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Application News

No. B102

Probe Electro Spray Ionization Mass Spectrometer

Direct Rapid Analysis of Tetrodotoxin Contained in Fugu Using DPiMS™-8060

Fugu (pufferfish) has long been a familiar to the Japanese as high-quality food fish, and is now consumed in other countries as well. Even though it is widely known that certain parts and species of fugu contain the deadly poison tetrodotoxin (TTX, also called fugu poison), incidents of fugu poisoning occur frequently due to careless control.

Establishment of a quick and simple detection method for TTX originating from fugu has been strongly desired, not only for sites involved in food hygiene and quality control, but also for general consumers, who have a heightened awareness of food safety.

This article introduces a quick TTX analysis method using the new Shimadzu DPiMS-8060 mass spectrometer (Fig. 1), which combines the new ionization method called probe electro spray ionization (PESI) and a tandem-type mass spectrometer. A direct rapid analysis method for TTX contained in fugu which does not require pretreatment is also introduced. This method is applicable not only to the liver and ovaries of poisonous fugu, which are widely known to contain TTX, but also to the skin and muscles, which may contain TTX depending on the species.

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Measurement of Tetrodotoxin Standard Sample

As a standard sample, Tetrodotoxin, from fugu (FUJIFILM Wako Pure Chemical Corporation) was prepared in a 50 % ethanol solution, and 10 µL of the sample solution was injected in the dedicated liquid sample plate of the DPiMS-8060 and measured.

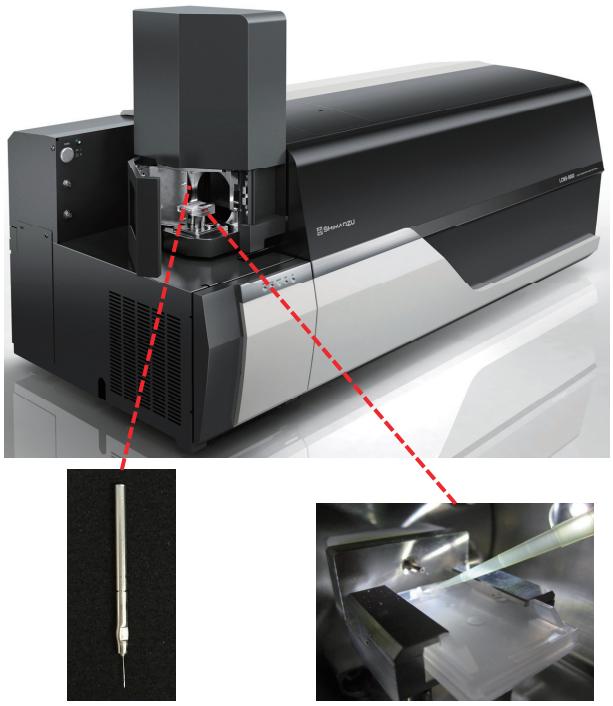
A product ion scan was carried out, conditions which enable confirmation of the characteristic fragment ion *m/z* 162.1 of TTX (Fig. 2) were studied, and the conditions shown in Table 1 were set. The results obtained by the product ion scan are shown in Fig. 3.

Next, 1, 5, 25, 50, 100, and 300 ng/mL of the TTX standard sample were prepared. The samples were measured under the MRM (Multiple Reaction Monitoring) condition, and a calibration curve was prepared.

Based on the results, the detection limit and the quantitative lower limit of TTX by DPiMS-8060 analysis were calculated. The calibration curves and the values of these limits are shown in Fig. 4.

Table 1 TTX Analysis Conditions for DPiMS-8060

Collision Energy	: -30 V
MRM Transition	: *m/z* : 320.2 > 162.1 (Monitoring conducted using proton adduct as precursor ion.)
Survey Event : Product Ion Scan MS Range	: *m/z* : 100-370
Scan Speed	: 5,000 u/sec
Event Time	: 0.06 sec
Desolvation Line	: 250 °C
Heat Block	50 °C
Polarity	: Positive
Acquisition time	: 0.5 min



Probe (Tip diameter 700 nm) Sample plate for liquids

Fig. 1 DPiMS™-8060

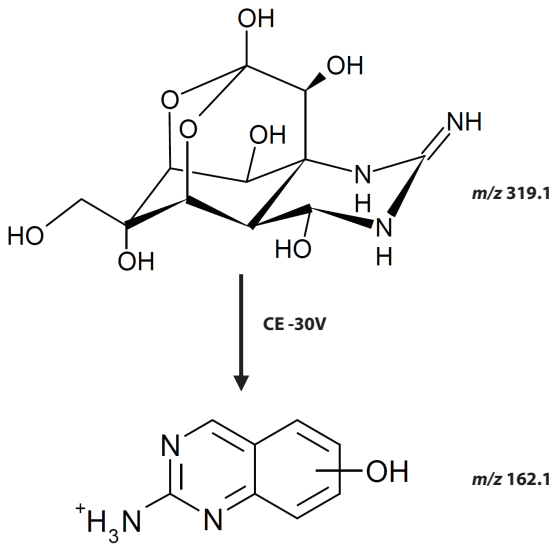


Fig. 2 TTX and Fragment Ion

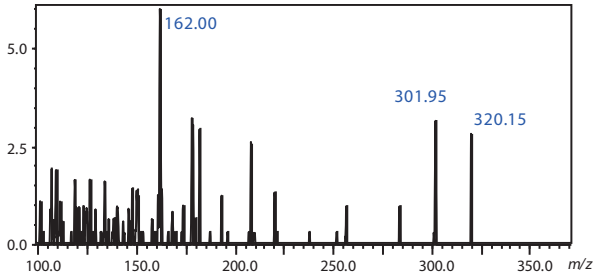


Fig. 3 Product Ion Scan of TTX Standard Sample

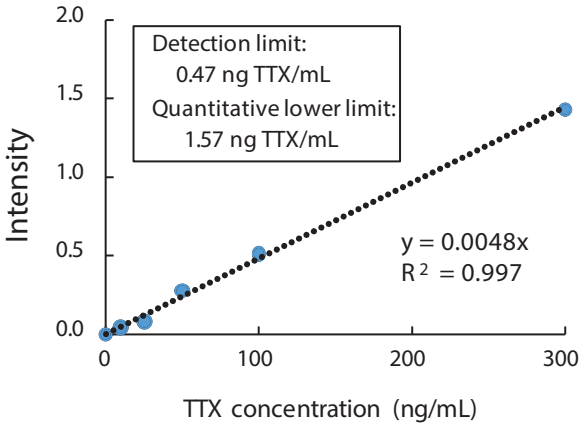


Fig. 4 Calibration Curve of TTX Standard Sample

*Values shown here are reference values and are not guaranteed values.

Detection of TTX Contained in Fugu

Real samples approximately 3 mm square were taken from the muscle, skin, liver, and ovary of the finepatterned puffer (*Takifugu poecilonotus*, Fig. 5), which is one species of poisonous fugu. The samples were inserted in the dedicated biological sample plate of the DPiMS-8060, 35 µL of the 50 % ethanol solution was dripped on the top part as an ionization accelerator, and a product ion scan was conducted. The results are shown in Fig. 6. Fragment ions of TTX were detected from all of the tissues. Furthermore, the fact that differences in detection sensitivity could also be seen, depending on the part, suggested that the magnitude of the TTX concentration contained in the respective parts of poisonous fugu can be measured simply without pretreatment by using the DPiMS-8060.

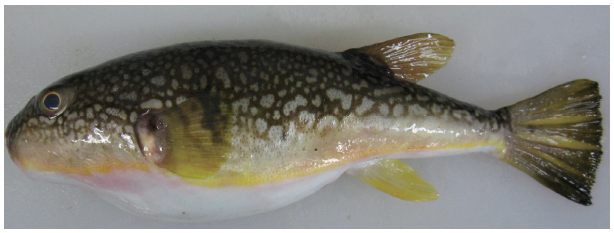


Fig. 5 Finepatterned Puffer

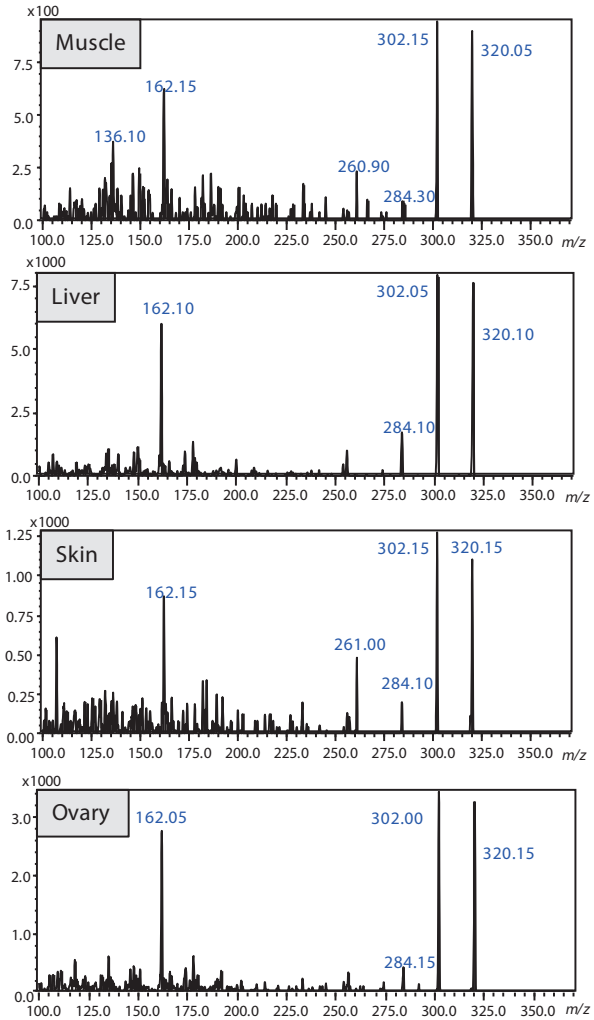


Fig. 6 Product Ion Scans of Various Parts of Poisonous Fugu (Finepatterned Puffer)

Conclusion

As a result of an analysis of a standard sample of tetrodotoxin (TTX), which is the deadly poisonous component contained in the tissue of fugu fish, it was shown that simple and high sensitivity mass spectrometry in analysis of TTX is possible by using the Shimadzu DPiMS-8060, even though analysis of this high polarity component by the conventional LCMS method tends to be complicated, for example, requiring sample pretreatment.

Moreover, quick and simple detection of TTX in fugu tissues was possible without pretreatment. This suggested the possibility that the DPiMS-8060 may become an effective analytical method in the field of inspections for protection of food safety.

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