
**Milk, milk products and infant
formula — Determination of
melamine and cyanuric acid by liquid
chromatography and tandem mass
spectrometry (LC-MS/MS)**

*Lait, produits laitiers et formule infantile — Détermination de la
teneur en mélamine et en acide cyanurique par chromatographie en
phase liquide couplée à la spectrométrie de masse en tandem (CL-SM/
SM)*



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Contents

Page

Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents	1
5.1 List of reagents	1
5.2 Preparation of stock solutions	2
5.3 Preparation of standard solutions	3
5.4 Preparation of calibration solutions	4
6 Apparatus	5
7 Procedure	6
7.1 Sample preparation	6
7.2 Extraction	6
7.2.1 General	6
7.2.2 Extraction procedure	6
7.3 Determination	6
8 System settings	7
8.1 HPLC parameters	7
8.2 HPLC-MS/MS parameters	7
8.3 UHPLC parameters	9
8.4 UHPLC-MS/MS parameters	9
8.5 Preparation of the LC-MS/MS system and samples	10
8.5.1 Tuning of the LC-MS/MS system	10
8.5.2 Checking of the instrument settings	10
8.5.3 Checking of the sensitivity of the system	10
8.5.4 Sample list	11
9 Calculation and expression of results	11
9.1 General	11
9.2 Calibration criteria	11
9.3 Identification criteria	11
9.4 Recovery	12
9.5 Calculation of results	13
10 Precision data	14
10.1 General	14
10.2 Repeatability	14
10.3 Reproducibility	14
11 Test report	15
Annex A (informative) Precision data	16
Bibliography	18

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).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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Milk, milk products and infant formula — Determination of melamine and cyanuric acid by liquid chromatography and tandem mass spectrometry (LC-MS/MS)

1 Scope

This document specifies a method for the determination of melamine and cyanuric acid with liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS). The method has been validated in an interlaboratory study via the analysis of spiked samples of milk-based infant formula, soy-based infant formula, milk powder, whole milk, soy drink and milk chocolate ranging from 0,71 mg/kg to 1,43 mg/kg for melamine and 0,57 mg/kg to 1,45 mg/kg for cyanuric acid. The limits of quantification (LOQ) for melamine and cyanuric acid in food are 0,05 mg/kg and 0,25 mg/kg, respectively. The upper limit of the working range is up to 10 mg/kg for melamine and up to 25 mg/kg for cyanuric acid.

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*

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

4 Principle

A test portion of the homogenous food sample is fortified with ^{13}C labelled internal standards (melamine and cyanuric acid). After incubation for at least 1 h, water is added to the sample and, after shaking, the slurry is dissolved in a mixture of acetonitrile and water. The sample is shaken and centrifuged. After the separation of supernatant from sediments, benzoguanamine is added as a recovery standard. An aliquot of the aqueous supernatant is injected into a LC-MS/MS system. The triple quadrupole mass spectrometer is coupled either to high performance liquid chromatography (HPLC) or to ultra high performance liquid chromatography (UHPLC). Chromatography is based on hydrophilic interaction liquid chromatography (HILIC). Ionization is achieved by electrospray ionization (ESI) in multiple reaction monitoring (MRM).

5 Reagents

5.1 List of reagents

Use only reagents of recognized analytical grade and water conforming to grade 1 of ISO 3696, unless otherwise specified. Use only reagents with purity suitable for melamine and cyanuric acid analysis.