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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 22388-2008

Determination of melamine in raw milk and dairy products

原料乳与乳制品中三聚氰胺检测方法

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1 Scope

GB/T 22388-2008 is the Chinese national standard on determination of melamine in raw milk and dairy products, in the field of food technology. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 7 October 2008 by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of China, and has been in force since 7 October 2008.

Classification: ICS 67.100, CCS C53. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This Standard specifies three determination methods for melamine in raw milk, dairy product as well as products containing dairy, i.e., high performance liquid chromatography (HPLC), liquid chromatography - mass spectrometry/mass spectrometry (LC-MS/MS) and gas chromatography - mass spectrometry [including gas chromatography-mass spectrometry (GC-MS), gas chromatography - mass spectrometry/mass spectrometry (GC-MS/MS)]. This Standard applies to the quantitative determination of melamine in raw milk, dairy products and products containing dairy. Liquid chromatography - mass spectrometry/mass spectrometry and gas chromatography - mass spectrometry (including gas chromatography - mass spectrometry/mass spectrometry) are applicable simultaneously to the qualitative confirmation of melamine in raw milk, dairy products, and products containing dairy. The limit of quantitation of HPLC of this Standard is 2 mg/kg. The limit of quantitation of liquid chromatography - mass spectrometry/mass spectrometry is 0.01 mg/kg. The limit of quantitation of gas chromatography - mass spectrometry is 0.05 mg/kg (the limit of quantitation of gas chromatography - mass spectrometry/mass spectrometry is 0.005 mg/kg).

2 Normative references

The following standards contain the provisions which, through reference in this Standard, constitute the provisions of this Standard. For dated references, subsequent amendments (excluding corrections) or revisions do not apply to this Standard. However, the parties who enter into agreement based on this Standard are encouraged to investigate whether the latest versions of these documents are applicable. For undated reference documents, the latest versions apply to this Standard.

GB/T 6682, Water for analytical laboratory use - Specification and test methods (GB/T 6682-2008, ISO 3696.1987, MOD)

3 Method One -- High performance liquid chromatography

3.1 Principle Extract sample with trichloroacetic acid solution-acetonitrile. After purification by cation exchange solid phase extraction column, determine by high performance liquid chromatography; quantify by external standard method.

3.2 Reagents and materials Unless otherwise specified, all reagents are analytically pure, and water is grade one according to GB/T 6682.

3.3 Instruments and equipment

3.3.1 High performance liquid chromatography (HPLC) instrument. equipped with UV Detector or diode array detector.

3.3.2 Analytical balance. resolution is 0.0001 g and 0.01 g.

3.5.1 HPLC reference conditions

3.5.2 Drawing of standard curve Use mobile phase to dilute the melamine standard stock solution grade by grade to obtain the standard working solutions with concentrations of 0.8, 2, 20, 40, 80 µg/mL. Test the concentration from low to high. With peak area- concentration, obtain the standard curve regression equation. See Figure A.1 in Annex A for HPLC chromatogram of matrix matching melamine sample.

3.7 Method limit of quantification The limit of quantification of this Method is 2 mg/kg.

3.8 Rate of recovery In the concentration range of 2mg/kg ~ 10mg/kg, the recovery rate is between 80% ~ 110%, and the relative standard deviation is less than 10%.

3.9 Allowable difference The absolute difference between two independent determinations obtained under repeatability conditions must not exceed 10% of the arithmetic mean.

4 Method Two -- Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS)

4.1 Principle Extract sample with trichloroacetic acid solution. After purification by cation exchange solid phase extraction column, determine by liquid chromatography- mass spectrometry/mass spectrometry; quantify by external standard method.

4.2 Reagents and materials Unless otherwise specified, all reagents are analytically pure, and water is grade one according to GB/T 6682.

4.3 Instruments and equipment

4.3.1 Liquid chromatography-mass spectrometry/mass spectrometry (LC- MS/MS) instrument. equipped with electron spray ionization (ESI).

4.3.2 Others same with 3.3.

4.4.2 Purification Transfer the liquid to be purified in

4.4.1 to the solid phase extraction column (3.2.13). Wash sequentially with 3 mL of water and 3 mL of methanol. After pumping to near dryness, elute with 6 mL of ammoniated methanol solution (3.2.9).

4.5.1 LC reference conditions

4.5.3 Drawing of standard curve Take blank sample and process it according to 4.4. Use the sample solution obtained to dilute the melamine standard stock solution (3.2.12) grade by grade to obtain the standard working solutions with concentrations of 0.01, 0.05, 0.1, 0.2, 0.5 µg/mL.

4.5.4 Quantitative determination The melamine response in the sample to be tested shall be within the linear range of the standard curve. When it exceeds the linear range, the injection analysis shall be performed after dilution.

4.6 Blank test Except that the sample is not weighed, it is performed according to the above measurement conditions and steps.

4.7 Method limit of quantification The limit of quantification of this Method is 0.01 mg/kg.

4.8 Rate of recovery In the concentration range of 0.01mg/kg ~ 0.5mg/kg, the recovery rate is between 80% ~ 110%, and the relative standard deviation is less than 10%.

4.9 Allowable difference The absolute difference between two independent determinations obtained under repeatability conditions must not exceed 15% of the arithmetic mean.

5 Method Three -- Gas chromatography-mass spectrometry (GC-MS and GC-MS/MS)

5.1 Principle The sample is subjected to ultrasonic extraction, solid phase extraction purification, and silanization derivatization. The derivatized products use selected ion monitoring mass spectrometry (SIM) or multiple reaction monitoring mass spectrometry (MRM). Determine the nature with compound retention time and abundance ratio of MS fragments. Quantify by external standard method.

5.2 Reagents and materials Unless otherwise specified, all reagents are analytically pure, and water is grade one according to GB/T 6682.

5.3 Instruments and equipment

5.3.1 Gas chromatography-mass spectrometry (GC-MS) instrument. equipped with electron impact ionization (EI).

5.3.2 Gas chromatography-mass spectrometry/mass spectrometry (GC- MS/MS) instrument. equipped with electron impact ionization (EI).

5.3.3 Electronic incubator.

5.3.4 Same with 3.3.