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Meat and meat products — Determination of chloramphenicol content — Reference method

Viande et produits à base de viande — Dosage du chloramphénicol — Méthode de référence

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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).

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).

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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.

The committee responsible for this document is Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

This second edition cancels and replaces the first edition (ISO 13493:1998), which has been technically revised.

The main changes compared to the previous edition are as follows:

— A new test method-liquid chromatography tandem mass spectrometry method(LC-MS/MS) method is added, and the provision that the test method of liquid chromatography asreference method is cancelled, accordingly, the test method of liquid chromatography tandem mass spectrometry method(LC-MS/MS) is stipulated as reference method (namely referee method).

— The determination scope is expanded to muscle, casing and liver of meat and meat products including livestock, poultry and sea food.

— The clauses order of the document has been rearranged.

— The introductory texts for “Foreword”, “Normative references” and “Terms and definitions” have been modified in accordance with the requirements of ISO/IEC Directives, Part 2.

— The title of the document has been modified.

— The scope of the document has been modified.

— The documents list of “Normative references” has been updated.

— In the “Chromatographic conditions” of the test method of liquid chromatography, “detector range”, “Recorder ranger” and “paper speed” have been deleted.

— The clauses of “Test method of liquid chromatography tandem mass spectrometry method (reference method)” have been added.

— The documents list of “Bibliography” has been updated.

Introduction

This document does not involve the use of patents.

Meat and meat products — Determination of chloramphenicol content — Reference method

1 Scope

This document specifies liquid chromatographic (LC) method for the determination of Chloramphenicol content of muscle tissue of meat, including livestock and poultry.

This document specifies liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the determination of Chloramphenicol content of muscle tissue, casing, liver of meat and meat products, including livestock and poultry.

This document specifies liquid chromatography tandem mass spectrometry method as the reference method.

Liquid chromatographic (LC) method is suitable for the determination of Chloramphenicol contents great than 6.5 mg/kg.

Liquid chromatography tandem mass spectrometry method (LC-MS/MS) is suitable for the determination of chloramphenicol contents great than 0.1 µg/kg.

Test samples which have deteriorated cannot be analyzed with this method.

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*

ISO 648, *Laboratory glassware — Single-volume pipettes*

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:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
Chloramphenicol content of meat and meat products
Mass fraction of chloramphenicol residue determined according to the procedure specified in this International Standard.

Note 1 to entry: The chloramphenicol content is expressed in micrograms per kilogram.

4 Principle

4.1 Liquid chromatographic method

A test portion is extracted with water. Filltration and solid-phase extraction are used to isolate the lipophillic components from the aquesous solution. The chloramphenicol is eluted from the cartridge with dichloromethane. The organic phase is evaporated and purified by liquid-liquid extraction with water and toluene. The chloramphenicol is measured with reverse-phase chromatography by ultraviolet (UV) detection.

4.2 Liquid chromatography tandem mass spectrometry method

The test portion is extracted with ethyl acetate, defatted with n-hexane and cleaned up with Hydrophile-Lipophile Balance (HLB) solid phase extraction. The Chloramphenicol is determined and confirmed by LC-MS/MS in multiple reaction monitoring (MRM) mode, operating in negative ionization.

5 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 17604[1].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Start from a representative sample of at least 200 g. Store the sample in such a way that deterioration and change in composition are prevented.

6 Preparation of test sample

Allow the sample to reach room temperature. Remove excess of fat and inedible parts.

Homogenize the laboratory sample with the appropriate equipment (7.2.3). Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared sample. Close the container and store in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as practicable, but always within 24 h after homogenization.

7 Test method of liquid chromatography

7.1 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

7.1.1 Water, complying with at least grade 3 in accordance with ISO 3696. The water shall be free of organic compounds.

7.1.2 Nitrogen, suitable for evaporating solvents.

7.1.3 Dichloromethane.

7.1.4 Toluene.

7.1.5 Acetate buffer, c(CH_3CO_2Na)=0,01 mol/l, pH=4,3.

Dissolve 0,82 g of anhydrous sodium acetate in about 970 mL of water. Adjust the pH to 4,3 with 50%(m/m) dilute acetic acid (CH_3CO_2H) using the pH-meter (7.2.1). Transfer the solution to a 1000 mL one-mark volumetric flask. Dilute to the mark with water and mix.

7.1.6 Acetonitrile, HPLC grade.

7.1.7 Mobile phase.

Add 750 mL of acetate buffer (7.1.5) to 250 mL of acetonitrile (7.1.6) and mix thoroughly.

Before use, filter the eluent through a 0,22µm filter (7.2.2) and degas.

7.1.8 Chloramphenicol stock solution, 100 µg/mL.

Weigh, to the nearest 0.1mg, 10 mg of chloramphenicol and transfer it to a 100 mL one-mark volumetric flask. Dilute to the mark with methanol and mix.

This stock solution is stable for 1 month when stored in the dark.

7.1.9 Chloramphenicol standard solutions.

Pipette 5,0 mL of the stock solution (7.1.8) into a 100 mL one-mark volumetric flask. Dilute to the mark with water and mix.

Prepare four standard solutions by diluting 1,0 mL, 2,0 mL, 5,0 mL and 15,0 mL of this solution to 100 mL with water to obtain solutions with a chloramphenicol content of 0,05 µg/mL, 0,10 µg/mL, 0,25 µg/mL and 0,75 µg/mL respectively.

These standard solutions are stable for 1 week when stored in the dark.

7.2 Apparatus

The usual laboratory apparatus and, in particular, the following.

7.2.1 pH-meter.

7.2.2 Membrane filter, of low dead volume and pore size 0,22µm.

7.2.3 Mechanical or electrical equipment capable of homogenizing the laboratory sample.

This includes a high-speed rotational cutter, or a mincer fitted with a plate with apertures not exceeding 4,0 mm in diameter.

7.2.4 Laboratory blender (e.g. Stomacher blender or vortex type).

7.2.5 Filter paper, quantitative, fast filtration rate, of diameter about 15 cm.

NOTE For example, Whatman 41 proved to be suitable.

7.2.6 Extraction cartridges, of capacity 20 mL, containing diatomaceous earth that extracts lipophilic components from aqueous solutions.

NOTE Extrelut®, manufactured by Merck, Darmstadt, Germany (No. 11737). proved to be suitable. of capacities 100 mL, 50 mL, 25 mL and 1mL complying with ISO 648, class B.

7.2.7 Water bath or heating block, capable of being maintained at (40±1) °C, with equipment for drying with nitrogen (7.1.2); or rotary vacuum evaporator.

7.2.8 Centrifuge tubes, of capacity 25 mL.

7.2.9 Vortex mixer, operating at a rotation frequency of about 700 rpm/min.

7.2.10 Centrifuge, operating at a radial acceleration of about 1000 g_n.

7.2.11 Micropipettes, of capacity 300 µL.

7.2.12 Liquid chromatograph, equipped with:

— a constant-flow pump;

— an injector.

— a reverse-phase C_8 or C_{18} column with an internal diameter of 3mm, length of 20 cm, and particle size of 5µm, or a column of equivalent quality;

— a UV/VIS detector suitable for measurements at a wavelength of 285 nm; if available, a diode array detector (for confirmation purposes);

7.3 Procedure

NOTE If it is required to check whether the repeatability limit (see 7.5.2) is met, carry out two single determinations in accordance with 7.3.1-7.3.6.

7.3.1 General

In conjunction with the analysis of the test solution (or a series of test solutions), analyse a spiked blank sample with a chloramphenicol content of 10 µg/kg and a blank sample.

7.3.2 Test portion

Weigh 10 g(m)of the prepared test sample (see clause 6) the nearest 0,1 g in a 100 mL conical flask.

7.3.3 Preparation of extract

7.3.3.1 Add 40,0 mL of water and mix vigorously for 3 min with the laboratory blender (7.2.4).

7.3.3.2 The volume(V1) of the water phase obtained is 40,0 mL plus the volume of water in the test portion (normally about 7,5 mL water int 10 g of sample).

7.3.3.3 Filter the sample through a filter paper (7.2.5).

7.3.4 Solid-phase extraction

7.3.4.1 Transfer 20,0 mL(V2) of the filtrate to an extraction cartridge (7.2.6).

7.3.4.2 After (15±0,2) min, elute the chloramphenicol with 70 mL of dichloromethane (7.1.3). Evaporate the organic phase to a volume of about 1 mL under a gentle stream of nitrogen (7.1.2) in the water bath (7.2.7)

7.3.4.3 Transfer the residue to a centrifuge tube (7.2.8) with about 10 mL of dichloromethane (7.1.3). Evaporate carefully to absolute dryness.

7.3.5 Liquid-liquid extraction

7.3.5.1 Add 400 µL (V3) of water and 2,0 mL of toluene (7.1.4) to the residue and mix gently for 1min at a rotation frequency of about 700 min^{-1} on the vortex mixer (7.2.9).

7.3.5.2 Centrifuge for 5 min at a radial acceleration of 1000 g_n in the centrifuge (7.2.10). Remove as much as possible of the organic phase with a pipette and discard it.

7.3.5.3 Add 1,5 mL of toluene and mix gently for 1 min at a rotation frequency of about 700 min^{-1} on the vortex mixer (7.2.9). Centrifuge for 5 min at a radial acceleration of 1000 g in the centrifuge (7.2.10).

7.3.5.4 Remove as much possible of the organic phase with a pipette and discard it. Transfer 300 µL of the aqueous phase to a suitable container using a micropipette (7.2.11).

7.3.6 Chromatographic analysis

7.3.6.1 Chromatographic conditions

Parameter	Setting
Wavelength	285 nm
Mobile phase (7.1.7) volume flow rate	0.6 mL/min
Injection volume	100 µL

NOTE The injection volume and the volume flow rate depend on the column dimensions.

7.3.6.2 Chromatographic procedure

Wait until the liquid chromatograph (7.2.12) system is stabilized. Inject the blank sample, the spiked blank sample, the four chloramphenicol standard solutions (7.1.9).

Check for chloramphenicol signals in the sample chromatograms at the retention time of chloramphenicol contents of these solutions.

7.3.6.3 Measurement

Measure the chloramphenicol peak heights or peak areas of the test solution and the chloramphenicol standard solutions.

The responses obtained for the chloramphenicol standard solutions shall be linearly related to the chloramphenicol contents of these solutions.

NOTE Confirmation can be carried out with a diode array detector for chloramphenicol contents exceeding 10 µg/kg.