
**Microbiology of the food chain —
Quantitative determination of emetic
toxin (cereulide) using LC-MS/MS**

*Microbiologie de la chaîne alimentaire — Détermination quantitative
de la toxine émétique (céreulide) par CL-SM/SM*

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Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 General principle	1
5 Reagents	1
6 Apparatus and equipment	3
7 Procedure	4
7.1 Sample preparation	4
7.2 Standard preparation	4
7.3 LC-MS analysis.....	5
7.3.1 LC conditions.....	5
7.3.2 MS conditions and tuning parameters.....	5
7.3.3 Transitions (multiple reaction monitoring, MRM).....	6
8 Calculation	6
9 Quality controls	7
10 Precision	8
10.1 General.....	8
10.2 Repeatability.....	8
10.3 Reproducibility.....	9
Annex A (informative) Results of the interlaboratory study	10
Annex B (informative) Possible transitions of cereulide in MS^[3]	12
Bibliography	14

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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Introduction

Cereulide, the emetic toxin produced in foods by certain strains of *Bacillus cereus*, is a heat and acid stable toxin that causes nausea and vomiting when ingested. In very rare cases, people can die after ingestion of the toxin. Due to its stability, the toxin may still be present even when *B. cereus* can no longer be detected. The presence of cereulide seems to be linked to starch-rich foods like rice (dishes) and pasta (dishes). However, recent data suggest that the occurrence of food borne outbreaks due to cereulide is more common to foods in general[9]. The toxin has a cyclic structure and consists of in total 12 monomers as a repeat of (D-O-Leucine-D-Alanine-L-O-Valine-L-Valine). Several methods have been developed for the detection and/or quantification of the toxin. Some of these methods are nonspecific bio-assays[3, 4] and other methods are specifically based on the chemical analysis using liquid chromatography with mass spectrometry (LC-MS/MS) for the detection and quantification of the toxin[5, 6, 7, 8]. The chemical methods are more specific for cereulide and have, therefore, been chosen as the starting point for standardization of a method for the quantification of cereulide. Recently, research has been done for the chemodiversity of cereulide. At least 18 cereulide variants were detected by UHPLC-TOFMS and ion-trap MSⁿ sequencing, among which the previously unknown isocereulides A–G[10].

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Microbiology of the food chain — Quantitative determination of emetic toxin (cereulide) using LC-MS/MS

1 Scope

This document describes the quantitative analysis of the emetic toxin cereulide using high performance liquid chromatography (HPLC) or ultra performance liquid chromatography (UHPLC) connected to a tandem mass spectrometer (LC-MS/MS).

This document is applicable to the analysis of the toxin in products intended for human consumption.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

cereulide

toxin cyclo[D-O-Leucine-D-Alanine-L-O-Valine-L-Valine]₃ produced by certain strains of the species of *B. cereus*

4 General principle

Cereulide is extracted from the food matrix by shaking the sample with acetonitrile. ¹³C₆-Cereulide is used as an internal standard. The components in the solution are separated using HPLC or UHPLC and subsequently detected using tandem mass spectrometry (LC-MS/MS). For MS, the electro spray ionization technique (ESI) is used, using the positive mode. The level of emetic toxin (cereulide) is expressed as µg cereulide/kg product.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Water, according to ISO 3696.

5.2 Acetonitrile, LC-MS grade.