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Meat and meat products — Determination of chloramphenicol content — Method using liquid chromatography

Viande et produits à base de viande — Dosage du chloramphénicol — Méthode par chromatographie en phase liquide

Reference number
ISO 13493:1998(E)

Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13493 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 6, *Meat and meat products.*

Annex A of this International Standard is for information only.

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Meat and meat products — Determination of chloramphenicol content — Method using liquid chromatography

1 Scope

This International Standard specifies a liquid chromatographic method for the determination of the chloramphenicol content of the muscle tissue of meat, including poultry.

The method is suitable for the determination of chloramphenicol contents greater than 6,5 µg/kg.

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

NOTE This International Standard may be applicable for the determination of the chloramphenicol content of all kinds of meat and meat products. However, materials other than muscle tissue were not included in the collaborative testing of the method.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

3 Definition

For the purposes of this International Standard, the following definition applies.

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 The chloramphenicol content is expressed in micrograms per kilogram.

4 Principle

A test portion is extracted with water. Filtration and solid-phase extraction are used to isolate the lipophilic components from the aqueous 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.

5 Reagents

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

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

5.2 Nitrogen, suitable for evaporating solvents.

5.3 Dichloromethane.

5.4 Toluene.

5.5 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 (6.1). Transfer the solution to a 1000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

5.6 Acetonitrile, suitable for UV spectroscopy.

5.7 Mobile phase.

Add 750 ml of acetate buffer (5.5) to 250 ml of acetonitrile (5.6) and mix thoroughly.

Before use, filter the eluent through a 0,22 µm filter (6.2) and degas.

5.8 Chloramphenicol stock solution, 100 µg/ml.

Weigh, to the nearest 0,1 mg, 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.

5.9 Chloramphenicol standard solutions.

Pipette 5,0 ml of the stock solution (5.8) 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.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 pH-meter.

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

6.3 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.

6.4 Laboratory blender (e.g. Stomacher blender[1)] or vortex type).

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

NOTE For example, Whatman 41 proved to be suitable[1)].

6.6 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[1)].

6.7 Water bath or **heating block,** capable of being maintained at (40 ± 1) °C, with equipment for drying with nitrogen (5.2); or **rotary vacuum evaporator.**

6.8 Centrifuge tubes, of capacity 25 ml.

6.9 Vortex mixer, operating at a rotation frequency of about 700 min^{-1}.

6.10 Centrifuge, operating at a radial acceleration of about 1 000 *g*.

6.11 Micropipettes, of capacity 300 µl.

6.12 Liquid chromatograph, equipped with:

— a constant-flow pump;
— an injector;
— a reverse-phase C_8 or C_{18} column with an internal diameter of 3 mm, length of 20 cm, and particle size of 5 µm, 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);
— a recorder with variable measuring range or an integrator.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 3100-1 [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.

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

1) These are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

8 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 (6.3). 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.

Store the sample, if necessary, at a temperature below −18 °C.

Analyse the sample as soon as practicable, but always within 24 h after homogenization.

9 Procedure

NOTE If it is required to check whether the repeatability limit (see 11.2) is met, carry out two single determinations in accordance with 9.1 to 9.6.

9.1 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.

9.2 Test portion

Weigh 10 g (m) of the prepared test sample (see clause 8) to the nearest 0,1 g in a 100 ml conical flask.

9.3 Preparation of extract

Add 40,0 ml of water and mix vigorously for 3 min with the laboratory blender (6.4).

The volume (V_1) of the water phase obtained is 40,0 ml plus the volume of water in the test portion (normally about 7,5 ml water in 10 g of sample).

Filter the sample through a filter paper (6.5).

9.4 Solid-phase extraction

Transfer 20,0 ml (V_2) of the filtrate to an extraction cartridge (6.6).

After (15 ± 0,2) min, elute the chloramphenicol with 70 ml of dichloromethane (5.3). Evaporate the organic phase to a volume of about 1 ml under a gentle stream of nitrogen (5.2) in the water bath (6.7) or using the rotary vacuum evaporator (6.7).

Transfer the residue to a centrifuge tube (6.8) with about 10 ml of dichloromethane (5.3).

Evaporate carefully to absolute dryness.

9.5 Liquid-liquid extraction

Add 400 µl (V_3) of water and 2,0 ml of toluene (5.4) to the residue and mix gently for 1 min at a rotation frequency of about 700 min^{-1} on the vortex mixer (6.9).

Centrifuge for 5 min at a radial acceleration of 1 000 *g* in the centrifuge (6.10).

Remove as much as possible of the organic phase with a pipette and discard it.

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 (6.9).

Centrifuge for 5 min at a radial acceleration of 1 000 *g* in the centrifuge (6.10).

Remove as much as 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 (6.11).

9.6 Chromatographic analysis

9.6.1 Chromatographic conditions

Parameter	Setting
Wavelength	285 nm
Detector range	absorbance of 0,005 to 0,01 at full scale of recorder
Recorder range	10 mV
Paper speed	1,0 cm/min
Mobile phase (5.7) 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.

9.6.2 Chromatographic procedure

Wait until the liquid chromatograph (6.12) system is stabilized. Inject the blank sample, the spiked blank sample, the four chloramphenicol standard solutions (5.9), the test solution obtained in 9.5, and again the chloramphenicol standard solutions (5.9).

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

9.6.3 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.

10 Calculation

Calculate the chloramphenicol content of the test sample using the equation:

$$w = \frac{h \times \rho \times V_1 \times V_3}{h_s \times m \times V_2}$$

where

w is the chloramphenicol content, in micrograms per kilogram, of the test sample;

h is the peak height or peak area, in length or area units, found for the test solution;

h_s is the peak height or peak area, in length or area units, found for one of the standard solutions (5.9);

ρ is the chloramphenicol content, in micrograms per millilitre, of the standard solution;

m is the mass, in grams, of the test portion (9.2);

V_1 is the volume, in millilitres, of the water phase obtained after mixing in 9.3 (V_1 = 40 ml + the volume of water in the test portion);

V_2 is the volume, in millilitres, of filtrate transferred in 9.4 to the extraction cartridge (V_2 = 20 ml);

V_3 is the volume, in microlitres, of water added in 9.5 to the residue (V_3 = 400 µl).

Report the result rounded to the nearest 0,1 µg/kg.

The result shall not be corrected for recovery. The recovery shall be specified in the test report (clause 12).

11 Precision

11.1 Interlaboratory tests

The precision of the method was established by an interlaboratory test carried out in accordance with ISO 5725 [2][2)].

The results of this interlaboratory test have been published (see reference [5]). The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

The results of another interlaboratory test, carried out in accordance with ISO 5725, show that recovery for meat, meat products and poultry is reproducible and approximately 55 %.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed 2,1 µg/kg for a chloramphenicol content of 10 µg/kg.

2) ISO 5725:1986 (now withdrawn) was used to obtain the precision results.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed 4,9 µg/kg for a chloramphenicol content of 10 µg/kg.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents that occurred which may have influenced the test result(s);

— the test result obtained, or the two test results obtained if the repeatability has been checked;

— the recovery.