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Application News

High Performance Liquid Chromatograph Mass Spectrometer

Analysis of Cereulide in Food Using Triple Quadrupole Mass Spectrometer

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User Benefits

- This method enables the rapid analysis of cereulide, the emetic toxin responsible for *Bacillus cereus* foodborne illness.
- Good quantification over a wide concentration range was achieved with the developed method.
- This analytical method achieves good recovery rates with a simple pretreatment.

■ Introduction

Bacillus cereus is a Gram-positive spore-forming rod that commonly contaminates various agricultural products and causes foodborne illness. Its vegetative cells and spores are frequently found in boiled or fried rice, pasta, vegetables, and dairy products[1].

B. cereus is known to cause two types of foodborne illnesses: diarrheal and emetic. The emetic type is caused by cereulide, a toxin produced by the bacterium. Cereulide is resistant to heat, acids, alkalis, and digestive enzymes, and due to its ability to easily proliferate at room temperature, there have been several reports of its detection in fried rice and pasta.

The measurements of vacuolation degeneration activity using HEp-2 cells and PCR are known methods for the detection of cereulide. However, these methods require several days of cell culture or cannot confirm the production of cereulide itself. Therefore, in recent years, there has been a growing demand for analytical methods that directly detect cereulide using LC-MS/MS[2].

We have developed a pretreatment method using methanol as an extraction solvent and an analytical method for cereulide in fried rice using an UHPLC-based LC-MS/MS (Fig. 1).

Fig. 1 Nexera™ and LCMS-8060RX

■ Sample Preparation and Analytical Conditions

The pretreatment method (Fig.2) was performed with reference to the Annual Report of Kumamoto Prefectural Institute of Public - Health and Environmental Science[3].

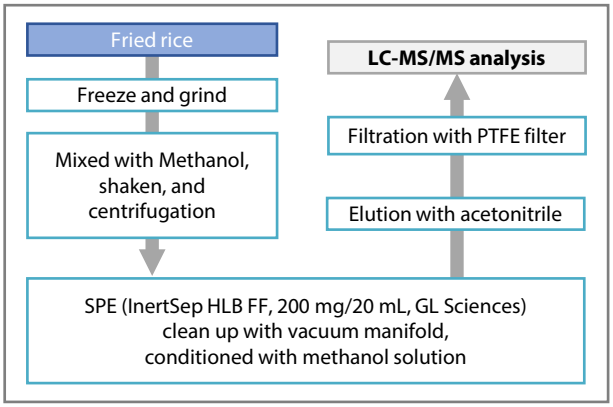

Fig. 2 Workflow of pretreatment

Fried rice sample preparation: 1 g of frozen pulverized fried rice was mixed with 10 mL of methanol and shaken for 5 min. After centrifugation at 3,000 rpm for 5 min, the supernatant was collected by decantation. The residue was mixed with 5 mL of methanol and subjected to 5 min of shaking treatment. After centrifugation at 3,000 rpm for 5 min, it was added to the extract and adjusted to a final volume of 15 mL with methanol.

SPE cleanup: The SPE cartridges (InertSep HLB FF, 200 mg/20 mL, GL Sciences), which have large particle sizes in the packing material and improved flowability to reduce clogging, were used to remove lipid components from the extracts of fried rice. Using the vacuum manifold, the SPE cartridges were conditioned with 10 mL of 75% methanol solution. The fried rice extract was then passed through an SPE cartridge and eluted with 10 mL of acetonitrile.

LC-MS/MS analysis: The collected eluate was filtered with a PTFE filter (TORAST Disc, 13 mm, 0.22 µm) and the solution was analyzed by LC-MS/MS. In this study, the LCMS-8060RX was used for analysis. The analytical conditions are shown in Table 1.

The LCMS-8060RX is equipped with the new CoreSpray technology, which enables a more consistent nebulizer flow compared to previous systems.

Table 1 LC-MS/MS conditions

[HPLC conditions] (Nexera X3)		
Column	:	Shim-pack Scepter™ C18-120*1 (50 mm x 2.1 mm I.D., 1.9 µm)
Mobile phase A	:	10 mM ammonium formate / 0.1% formic acid / water
Mobile phase B	:	Acetonitrile
Flow rate	:	0.4 mL/min
Gradient program	:	B conc. 80% – 95% (6-10 min) – 80% (10.01-15 min)
Column temp.	:	40°C
Injection volume	:	3 µL
[MS conditions] (LCMS-8060RX)		
Ionization	:	ESI, Positive mode
Mode	:	MRM (*m/z* 1170.70 > 357.30)
Interface voltage	:	+1 kV
Focus voltage	:	+2 kV
Nebulizing gas flow	:	2.0 L/min
Drying gas flow	:	5.0 L/min
Heating gas flow	:	15.0 L/min
Interface temp.	:	300°C
DL temp.	:	300°C
Heat Block temp.	:	500°C
Probe position	:	+1 mm

*1 P/N：227-31012-03

■ Calibration Curve and Mass Chromatograms

The calibration curve is shown in Fig. 3. Additionally, Fig. 4 shows the Mass chromatograms of cereulide in the standard and recovery test samples. A calibration curve with good linearity (R^2 > 0.999) in the range of 0.005 to 50 µg/L (converted in food: 0.05-500 ng/g) was obtained.

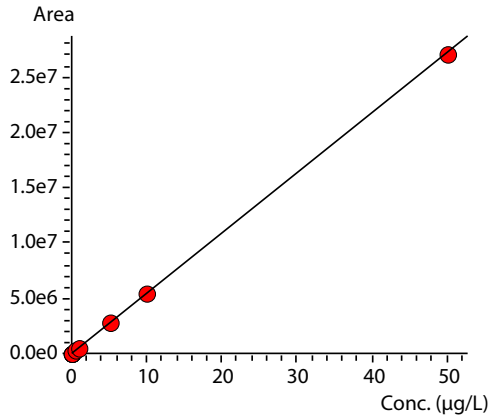

Fig. 3 Calibration curve of cereulide

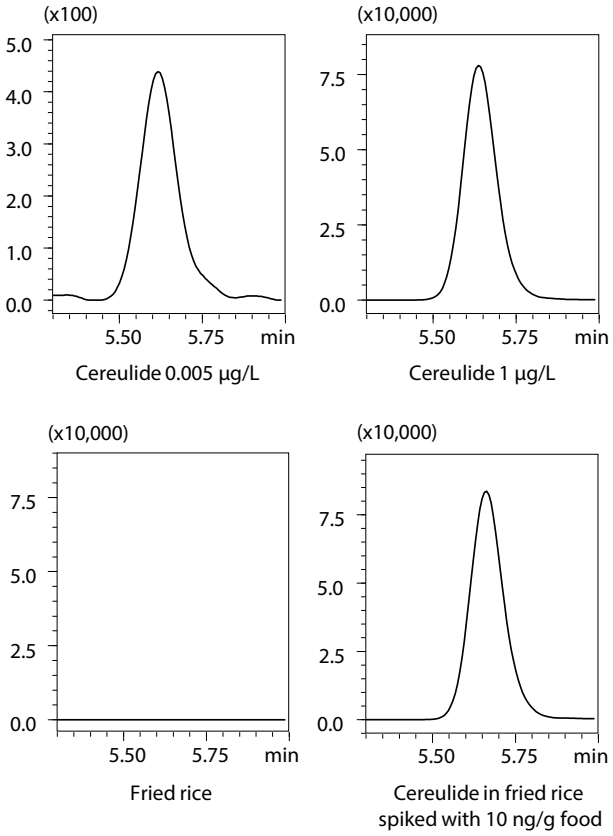

Fig. 4 Mass chromatograms of cereulide

■ Quantification and Recovery Test

Determination of cereulide was performed on fried rice. A spike and recovery test was also conducted using cereulide-free fried rice. The amount of cereulide standard solution was added to reach 10 ng/L (converted in food: 100 ng/g). A sample of 1 g of frozen, pulverized fried rice to which cereulide was added, extracted, and purified in solid phase was analyzed by the LCMS-8060RX, and a good recovery rate of 110.1% was obtained.

Table 2 Recovery rate

Food	Recovery rate (%)
Fried rice	110.1

■ Conclusion

The analytical method for cereulide using LC-MS/MS achieves analysis in a short time of 15 minutes. Therefore, compared to conventional methods such as measuring vacuolation degeneration activity using HEp-2 cells and PCR, this method allows for rapid processing from sample pretreatment to measurement and analysis.

The recovery test was conducted at 10 ng/g, which is lower than the level reported in a food poisoning incident, using the implicated food as a reference. Using the optimized analytical method, a good recovery rate within the range of 80-120% was achieved in fried rice with simple pretreatment.

<References>

1) U.S. Food and Drug Administration (2020). Bacteriological Analytical Manual Chapter 14: Bacillus cereus
2) Julien Masquelier et al. (2023). Validation of a Targeted LC–MS/MS Method for Cereulide and Application in Food and Faeces: Toxins, 16(1), 13
3) Kazuma Yagi et al. (2021). Development of rapid analytical method for cereulide by LC-MS/MS: Annual Report of Kumamoto Prefectural Institute of Public-Health and Environmental Science, No.51

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