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Eggs and egg products — Determination of nitroimidazole residues — Liquid chromatography-tandem mass spectrometry method

Œufs et ovoproduits — Dosage des résidus de nitroimidazole — Méthode de chromatographie en phase liquide couplée à la spectrométrie de masse en tandem

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Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

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.

Eggs and egg products — Determination of nitroimidazole residues — Liquid chromatography-tandem mass spectrometry method

1 Scope

This document specifies a liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the determination of nitroimidazole residues in eggs and egg products.

This document is applicable to eggs and egg products, including whole eggs (fresh, frozen or pasteurized), egg yolk (liquid, frozen, dried or pasteurized), egg white (liquid, frozen, dried or pasteurized), whole egg powder, egg white powder, egg yolk powder, and liquid or dried albumin preparations.

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

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

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/

3.1
nitroimidazole residues
compounds including metronidazole, dimetridazole, ronidazole, ipronidazole, hydroxymetronidazole, hydroxydimetridazole and hydroxyipronidazole

4 Principle

The test sample is extracted using acetonitrile. The extracted solution is purified through solid-phase extraction. The nitroimidazole residues are determined and confirmed by LC-MS/MS, and quantified with an internal standard method.

5 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in CAC/GL 50-2004.

It is essential that the laboratory receives a sample which is truly representative and remains unaffected by damage or change during transport or storage.

Start from a representative sample of at least 1 kg.

Store the sample in such a way that deterioration and change in composition are prevented.

6 Preparation of test sample

6.1 Preparation of sample

6.1.1 Fresh eggs and egg products with shell

Take 16 (about 1 kg) fresh eggs or egg products and remove the shells. Homogenize the egg contents sample with the appropriate equipment. Transfer the homogenized sample into a polyethylene bottle.

6.1.2 Other egg products

Homogenize the egg products samples thoroughly. Transfer the homogenized sample into a polyethylene bottle.

For concentrated or dried egg products, weigh 5 g (to the nearest 0,01 g) of the homogenized sample into a 50 ml centrifuge tube, add 20 ml of water for reconstitution, vortex for 10 min, and then sonicate for 10 min. Ensure that the sample is evenly dispersed to obtain the reconstituted egg solutions.

NOTE This step is necessary to reconstitute dried (e.g. egg powder) or concentrated egg products to their ready-to-use form by diluting them to the required concentration. Liquid and frozen products are already in a suitable state for analysis and can bypass this procedure.

6.2 Storage

Store the test sample and standby sample at a temperature between −20 °C and −16 °C.

7 Reagents and materials

Reagents shall be of recognized analytical grade, unless otherwise specified.

7.1 Reagents

7.1.1 Water, grade 1 in accordance with ISO 3696.

7.1.2 Acetonitrile (CH_3CN), HPLC grade.

7.1.3 Methanol (CH_3OH), HPLC grade.

7.1.4 Formic acid (HCOOH), HPLC grade.

7.1.5 Sodium chloride (NaCl).

7.1.6 Dimethyl sulfoxide (DMSO).

7.1.7 *n*-Hexane (C_6H_{14}).

7.2 Preparation of solution

7.2.1 10 % Methanol aqueous solution (containing 0,1 % formic acid).

Pipette 10 ml of methanol and 0,1 ml of formic acid into a 100 ml one-mark volumetric flask. Dilute to the mark with water and mix.

7.2.2 0,1 % Aqueous formic acid solution.

Pipette 1 ml of formic acid to a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix thoroughly.

7.2.3 Acetonitrile-saturated *n*-hexane.

Obtain the supernatant from the solution of acetonitrile and *n*-hexane (v/v = 18/82) after sufficient mixture.

7.3 Standards

7.3.1 Nitroimidazoles, purity ≥ 95 %, see Annex A.

7.3.2 Nitroimidazoles isotope internal standard.

Stock solutions of 100 mg/l for the following compounds: metronidazole-d_3, dimetridazole-d_3, ronidazole-d_3, ipronidazole-d_3, hydroxymetronidazole-d_2, hydroxydimetridazole-d_3 and hydroxyipronidazole-d_3.

7.4 Preparation of standard solution

7.4.1 Standard stock solution, 1,00 mg/ml.

Weigh 10 mg (to the nearest 0,1 mg) of metronidazole, dimetridazole, ronidazole, ipronidazole, hydroxymetronidazole, hydroxydimetridazole and hydroxyipronidazole. Transfer each solution to a separate 10 ml volumetric flask. Dilute to the mark with methanol and mix thoroughly.

The prepared stock solutions are stable for six months when stored in the dark at −18 °C.

7.4.2 Mixed standard intermediate solution I, 10,0 µg/ml.

Pipette 0,1 ml of each of the standard stock solution (7.4.1) into a 10 ml one-mark volumetric flask. Dilute to the mark with methanol and mix thoroughly.

This solution is stable for one month when stored in the dark at −18 °C.

7.4.3 Mixed standard intermediate solution II, 200 ng/ml.

Pipette 0,2 ml mixed standard intermediate solution I (7.4.2) into a 10 ml one-mark volumetric flask. Dilute to the mark with methanol and mix.

The solution is for the current use.

7.4.4 Isotope internal standard intermediate solution, 1,00 µg/ml.

Pipette 0,1 ml of the isotope internal standard: stock solutions (7.3.2) into a 10 ml one-mark volumetric flask. Dilute to the mark with methanol and mix thoroughly.

This solution is stable for one month when stored in the dark at −18 °C.

7.4.5 Mixed standard working solution.

Pipette a certain amount of mixed standard intermediate solution II (7.4.3) and isotope internal standard intermediate solution (7.4.4) into seven volumetric flasks. Dilute with 10 % methanol aqueous solution (7.2.1) to obtain mixed standard solutions with target nitroimidazoles concentrations of 1,00 ng/ml, 2,00 ng/ml, 5,00 ng/ml, 10,0 ng/ml, 20,0 ng/ml, 50,0 ng/ml and 100 ng/ml. The concentration of each isotope internal standard is 20,0 ng/ml.