
Krma: metode vzorčenja in analize - Določevanje melamina in cianurne kisline z metodo tekočinske kromatografije z masno spektrometrično detekcijo (LC-MS/MS)

Animal Feeding stuffs: Methods of sampling and analysis - Determination of melamine and cyanuric acid content by liquid chromatographic method with mass spectrometric detection (LC-MS/MS)

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung des Gehaltes von Melamin und Cyanursäure mittels flüssigkeitschromatographischen Verfahrens mit massenspektrometrischem Nachweis (LC-MS/MS)

Aliments des animaux : Méthodes d'échantillonnage et d'analyse - Détermination des teneurs en mélamine et en acide cyanurique par chromatographie liquide couplée à la spectrométrie de masse (LC-MS/MS)

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Animal Feeding stuffs - Methods of sampling and analysis - Determination of melamine and cyanuric acid content by liquid chromatographic method with mass spectrometric detection (LC-MS/MS)

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d'analyse - Détermination des teneurs en mélanine et
en acide cyanurique par chromatographie liquide
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von Melamin und Cyanursäure mittels
flüssigkeitschromatographischem Verfahren mit
massenspektrometrischem Nachweis (LC-MS/MS)

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CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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EN 17212:2019 (E)**European foreword**

This document (EN 17212:2019) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs: Methods of sampling and analysis”, the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by March 2020, and conflicting national standards shall be withdrawn at the latest by March 2020.

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

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Introduction

This document was developed in response to the demand for a reliable method to detect and quantify melamine and cyanuric acid in feeding stuffs. Both substances were illegally mixed into feed.

WARNING — The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

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EN 17212:2019 (E)**1 Scope**

This document specifies a high-performance liquid chromatographic (HPLC) mass spectrometric (MS) method for screening and quantification of melamine and cyanuric acid in the concentration range between 1 mg/kg and 100 mg/kg feed.

The method is validated in an international collaborative trial for melamine in complete feed, complementary feed, feed material, milk replacer and pet food including canned pet food in the range between 1 mg/kg and 80 mg/kg with particular regard to the maximum level of 2,5 mg/kg as established by the European Commission.

Laboratory experiences have shown that the method is also applicable for cyanuric acid in the same concentration range in complete feed ($n = 7$), complementary feed ($n = 6$), feed material ($n = 7$, resp. 9), milk replacer ($n = 7$) and pet food ($n = 7$) including canned pet food.

Since the LC-MS/MS sensitivity for cyanuric acid is lower than for melamine, it has to be ensured that the LC-MS/MS system is in excellent working order. The method is applicable to feeding stuffs but not tested for pre-mixtures and feed additives.

Quantification of concentrations above 100 mg/kg is possible, but the method has to be validated by the operator.

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.

EN ISO 6497, *Animal feeding stuffs - Sampling (ISO 6497)*

EN ISO 6498:2012, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498:2012)*

EN ISO/IEC 17043:2010, *Conformity assessment — General requirements for proficiency testing (ISO/IEC 17043:2010)*

ISO 3534-2:2006, *Statistics — Vocabulary and symbols — Part 2: Applied statistics*

ISO 16577:2016, *Molecular biomarker analysis — Terms and definitions*

ISO 24276:2006, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

ISO/IEC GUIDE 99:2007, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

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

calibration

complete set of operations which estimates under specified conditions the calibration function from observations of the response variable obtained on reference states

[SOURCE: ISO 3534-2:2006, 3.5.13]

3.2

calibration function

functional relationship between the expected value of the response variable and the value of the net state variable

[SOURCE: ISO 3534-2:2006, 3.5.12]

3.3

canned pet food

feed product for pets which has been processed, packaged, sealed and sterilized for preservation in cans or similar containers

[SOURCE: EN ISO 6498:2012, 2.3.15]

3.4

collaborative trial

interlaboratory comparisons

organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions

Note 1 to entry: Interlaboratory comparisons are widely used for a number of purposes and their use is increasing internationally. Typical purposes for interlaboratory comparisons include the evaluation of the performance characteristics of a method – often described as collaborative trials [SOURCE: EN ISO/IEC 17043:2010, Introduction].

[SOURCE: EN ISO/IEC 17043:2010, 3.4]

3.5

complementary feed

compound feed which has a high content of certain substances but which, by reason of its composition, is sufficient for a daily ration only if used in combination with other feed

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1j)] [1]

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EN 17212:2019 (E)**3.6****complete feed**

compound feed which, by reason of its composition, is sufficient for a daily ration

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1i)] [1]

3.7**compound feed**

mixture of at least two feed materials, whether or not containing feed additives, for oral animal feeding in the form of complete or complementary feed

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1h)] [1]

3.8**contaminated materials**

feed containing a level of undesirable substances in excess of that which is acceptable under Directive 2002/32/EC

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1p)] [1]

3.9**detection limit****limit of detection**

measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β , given a probability α of falsely claiming its presence

Note 1 to entry: IUPAC recommends default values for α and β equal to 0,05.

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Note 2 to entry: The abbreviation LOD is sometimes used.

Note 3 to entry: The term 'sensitivity' is discouraged for 'detection limit'.

[SOURCE: ISO/IEC Guide 99:2007, 4.18]

3.10**feed material**

products of vegetable or animal origin, whose principal purpose is to meet animals' nutritional needs, in their natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, whether or not containing feed additives, which are intended for use in oral animal-feeding either directly as such, or after processing, or in the preparation of compound feed, or as carrier of premixtures

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1g)] [1]

3.11**HorRat**

ratio of the reproducibility relative standard deviation to that calculated from the Horwitz equation

Note 1 to entry: Predicted relative standard deviation $PRSD_R = 2 C^{-0,15}$

$$\text{HorRat}_R = RSD_R / PRSD_R \quad (1)$$

$$\text{HorRat}_r = RSD_r / PRSD_R \quad (2)$$

Note 2 to entry: If applied to within-laboratory studies, the normal range of HorRat(r) is 0,30 – 1,30.

Note 3 to entry: To check proper calculation of $PRSD_R$, a C of 10^{-6} should give a $PRSD_R$ of 16 %. C is concentration expressed as a mass fraction (both numerator and denominator expressed in the same units). The HorRat is indicative of method performance for a large majority of methods in chemistry. Normal values lie between 0,50 and 2,00.

[SOURCE: ISO 16577:2016, 3.75]

3.12**milk replacer**

compound feed administered in dry form or after dilution in a given quantity of liquid for feeding young animals as a complement to, or substitute for, post-colostral milk or for feeding young animals such as calves, lambs or kids intended for slaughter

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1)] [1]

3.13**precision**

closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The 'specified conditions' can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1) [2].

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility.

Note 4 to entry: Sometimes 'measurement precision' is erroneously used to mean 'measurement accuracy'.

[SOURCE: ISO-IEC Guide 99:2007, 2.15]

3.14**repeatability**

precision under repeatability conditions

Note 1 to entry: Repeatability can be expressed quantitatively in terms of the dispersion characteristics of the results.

[SOURCE: ISO 3534-2:2006, 3.3.5]

EN 17212:2019 (E)**3.15****repeatability conditions**

observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in the same test or measuring facility by the same operator using the same equipment within short intervals of time

Note 1 to entry: Repeatability conditions include:

- the same measurement procedure or test procedure;
- the same operator;
- the same measuring or test equipment used under the same conditions;
- the same location;
- repetition over a short period of time.

[SOURCE: ISO 3534-2:2006, 3.3.6]

3.16**repeatability limit**

r

repeatability critical difference for a specified probability of 95 %

[SOURCE: ISO 3534-2:2006, 3.3.9]

3.17**repeatability standard deviation**

standard deviation of test results or measurement results obtained under repeatability conditions

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under repeatability conditions.

Note 2 to entry: Similarly, “repeatability variance” and “repeatability coefficient of variation” can be defined and used as measures of the dispersion of test or measurement results under repeatability conditions.

[SOURCE: ISO 3534-2:2006, 3.3.7]

3.18**reproducibility**

precision under reproducibility conditions

Note 1 to entry: Reproducibility can be expressed quantitatively in terms of the dispersion characteristics (σ) of the results.

Note 2 to entry: Results are usually understood to be corrected results.

[SOURCE: ISO 3534-2:2006, 3.3.10]

3.19**reproducibility conditions**

observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment