



**SLOVENSKI STANDARD**  
**SIST EN ISO 18465:2017**

**01-april-2017**

---

**Mikrobiologija v prehranski verigi - Kvantitativno določanje emetičnih toksinov (cereulide) z uporabo LC-MS/MS (ISO 18465:2017)**

Microbiology of the food chain - Quantitative determination of emetic toxin (cereulide) using LC-MS/MS (ISO 18465:2017)

Mikrobiologie der Lebensmittelkette - Quantitative Bestimmung von emetischem Toxin (Cereulid) mittels LC-MS/MS (ISO 18465:2017)

Microbiologie de la chaîne alimentaire - Détermination quantitative de la toxine émétique (céréulide) par CL-SM/SM (ISO 18465:2017)

<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>

**Ta slovenski standard je istoveten z: EN ISO 18465:2017**

---

**ICS:**

07.100.30      Mikrobiologija živil      Food microbiology

**SIST EN ISO 18465:2017**      en

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 18465:2017](#)

<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>

EUROPEAN STANDARD

EN ISO 18465

NORME EUROPÉENNE

EUROPÄISCHE NORM

February 2017

ICS 07.100.30

English Version

Microbiology of the food chain - Quantitative  
determination of emetic toxin (cereulide) using LC-MS/MS  
(ISO 18465:2017)

Microbiologie de la chaîne alimentaire - Détermination  
quantitative de la toxine émétique (céréulide) par CL-  
SM/SM (ISO 18465:2016)

Mikrobiologie der Lebensmittelkette - Quantitative  
Bestimmung von emetischem Toxin (Cereulid) mittels  
LC-MS/MS (ISO 18465:2017)

This European Standard was approved by CEN on 28 December 2016.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

**iTeh STANDARD PREVIEW**

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

<b>Contents</b>	<b>Page</b>
<b>European Foreword.....</b>	<b>3</b>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 18465:2017](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017)  
<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>

## European Foreword

This document (EN ISO 18465:2017) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2017 and conflicting national standards shall be withdrawn at the latest by August 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

[SIST EN ISO 18465:2017](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b7502/sist-en-iso-18465-2017)

[https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b7502/sist-en-iso-18465-2017)

[77a0ab7b7502/sist-en-iso-18465-2017](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b7502/sist-en-iso-18465-2017)

### Endorsement notice

The text of ISO 18465:2017 has been approved by CEN as EN ISO 18465:2017 without any modification.

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 18465:2017](#)

<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>

INTERNATIONAL  
STANDARD

ISO  
18465

First edition  
2017-01

---

---

**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*

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 18465:2017](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017)

<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>



Reference number  
ISO 18465:2017(E)

© ISO 2017

## iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST EN ISO 18465:2017](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017)

<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>



### **COPYRIGHT PROTECTED DOCUMENT**

© ISO 2017, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
Ch. de Blandonnet 8 • CP 401  
CH-1214 Vernier, Geneva, Switzerland  
Tel. +41 22 749 01 11  
Fax +41 22 749 09 47  
copyright@iso.org  
www.iso.org



# Contents

Page

Foreword.....	iv
Introduction.....	v
<b>1 Scope.....</b>	<b>1</b>
<b>2 Normative references.....</b>	<b>1</b>
<b>3 Terms and definitions.....</b>	<b>1</b>
<b>4 General principle.....</b>	<b>1</b>
<b>5 Reagents.....</b>	<b>1</b>
<b>6 Apparatus and equipment.....</b>	<b>3</b>
<b>7 Procedure.....</b>	<b>4</b>
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
<b>8 Calculation.....</b>	<b>6</b>
<b>9 Quality controls.....</b>	<b>7</b>
<b>10 Precision.....</b>	<b>8</b>
10.1 General.....	8
10.2 Repeatability.....	8
10.3 Reproducibility.....	9
<b>Annex A (informative) Results of the interlaboratory study.....</b>	<b>10</b>
<b>Annex B (informative) Possible transitions of cereulide in MS<sup>3</sup>.....</b>	<b>12</b>
<b>Bibliography.....</b>	<b>14</b>

## ISO 18465:2017(E)

### 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://www.iso.org/iso/foreword.html).

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food Analysis — Horizontal methods*, in collaboration with ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

## 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<sup>[9]</sup>. 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<sup>[3, 4]</sup> 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<sup>[5, 6, 7, 8]</sup>. 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 MS<sup>n</sup> sequencing, among which the previously unknown isocereulides A-G<sup>[10]</sup>.

## iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST EN ISO 18465:2017](https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017)

<https://standards.iteh.ai/catalog/standards/sist/44d453e5-0ca5-4af3-a4f1-77a0ab7b75f2/sist-en-iso-18465-2017>