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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](http://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](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, 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 [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by 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 with the previous edition are as follows:

- A new test method, the liquid chromatography tandem mass spectrometry method (LC-MS/MS) has been added. The reference test method has been changed from liquid chromatography to LC-MS/MS.
- The Scope has been expanded to include muscle, casing and the liver of meat and meat products, including livestock, poultry and seafood.
- The title of the document has been modified.
- The document structure has been rearranged.
- The introductory texts for the Foreword, [Clause 2](#) and [Clause 3](#) have been modified.
- [Clause 2](#) has been updated.
- In [7.3.6.1](#), “Chromatographic conditions”, “detector range”, “recorder ranger” and “paper speed” have been deleted.
- [Clause 8](#) “Test method of liquid chromatography tandem mass spectrometry method (reference method)” has been added.
- The Bibliography has been updated.

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](http://www.iso.org/members.html).

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

## 1 Scope

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

This document specifies the 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 LC-MS/MS as the reference method.

The LC method is suitable for the determination of chloramphenicol content greater than 6,5 mg/kg.

LC-MS/MS is suitable for the determination of chloramphenicol content greater than 0,1 µg/kg.

Test samples which have deteriorated cannot be analysed 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*

## 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

mass fraction of chloramphenicol residue in meat and meat products

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

Note 2 to entry: The chloramphenicol content is determined according to the procedure specified in this document.

## 4 Principle

### 4.1 Liquid chromatographic method

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.

## 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 document. A recommended sampling method is given in ISO 17604.

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 of homogenization.

## 7 Test method of liquid chromatography

### 7.1 Reagents

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

**7.1.1 Water**, conforming to 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**,  $c(\text{CH}_3\text{CO}_2\text{Na}) = 0,01 \text{ mol/l}$ ,  $\text{pH} = 4,3$ .

Dissolve 0,82 g of anhydrous sodium acetate in about 970 ml of water. Adjust the pH to 4,3 with a mass fraction of 50 % dilute acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ) using the pH-meter (7.2.1). Transfer the solution to a 1 000 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 0,22 µm filter (7.2.2) and degas.

### 7.1.8 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 one 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 one week when stored in the dark.

## 7.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

### 7.2.1 pH-meter.

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

### 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<sup>1)</sup> or vortex type).

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

NOTE For example, Whatman 41, 1441-047-GE Whatman Grade 41, proved to be suitable<sup>1)</sup>.

### 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<sup>1)</sup>.

### 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 r/min.

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

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

**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<sub>8</sub> or C<sub>18</sub> 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).

## 7.3 Procedure

### 7.3.1 General

If it is necessary to check whether the repeatability limit (see [7.5.2](#)) is met, carry out two single determinations in accordance with [7.3.1](#) to [7.3.6](#).

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 (*V*<sub>1</sub>) 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).

**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 (*V*<sub>2</sub>) 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 (*V*<sub>3</sub>) of water and 2,0 ml of toluene ([7.1.4](#)) to the residue and mix gently for 1 min at a rotation frequency of about 700 r/min on the vortex mixer ([7.2.9](#)).



**7.3.5.2** Centrifuge for 5 min at a radial acceleration of 1 000*g* 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 r/min on the vortex mixer (7.2.9). Centrifuge for 5 min at a radial acceleration of 1 000*g* in the centrifuge (7.2.10).

**7.3.5.4** 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 (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 and 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.

### 7.4 Calculation

Calculate the chloramphenicol content of the test sample using [Formula \(1\)](#):

$$w = \frac{h \times \rho \times V_1 \times V_3}{h_s \times m \times V_2} \quad (1)$$

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<sub>s</sub>* is the peak height or peak area, in length or area units, found for one of the standard solutions (7.1.9);

- $\rho$  is the chloramphenicol content, in micrograms per millilitre, of the standard solution;
- $m$  is the mass, in grams, of the test portion (7.3.2);
- $V_1$  is the volume, in millilitres, of the water phase obtained after mixing in 7.3.3.2 ( $V_1 = 40$  ml + the volume of water in the test portion);
- $V_2$  is the volume, in millilitres, of filtrate transferred in 7.3.4.1 to the extraction cartridge ( $V_2 = 20$  ml);
- $V_3$  is the volume, in microlitres, of water added in 7.3.5.1 to the residue ( $V_3 = 400$   $\mu$ l).

Report the result rounded to the nearest 0,1  $\mu$ g/kg.

The result shall not be corrected for recovery. The recovery shall be specified in the test report (see Clause 9).

## 7.5 Precision

### 7.5.1 Interlaboratory test

The precision of the method was established by interlaboratory tests carried out in accordance with ISO 5725:1986<sup>2)</sup>.

The results of interlaboratory tests have been published, see Reference [5]. The values derived from this test will possibly not be applicable to concentration ranges and matrices other than those given.

The results of another interlaboratory test, carried out in accordance with ISO 5725:1986, show that recovery for meat, meat products and poultry is reproducible and approximately 55 %.

### 7.5.2 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  $\mu$ g/kg for a chloramphenicol content of 10  $\mu$ g/kg.

### 7.5.3 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  $\mu$ g/kg for a chloramphenicol content of 10  $\mu$ g/kg.

## 8 Test method of liquid chromatography tandem mass spectrometry method (reference method)

### 8.1 Reagents and materials

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

**8.1.1 Water**, conforming to at least grade 3 in accordance with ISO 3696.

**8.1.2 Methanol**, LC-MS/HPLC grade.

**8.1.3 Acetonitrile**, LC-MS/HPLC grade.

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2) Cancelled and replaced by the ISO 5725 series.

**8.1.4 Ethyl acetate.**

**8.1.5 n-hexane.**

**8.1.6 Ammonium hydroxide:** analysis grade.

**8.1.7  $\beta$ -glucuronidase,** about 40 000 enzyme activity unit.

**8.1.8 Methanol and water solution I (volume ratio 2:8).**

Add 20 ml of methanol (8.1.2) and 80 ml of water into a 100 ml one-mark volumetric flask and mix.

**8.1.9 Methanol and water solution II (volume ratio 1:1).**

Add 50 ml of methanol (8.1.2) and 50 ml of water into a 100 ml one-mark volumetric flask and mix.

**8.1.10 Chloramphenicol,** purity > 99 %, reference substance.

**8.1.11 Chloramphenicol stock solution,** 100  $\mu\text{g/ml}$ .

Weigh, to the nearest 0,1 mg, 10,0 mg of chloramphenicol (8.1.10) and transfer it to a 100 ml one-mark volumetric flask. Dilute to the mark with methanol (8.1.2) and mix.

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

**8.1.12 Chloramphenicol (CAP) working solution,** 1,0  $\mu\text{g/ml}$ .

Pipette 1,0 ml of the stock solution (8.1.11) into a 100 ml one-mark volumetric flask. Dilute to the mark with methanol and water solution II (8.1.9) and mix.

**8.1.13 Chloramphenicol- $\text{d}_5$ , internal standard,** purity  $\geq$  99 %, reference substance.

**8.1.14 Internal standard chloramphenicol- $\text{d}_5$  (CAP- $\text{d}_5$ ) stock standard solution,** 100  $\mu\text{g/ml}$ .

Weigh, to the nearest 0,1 mg, 10,0 mg of CAP- $\text{d}_5$  standard (8.1.13) and transfer it to a 100 ml one-mark volumetric flask. Dilute to the mark with methanol (8.1.2) and mix.

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

**8.1.15 Internal standard chloramphenicol- $\text{d}_5$  (CAP- $\text{d}_5$ ) working solution,** 1,0  $\mu\text{g/ml}$ .

Pipette 1,0 ml of stock standard solution of CAP- $\text{d}_5$  (8.1.14) into a 100 ml one-mark volumetric flask. Dilute to the mark with methanol and water solution II (8.1.9) and mix.

**8.1.16 Calibration curve standard working solutions.**

Prepare five standard solutions by pipetting 0,01 ml, 0,05 ml, 0,1 ml, 0,2 ml and 0,5 ml of CAP working solution (8.1.12) and 0,1 ml of CAP- $\text{d}_5$  standard working solution (8.1.15) into five 100 ml volumetric flasks. Dilute with methanol and water solution II (8.1.9) to obtain solutions with a CAP content of 0,1 ng/ml, 0,5 ng/ml, 1,0 ng/ml, 2,0 ng/ml and 5,0 ng/ml with CAP- $\text{d}_5$  concentration of 1,0 ng/ml.

**8.1.17 Acetonitrile and water solution (volume ratio 1:9).**

Add 10 ml of acetonitrile (8.1.3) and 90 ml of water to a 100 ml one-mark volumetric flask and mix.

## 8.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

### 8.2.1 LC-MS/MS equipment.

Liquid chromatography combined with electrospray ionization mass spectrometry.

### 8.2.2 Rotary vacuum evaporator.

### 8.2.3 Homogenizer, capable of spinning at a rotational frequency of 14 000 r/min.

### 8.2.4 Vortex mixer, operating at a rotation frequency of about 2 000 r/min.

### 8.2.5 Centrifuge, operating at a radial acceleration of about 2 000g.

### 8.2.6 Centrifuge tubes, of capacity 50 ml.

### 8.2.7 Solid-phase extraction cartridge of Oasis HLB column, 200 mg/6 ml, or equivalent.

Condition with 5 ml methanol followed by 5 ml water to activate and balance the column.

### 8.2.8 Constant thermostat water bath.

## 8.3 Procedure

### 8.3.1 General

If it is necessary to check whether the repeatability limit is met, carry out two single determinations in accordance with [8.3.1](#) to [8.3.5](#).

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 0,2 µg/kg and a blank sample.

### 8.3.2 Test portion

Weigh, to the nearest 0,1 g, 5,0 g of the prepared test sample (see [Clause 6](#)) into a 50 ml centrifuge tube ([8.2.6](#)).

### 8.3.3 Preparation of extract

#### 8.3.3.1 Muscle tissue and casing

**8.3.3.1.1** Add 0,1 ml CAP-d<sub>5</sub> working solution ([8.1.15](#)), 0,5 ml ammonium hydroxide ([8.1.6](#)) and 15 ml ethyl acetate. Homogenize for 30 s at 14 000 r/min ([8.2.3](#)).

**8.3.3.1.2** Centrifuge for 5 min at a radial acceleration of 2 000g ([8.2.5](#)). Transfer the supernatant to another centrifuge tube.

**8.3.3.1.3** Repeat the above procedures with 15 ml ethyl acetate ([8.1.4](#)). Combine two extracting solutions and evaporate ([8.2.2](#)) them to nearly dryness in the water bath below 50 °C.

**8.3.3.1.4** Add 4 ml n-hexane (8.1.5) and 4 ml water to dissolve residues. Mix for 1 min at 2 000 r/min in the vortex mixer (8.2.4). Centrifuge (8.2.5) for 3 min at 2 000g and discard the supernatant. Add 4 ml n-hexane and repeat the procedure.

### 8.3.3.2 Liver

Add 0,2 ml  $\beta$ -glucuronidase (8.1.7) and 15 ml water. Mix for 1 min at 2 000 r/min on the vortex mixer (8.2.4). Homogenize for 30 s at 14 000 r/min (8.2.3). Hydrolyse at 37 °C in a constant thermostat water bath for 12 h. Follow the same procedures as in 8.3.3.1.1 to 8.3.3.1.4.

### 8.3.4 Solid-phase extraction

**8.3.4.1** Transfer the above solution into the Oasis HLB column (8.2.7). Wash the centrifuge tube twice with 5 ml water, one time with 5 ml methanol and water solution I (8.1.8) and pass the washes through the Oasis HLB column. Discard the eluted solution.

**8.3.4.2** Evacuate the cartridge to dryness under vacuum (8.2.2). Elute the column with 6 ml methanol (8.1.2) and collect the eluted solution.

**8.3.4.3** Evaporate the organic phase to dryness under vacuum (8.2.2). The dried extracts are reconstituted into 5 ml acetonitrile solution (8.1.17) by vortexing (8.2.4) for 5 min to dissolve residue, and syringe-filtered for injection into LC-MS system (8.2.1).

### 8.3.5 LC-MS/MS analysis

#### 8.3.5.1 HPLC conditions

Mobile phase A: water (8.1.1)  
 Mobile phase B: acetonitrile (8.1.3)  
 Column: C<sub>18</sub>, 2,1 mm × 150 mm, 5 µm, or the equivalent  
 Column temperature: 30 °C  
 Flow rate: 0,3 ml/min  
 Gradient: LC gradient as described in Table 1  
 Injection volume: 20 µl

**Table 1 — Gradient of mobile phase**

LC gradient used for the analysis of chloramphenicol time min	A %	B %
0	90	10
2	90	10
6	20	80
7	20	80
7,1	90	10
10	90	10

## 8.3.5.2 MS parameters

Ionization type:	electrospray (ESI)
Polarity:	negative ionization
Mode:	MRM
Spray voltage:	–4 500 V
Curtain gas:	27 Pa
Ion source gas 1:	38 Pa
Ion source gas 2:	45 Pa
Source block temperature:	525 °C
Declustering potential (DP):	–65 V
Scan time (for each transition):	100 ms

Transitions for confirmation and quantification, CE and CXP are shown in [Table 2](#).

NOTE Figures of chloramphenicol standard solution and chloramphenicol-d<sub>5</sub> standard solution are shown in [Annex A](#).

**Table 2 — Transitions for confirmation and quantification, CE and CXP**

Compound	Transitions for confirmation m/z	Transitions for quantification m/z	CE/eV	CXP/V
CAP	321,0/256,9	321,0/152,0	–16	–10
	321,0/152,0		–25	–10
CAP-d <sub>5</sub> (IS)	326,0/157,1	326,0/157,1	–25	–10

## 8.3.5.3 Blank test

The operation of the blank test is the same as the description in the method of determination, but without sample addition.

## 8.3.5.4 Quantification and confirmation of LC-MS/MS

Under LC-MS/MS conditions (see [8.3.5.1](#) to [8.3.5.2](#)), the standard working solution and sample solution are injected. The calibration curve with isotope internal standard method is used for quantitative measurement, on the condition of the retention times of sample chromatogram peaks being consistent with those of the standard solution. The relative intensities of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of the standard solution shall correspond with that of the sample solution. The permitted tolerances listed in [Table 3](#) and the corresponding analyte can be present in the sample.

**Table 3 — Maximum permitted tolerances for relative ion intensities during confirmation**

Relative intensity	> 50 %	> 20 to 50 %	> 10 to 20 %	≤ 10 %
Permitted tolerances	±20 %	±25 %	±30 %	±50 %

## 8.4 Calculation

Quantification software of the LC-MS/MS instrument enables the construction of a customized template to check the linearity of calibration curves, calculate the peak area ratios obtained from the transition reactions, confirm the presence or absence of the analyte and reach the final results, expressed in micrograms per kilogram ( $\mu\text{g/kg}$ ). The blank value shall be subtracted from the above result of calculation. See [Formula \(2\)](#):

$$w = K \cdot \frac{(A / A_{\text{IS}} - b)}{a} \cdot \frac{V}{m} \quad (2)$$

where

- $w$  is the chloramphenicol content, in micrograms per kilogram, of the test sample ( $\mu\text{g/kg}$ );
- $K$  is the analyte mass concentration of the internal standard solution in the unknown sample, in nanograms per millilitre ( $K = 0,2 \text{ ng/ml}$ );
- $A$  is the peak area of the chloramphenicol to sample ([8.1.12](#));
- $A_{\text{IS}}$  is the peak area of the interior standard (IS) to sample ([8.1.15](#));
- $a$  is the slope of the regression line of peak area ratio versus concentration ratio;
- $b$  is the intercept of the regression line of peak area ratio versus concentration ratio;
- $V$  is the final volume of sample preparation, in millilitres (ml) ([8.1.17](#));
- $m$  is the sample mass in grams (g) ([8.3.2](#)).

## 8.5 Precision

### 8.5.1 Interlaboratory test

The precision of the method was established by interlaboratory tests carried out in accordance with ISO 5725:1986. The values derived from the test will possibly not be applicable to concentration ranges and matrices other than those given.

### 8.5.2 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  $0,01 \mu\text{g/kg}$  for a chloramphenicol content of  $0,1 \mu\text{g/kg}$ .

### 8.5.3 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  $0,025 \mu\text{g/kg}$  for a chloramphenicol content of  $0,1 \mu\text{g/kg}$ .

## 9 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 document, i.e. ISO 13493;
- all operating details not specified in this document, or regarded as optional, together with details of any incidents which have possibly influenced the test result(s);
- the test result obtained, or the two test results obtained if the repeatability has been checked.
- the recovery;
- the date of the test.

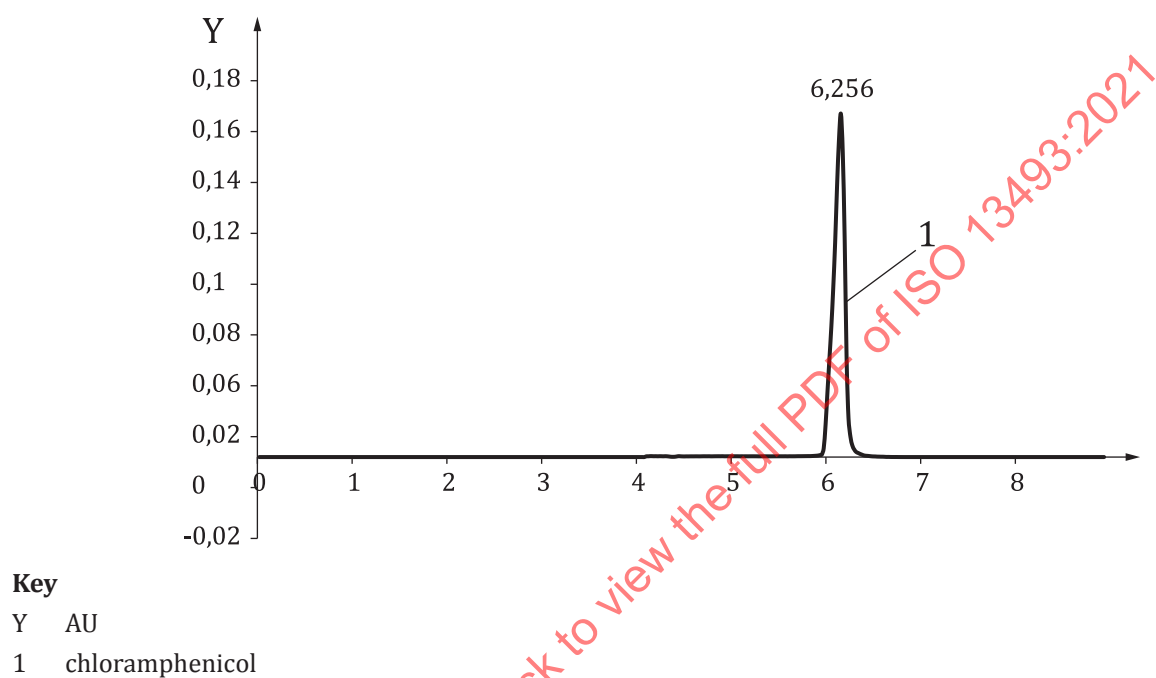
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## Annex A

(informative)

### HPLC chromatogram of chloramphenicol standard solution and LC-MS/MS chromatogram of chloramphenicol standard solution



**Figure A.1 — HPLC chromatogram of a chloramphenicol standard solution at a concentration of 5,0 µg/ml**