzle voltage of 500 V. Analysis were performed in multiple reaction monitoring (MRM) acquisition mode, selecting two transitions per molecule. The MS/MS transitions for the screening of lipophilic toxins were previously described [9]. Certified reference materials were provided by Cifga (Lugo, Spain) and The Institute for Marine Biosciences, National Research Council, Halifax, NS, Canada.

3. Results

Chromatograms of dietary supplements of *P. canaliculus* showed the existence of two peaks in the minute 2.8 and minute 3. Figure 1 represent the total ion chromatogram of a sample intended for human consumption.

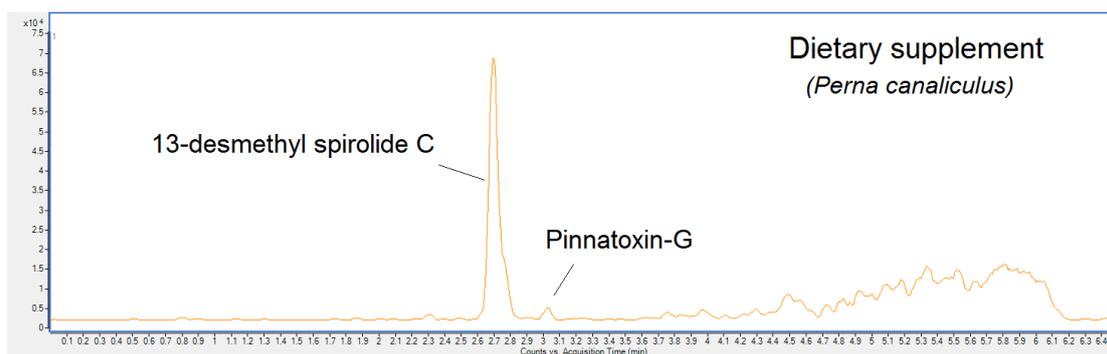


Figure 1. Total ion chromatogram (TIC) of dietary supplements (Green lipped mussels) for humans.

Analysing these peaks by multiple reaction monitoring (MRM) mode and using two transitions per molecule, it was observed that the first peak correspond to 13-desmethyl spirolide C (Figure 2) at levels around 90 µg/kg (dry product) and the second peak is pinnatoxin G (Figure 3) in trace amounts. These analyses confirm the presence of PnTXs in dietary supplements of *P. canaliculus*. The maximum level of 13-desmethyl spirolide C in these supplements was 98 µg/kg. Both toxins belong to the cyclic imine (CI) group, toxins which are not under legislation.

13-desmethyl spirolide C was quantified by an external standard calibration and considering the matrix effect which were calculated using matrix in the absence of toxins as a blank sample. There was a negative effect which entailed a suppression of signal (−2.5%). Linearity was assessed by calibration curves (nine points) prepared in both methanol and matrix within the range of 0.1 ng/mL to 25 ng/mL, obtaining a proper adjusted linear regression in both cases ($r^2 \geq 0.998$).

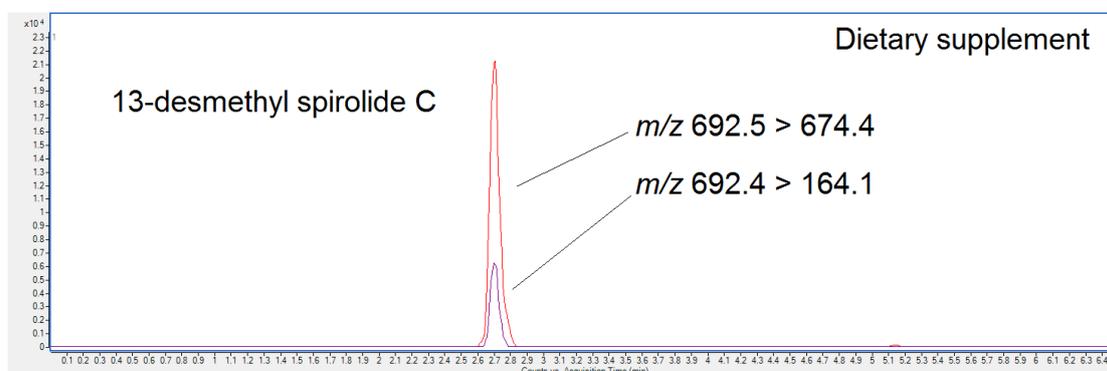


Figure 2. 13-desmethyl spirolide C analysis by Multiple Reaction Monitoring Chromatogram (MRM) from dietary supplements (Green lipped mussels) for humans.

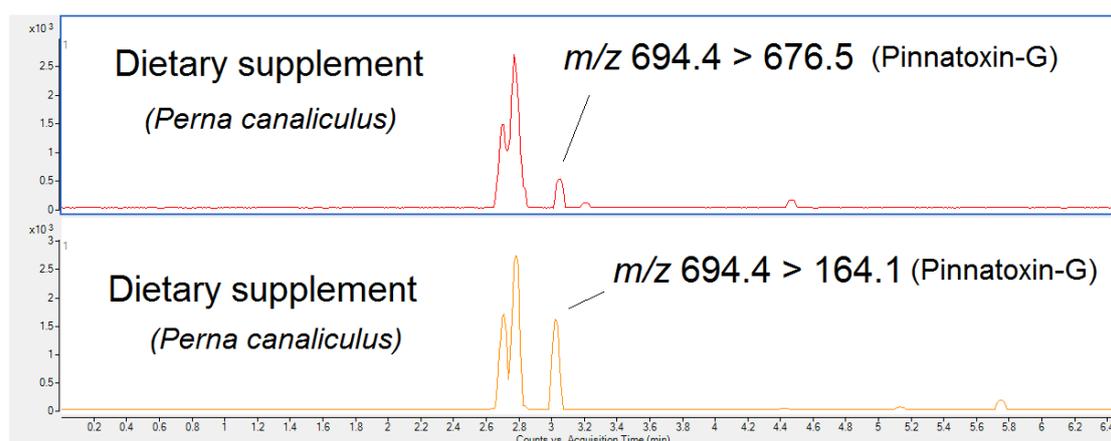


Figure 3. Pinnatoxin-G analysis by Multiple Reaction Monitoring (MRM) (two channels) from dietary supplements (Green lipped mussels) for humans.

Food supplement samples of green lipped mussels for dogs showed just the presence of 13-desmethyl spirolide C ranging from 33 to 39 $\mu\text{g}/\text{kg}$ (dry product).

4. Conclusions

Despite the fact that several toxic substances have been recorded in marketed samples of food supplements, these samples constitute the first reported analyses of emerging marine CI toxins in mussel-based supplements. 13-desmethyl spirolide C was found in dietary products intended for both human and animal consumption at levels up to 98 $\mu\text{g}/\text{kg}$ and 39 $\mu\text{g}/\text{kg}$, respectively. Besides, pinnatoxin-G was confirmed at trace levels. Moreover, there is no other information regarding the presence of CIs in fresh *P. canaliculus*.

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Conflicts of Interest: The authors declare no conflict of interest

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