SHIMADZU
Excellence in Science

Liquid Chromatograph Mass Spectrometer

Application News

# Analysis of Cereulide in Reconstituted Infant Formula Using Triple Quadrupole LC-MS/MS

Nozomi Maeshima, Saho Yoshioka

## User Benefits

- Accurate quantification is achieved even at the strict safety levels of the European Food Safety Authority (EFSA).
- Highly accurate and reproducible quantification without an internal standard was confirmed for reconstituted infant formula, using pretreatment methods based on ISO 18465 and the QuEChERS approach.

## ■ Introduction

Cereulide is a heat-stable emetic toxin produced by *Bacillus cereus*. Since even the ingestion of small amounts raises concerns about health effects, appropriate management of food is required. In particular, infants may experience vomiting and diarrhea from ingesting very small amounts. Therefore, the safety requirements for infant formula are extremely high, necessitating analytical methods capable of reliable detection and quantification even at low concentration ranges.

The European Food Safety Authority (EFSA) proposed an acute reference dose (ARfD) of 0.014 µg/kg body weight for infants in a rapid risk assessment, indicating that cereulide concentrations exceeding 0.054 µg/L in reconstituted infant formula may surpass safety thresholds[1]. However, since infant formula is a complex matrix rich in lipids and proteins, selecting an appropriate sample preparation method is crucial.

This Application News introduces an example of analyzing cereulide in infant formula using triple quadrupole LC-MS/MS, comparing sample preparation methods based on ISO 18465 and the QuEChERS approach. Spike-and-recovery tests yielded good recoveries for both methods, but the QuEChERS approach demonstrated a higher recovery rate.

## ■ Sample and Preparation

### Sample

A commercially available infant formula, reconstituted according to the manufacturer's instructions, was used as the sample.

### Method based on ISO 18465

First, 2.5 mL of the sample was placed in a 50 mL tube and mixed with 30 mL of acetonitrile. After shaking for 1 hour on a shaker, the mixture was centrifuged at 3,000 rpm for 5 minutes. The collected supernatant was filtered using a TORAST Disc (hydrophilic PTFE, 13 mm diameter, 0.45 µm pore size, P/N: GLCTD-HPTFE1345) to prepare the sample for LC-MS/MS analysis. The sample preparation flow is shown in Fig. 1 (a).

### QuEChERS Method

First, 2 mL of the sample was placed in a 50 mL tube, and 8 mL of water, 150 µL of formic acid, and 10 mL of acetonitrile were added, followed by shaking for 10 seconds. One extraction salt packet (Supel QuE, P/N: 55295-U) was added, and the tube was immediately shaken vigorously by hand for 10 seconds. After shaking for 5 minutes on a shaker, the mixture was centrifuged at 3,000 rpm for 5 minutes. The collected supernatant was used as the sample for LC-MS/MS analysis. The sample preparation flow is shown in Fig. 1 (b).

## ■ Analytical Conditions

Quantitative analysis was performed using a triple quadrupole mass spectrometer LCMS-8060RX coupled with a Nexera™ X3 UHPLC system. A Shim-pack Scepter™ was used as the analytical column, and the analysis was carried out with a 13-minute method including column washing and equilibration. The analytical conditions are shown in Table 1, and the MRM conditions in Table 2. The transitions recommended in ISO 18465 were used for quantification.

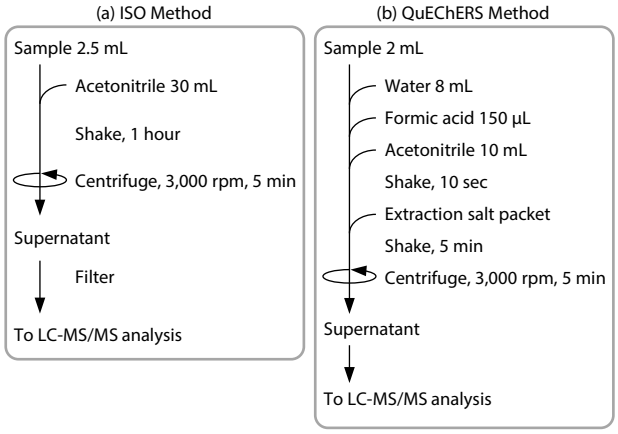


Fig. 1 Sample Preparation Flow

Table 1 LC-MS/MS Analytical 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% (0-6min) → 95% (6-10 min) → 80% (10.01-13 min) The flow was introduced into the mass spectrometer between 3 to 8 min using a flow switching valve. |
| Column temp. | : | 40˚C |
| Injection volume | : | 5 µL |
| MS conditions (LCMS-8060RX) | | |
| Ionization | : | ESI, Positive mode |
| Mode | : | MRM |
| 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

Table 2 MRM Transitions

| Quantifier Ion | Qualifier Ion |
|---|---|
| 1170.70>314.15 | 1170.70>172.05 |
| | 1170.70>357.30 |
| | 1170.70>499.10 |

## ■ Spike-and-Recovery Test

As sample preparation methods for quantifying cereulide in reconstituted infant formula, a method based on ISO 18465 and the QuEChERS method were compared.

To evaluate the validity of both methods, spike-and-recovery tests were conducted using each method. For the spiked samples, a cereulide standard was added to achieve a concentration of 0.054 µg/L in the reconstituted infant formula, based on the risk assessment criteria proposed by EFSA. After adding the standard, the samples were prepared using each method, and analysis was conducted using triple quadrupole LC-MS/MS.

Quantification was carried out by the external standard method, and a calibration curve was constructed in the range of 0.002 to 0.1 µg/L (Fig. 2). The accuracy of all calibration points ranged from 98.1% to 105.1%.

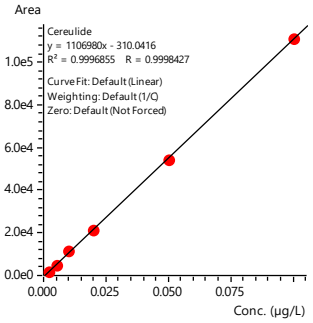


Fig. 2 Calibration Curve Using Cereulide Standard

The final concentration of the prepared sample (in the vial) was 0.0045 µg/L for the ISO 18465-based method and 0.0108 µg/L for the QuEChERS method. Samples were prepared in triplicate (n = 3) for each method to confirm reproducibility. The results are shown in Table 3. The recovery rates were 90.6% for the ISO 18465-based method and 100.1% for the QuEChERS method. The reproducibility (%RSD) values were 9.7% and 2.3%, respectively. Mass chromatograms of cereulide in the standard solution and spiked samples are shown in Fig. 3.

Table 3 Comparison of Spike-and-Recovery Test Results using ISO and QuEChERS Methods

| | | ISO Method | QuEChERS Method |
|---|---|---|---|
| Pre-spiked sample | Recovery (n=3) | 90.6 | 100.1 |
| | %RSD | 9.7 | 2.3 |
| Post-spiked sample | Recovery (n=1) | 109.3 | 108.7 |

In addition, the matrix effect on the ionization of cereulide was evaluated. The recovery rates of post-spiked samples—prepared by adding the standard to unspiked sample extracts just before LC-MS/MS analysis—were determined. As a result, the recovery rates for both sample preparation methods ranged from 108.7% to 109.3% (Table 3).

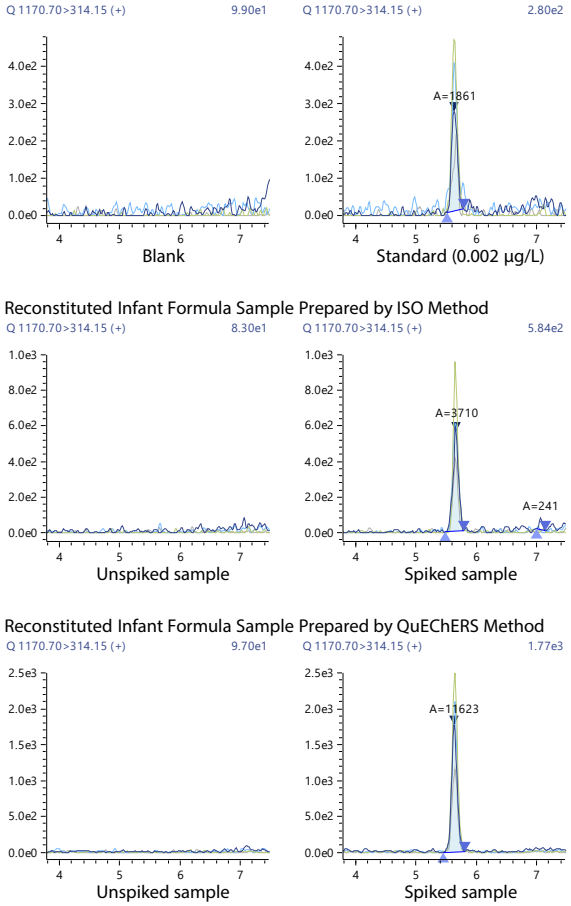


Fig. 3 Mass Chromatograms of Cereulide

## ■ Conclusion

This Application News introduced methods capable of highly sensitive and accurate quantification of cereulide in reconstituted infant formula using a triple quadrupole LC-MS/MS system. Spike-and-recovery tests conducted using a method based on ISO 18465 and the QuEChERS method yielded good recovery rates ranging from 90.6% to 100.1%. These methods enable the accurate quantification of cereulide even at the low concentration of 0.054 µg/L, which is the safety standard proposed by EFSA.

<References>

1) European Food Safety Authority (EFSA), Eskes C, *et al*. Rapid risk assessment on acute reference dose (ARfD) of cereulide in infants and information on acute consumption of infant formulae. EFSA Journal, 2026.
https://doi.org/10.2903/j.efsa.2026.9941
2) ISO 18465. Microbiology of the food chain - Quantitative determination of emetic toxin (cereulide) using LC-MS/MS. 2017.



01-01209-EN First Edition: May 2026

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

› Please fill out the survey

## Related Products Some products may be updated to newer models.


› LCMS-TQ RX Series Triple Quadrupole LC-MS/MS
Triple Quadrupole LC-MS/MS

## Related Solutions

› Food and Beverages

› Food Contamination

› Price Inquiry

› Product Inquiry

› Technical Service / Support Inquiry

› Other Inquiry