

BS ISO 6495-1:2015



BSI Standards Publication

# Animal feeding stuffs — Determination of water-soluble chlorides content

Part 1: Titrimetric method

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**National foreword**

This British Standard is the UK implementation of ISO 6495-1:2015. It supersedes BS 5766-5:1999 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

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ISBN 978 0 580 58083 3

ICS 65.120

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 July 2015.

**Amendments issued since publication**

Date	Text affected
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INTERNATIONAL  
STANDARD

**ISO**  
**6495-1**

First edition  
2015-06-15

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**Animal feeding stuffs —  
Determination of water-soluble  
chlorides content —**

**Part 1:  
Titrimetric method**

*Aliments des animaux — Détermination de la teneur en chlorures  
solubles dans l'eau —*

*Partie 1: Méthode titrimétrique*



Reference number  
ISO 6495-1:2015(E)

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Published in Switzerland

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## Foreword

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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)).

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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 WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 34, *Food Products*, Subcommittee SC 10, *Animal feeding stuffs*.

This first edition of ISO 6495-1 cancels and replaces ISO 6495:1999, which has been technically revised.

ISO 6495 consists of the following parts, under the general title *Animal feeding stuffs — Determination of water-soluble chloride content*:

— *Part 1: Titrimetric method*

# Animal feeding stuffs — Determination of water-soluble chlorides content —

## Part 1: Titrimetric method

### 1 Scope

This part of ISO 6495 specifies a method for the determination of water-soluble chloride content, expressed as sodium chloride, of animal feeding stuffs.

This method is applicable to animal feeding stuffs containing water-soluble chloride content, expressed as sodium chloride,  $\geq 0,05$  %.

### 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable to its application. 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 6497, *Animal feeding stuffs — Sampling*

ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation*

### 3 Principle

The chloride present in a test portion are dissolved in water. The solution is clarified if the product contains organic matter. It is then slightly acidified with nitric acid and the chlorides are precipitated as silver chloride by means of standard volumetric solution of silver nitrate. The excess silver nitrate is titrated with a standard volumetric solution of ammonium thiocyanate or potassium thiocyanate, by Volhard's method.

### 4 Reagents

Use only reagents of recognized analytical grade.

**4.1 Water**, complying with at least grade 3 in accordance with ISO 3696.

**4.2 Acetone**.

**4.3 *n*-Hexane**.

**4.4 Nitric acid**, mass concentration  $\rho_{20}(\text{HNO}_3) = 1,38$  g/ml.

**4.5 Dilute nitric acid**, volume fraction  $\rho(\text{HNO}_3) = 2$  %.

Dilute 20 ml nitric acid (4.4) to 1000 ml with water (4.1).

**4.6 Potassium chromate solution**, mass concentration  $\rho(\text{K}_2\text{CrO}_4) = 5 \%$  in water (4.1).

**4.7 Ammonium iron(III) sulfate**, saturated solution. Prepare from  $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  dissolved in water (4.1) until saturation. Approximately 125 g ammonium iron(III) sulfate per 100 ml water is required.

**4.8 Activated carbon**, free from chloride and not capable of adsorbing chloride.

**4.9 Carrez I solution.**

Dissolve 10,6 g of potassium hexacyanoferrate(II) trihydrate  $[\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}]$  in water (4.1). Dilute to 100 ml with water (4.1).

**4.10 Carrez II solution.**

Dissolve 21,9 g of zinc acetate dihydrate  $[\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}]$  in water (4.1), and add 3 ml of glacial acetic acid. Dilute to 100 ml with water (4.1).

**4.11 Sodium chloride**, standard volumetric solution,  $c(\text{NaCl}) = 0,1 \text{ mol/l}$ . The molarity of the standard volumetric solution shall be known to 0,000 1 mol/l.

Put about 20 g of finely pulverized sodium chloride in a thin layer on watch glass. Dry at about 250 °C for 1 h to 2 h. Let it cool in a desiccator, accurately weigh 5,8 g, recorded to 0,000 1 g (m), and dissolve in water (4.1). Dilute to the mark in a 1 000 ml volumetric flask with water (4.1) and mix well.

The concentration of standard sodium chloride solution can be calculated using Formula (1):

$$c_{\text{NaCl}} = \frac{m}{58,44} \quad (1)$$

where

$c_{\text{NaCl}}$  is the concentration of standard sodium chloride solution, in mol/l;

$m$  is the mass of sodium chloride, in grams.

**4.12 Silver nitrate**, standard volumetric solution,  $c_s = 0,1 \text{ mol/l}$ .

The molarity of the standard volumetric solution should be known to 0,000 1 mol/l, and duplicate titrations shall agree within  $\pm 0,1 \text{ ml}$ .

Put about 20 g of finely pulverized silver nitrate in a thin layer on watch glass. Dry at about 80 °C for 2 h to 3 h. Let it cool in a desiccator, weigh about 17,0 g and dissolve in water (4.1). Dilute to the mark in a 1 000 ml volumetric flask with water (4.1) and mix well.

Pipette 20 ml of standard sodium chloride solution (4.11) into a 200 ml conical flask. Add 1 ml of potassium chromate solution (4.6) and titrate while shaking vigorously with standard silver nitrate (4.12) until a reddish brown colour persists for at least 30 s.



The concentration of standard silver nitrate solution can be calculated using Formula (2):

$$c_s = \frac{20 \times c_{\text{NaCl}}}{V_s} \quad (2)$$

where

$c_s$  is the concentration of standard silver nitrate solution (4.12), in mol/l;

$c_{\text{NaCl}}$  is the concentration of standard sodium chloride solution (4.11), in mol/l;

$V_s$  is the volume of standard silver nitrate solution (4.12) used for titration, in ml.

**4.13 Ammonium thiocyanate or potassium thiocyanate**, standard volumetric solution,  $c_t = 0,1$  mol/l.

The molarity of standard volumetric solution shall be known to 0,000 1 mol/l, and duplicate titrations shall agree within  $\pm 0,1$  ml.

Weigh 7,6 g of ammonium thiocyanate, or 9,7 g of potassium thiocyanate and dissolve in water (4.1). Dilute to the mark in 1 000 ml volumetric flask and mix well.

Pipette 20 ml standard silver nitrate (4.12) into a 200 ml conical flask. Add 10 ml of dilute nitric acid (4.5) and 2 ml of saturated ammonium iron(III) sulfate solution (4.7). Titrate while shaking vigorously with standard ammonium, or potassium thiocyanate solution until a reddish brown colour persists for at least 30 s.

The concentration of standard ammonium or potassium thiocyanate solution can be calculated using Formula (3):

$$c_t = \frac{20 \times c_s}{V_t} \quad (3)$$

where

$c_t$  is the concentration of standard ammonium or potassium thiocyanate solution (4.13), in mol/l;

$c_s$  is the concentration of standard silver nitrate;

$V_t$  is the volume of standard ammonium or potassium thiocyanate solution (4.13) used for titration, in ml.

## 5 Apparatus

Usual laboratory apparatus and, in particular, the following.

**5.1 Rotary shaker**, operating at a rotation frequency of approximately 35 min<sup>-1</sup> to 40 min<sup>-1</sup>.

**5.2 Volumetric flasks**, class A of capacities 200 ml, 500 ml, and 1 000 ml.

**5.3 Pipettes**, class A of appropriate capacities.

**5.4 Burettes**, class A of appropriate capacities.

**5.5 Analytical balance**, capable of weighing to 0,000 1 g.

**5.6 Filter paper**, of appropriate porosity.

## 6 Sampling

Sampling is not part of the method specified in this part of ISO 6495. A recommended sampling method is given in ISO 6497.

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

## 7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

If solid, grind a representative part, 100 g to 150 g of the laboratory sample (usually 500 g) so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

## 8 Procedure

### 8.1 Preparation of test solution

#### 8.1.1 General

If the test sample is free from organic matter, proceed in accordance with [8.1.2](#).

If the test sample contains organic matter, proceed in accordance with [8.1.3](#), unless the test sample concerns cooked feeding stuffs, flax cakes and flour, products rich in flax flour, and other products rich in mucilage or in colloidal substances (e.g. dextrinated starch). In this case proceed in accordance with [8.1.4](#).

#### 8.1.2 Preparation of test solution of sample free from organic matter

Accurately weigh, to 0,000 1 g, not more than 10 g of the test sample ([Clause 7](#)) containing not more than 3 g of chloride. Transfer to a 500 ml volumetric flask ([5.2](#)) and add about 400 ml of water ([4.1](#)) at a temperature of approximately 20 °C.

Mix for 30 min in the rotary shaker ([5.1](#)), dilute to the mark with water ([4.1](#)), then mix, and filter through the filter paper ([5.6](#)).

Proceed in accordance with [8.2](#).

#### 8.1.3 Preparation of test solution of sample containing organic matter, excluding the products listed in 8.1.4

Accurately weigh, to 0,000 1 g, approximately 5 g of the test sample ([Clause 7](#)) containing not more than 3 g of chloride. Transfer to a 500 ml volumetric flask ([5.2](#)). Add 1 g of activated carbon ([4.8](#)), about 400 ml of water ([4.1](#)) at a temperature of approximately 20 °C, and 5 ml of Carrez I solution ([4.9](#)). Then mix and add 5 ml of Carrez II solution ([4.10](#)).

Mix for 30 min on the rotary shaker ([5.1](#)). Dilute to the mark with water ([4.1](#)), then mix and filter through the filter paper ([5.6](#)).

Proceed in accordance with [8.2](#).

#### 8.1.4 Cooked feeding stuffs, flax cakes and flour, products rich in flax flour, and other products rich in mucilage or in colloidal substances

Accurately weigh, to 0,000 1 g, approximately 5 g of the test sample ([Clause 7](#)) containing not more than 3 g of chloride. Transfer to a 500 ml volumetric flask ([5.2](#)). Add 1 g of activated carbon ([4.8](#)), about 400 ml of water ([4.1](#)) at a temperature of approximately 20 °C, and 5 ml of Carrez I solution ([4.9](#)). Then mix and add 5 ml of Carrez II solution ([4.10](#)).

Mix for 30 min on the rotary shaker (5.1). Dilute to the mark with water (4.1), then mix.

Decant (if necessary, centrifuge). By means of a pipette (5.3), transfer 100 ml of the supernatant to a 200 ml volumetric flask (5.2).

Dilute to the mark with acetone (4.2), then mix, bring the volume to the mark with acetone or water, remix and filter through the filter paper (5.6).

Proceed in accordance with 8.2.

## 8.2 Titration

By means of a pipette (5.3), transfer to a conical flask an aliquot portion of between 25 ml and 100 ml ( $V_a$ ) of the filtrate. The aliquot portion shall not contain more than 150 mg of chloride.

Dilute, if necessary, to a volume of not more than 100 ml with water. Add 5 ml of dilute nitric acid (4.5), 2 ml of saturated ammonium iron(III) sulfate solution (4.7) and 2 drops of standard ammonium or potassium thiocyanate solution (4.13) from a burette (5.4) filled to the zero mark.

Add standard silver nitrate solution (4.12) from another burette (5.4), while shaking until the reddish brown tint disappears, then add an excess of 5 ml standard silver nitrate solution (total volume of  $\text{AgNO}_3 = V_{s1}$ ). Shake vigorously to coagulate the precipitate. If necessary, 5 ml of n-hexane (4.3) may be added to assist coagulation.

Titrate the excess standard silver nitrate solution (4.12) with standard ammonium or potassium thiocyanate solution (4.13) from the burette until a reddish brown tint persists for at least 30 s (total volume of including 2 drops =  $V_{t1}$ ).

## 8.3 Blank test

Carry out a blank test in parallel with the determination, using the same procedure and the same reagents, but omitting the test portion.

## 9 Expression of results

Calculate the water-soluble chlorides content, expressed as sodium chloride, by Formula (4):

$$W_{wc} = \frac{M[(V_{s1} - V_{s0})c_s - (V_{t1} - V_{t0})c_t]}{w} \times \frac{V_i}{V_a} \times F \times 100 \quad (4)$$

where

$W_{wc}$  is the water-soluble chlorides content, expressed as sodium chloride, as percentage by mass of the test sample, in %;

$M$  is the molar mass of sodium chloride ( $M = 58,44$ ), in g/mol;

$V_{s1}$  is the volume of the silver nitrate solution added for the titration of the test solution (8.2), in ml;

$V_{s0}$  is the volume of the silver nitrate solution added for the titration of the blank solution (8.3), in ml;

$c_s$  is the concentration of the silver nitrate solution (4.12), in mol/l;

$V_{t1}$  is the volume of the ammonium or potassium thiocyanate solution used in the titration of the test solution (8.2), in ml;

$V_{t0}$  is the volume of the ammonium or potassium thiocyanate solution used in the titration of the blank solution (8.3), in ml;

$c_t$  is the concentration of the ammonium or potassium thiocyanate solution (4.13), in mol/l;

$w$  is the mass of the test portion used in 8.1.2, 8.1.3, and 8.1.4, in mg;

$V_i$  is the volume of the test solution prepared in 8.1.2, 8.1.3, and 8.1.4 ( $V_i = 500$ ), in ml;

$V_a$  is the volume of the aliquot portion of filtrate taken (8.2), in ml;

$F$  is a dilution factor.

$F = 1$  for other animal feeding stuffs (8.1.2 to 8.1.3), and

$F = 2$  for cooked feeding stuffs, flax cakes and flour, products rich in flax flour and other products in mucilage or in colloidal substances (see 8.1.4).

## 10 Precision

### 10.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentrations ranges and matrices other than those given.

## 10.2 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 the repeatability limit ( $r$ ) derived from Formula (5):

$$r = 0,134(w_{wc})^{0,521} \quad (5)$$

where

$r$  is the repeatability limit, expressed as percentage by mass;

$w_{wc}$  is the mean of the two single test results, expressed as percentage by mass.

## 10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit ( $R$ ) derived Formula (6):

$$R = 0,552 \% + 0,135w_{wc} \quad (6)$$

where

$R$  is the reproducibility limit, expressed as percentage by mass;

$w_{wc}$  is the mean of the two single test results, expressed as percentage by mass.

## 11 Test report

The test report shall specify the following:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this part of ISO 6495, i.e. ISO 6495-1;
- all operating details not specified in this part of ISO 6495, or regarded as optional, together with details of any incidents which may have influenced the test results;
- the test result(s) obtained; or if the repeatability has been checked, the final quoted result obtained.

## Annex A (informative)

### Results of interlaboratory test

An interlaboratory test was organized by ISO/TC 34/SC 10 “Animal feeding stuffs”, in 1987 and carried out in accordance with ISO 5725:1986.<sup>[1]</sup> The final statistical analysis was carried out in accordance with ISO 5725-2:1994.<sup>[3]</sup> In the test, 24 laboratories participated.

**Table A.1 — Statistical results of the interlaboratory test parameter (after removing of outliers)**

Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number of laboratories retained after eliminating outliers	22	21	21	22	21	22
Mean (%)	3,226	1,62	14,3	3,52	2,49	1,07
Repeatability standard deviation, $s_r$ , %	0,112	0,056	0,21	0,10	0,08	0,03
Repeatability coefficient of variation, $CV_r$ (%)	3,5	3,5	1,46	2,92	3,11	2,9
Repeatability limit, $r$ ( $r = 2,8 \times s_r$ ), %	0,312	0,156	0,586	0,287	0,217	0,087
Horror value ( $Ho_r$ )	1,6	1,4	0,8	1,3	1,4	1,1
Reproducibility standard deviation, $S_R$ , %	0,22	0,27	0,55	0,406	0,192	0,16
Reproducibility coefficient of variation, $CV_R$ (%)	7	16	4	12	8	15
Reproducibility limit, $R$ ( $R = 2,8 \times S_R$ ), %	0,612	0,744	1,537	1,138	0,537	0,443
Horror value ( $Ho_R$ )	2,0	4,4	1,4	3,5	2,2	3,7

Sample 1: Fish meal ([8.1.3](#))

Sample 4: Mixed feed stuff concentrate ([8.1.4](#))

Sample 2: Yeast ([8.1.3](#))

Sample 5: Mixed feed stuff concentrate ([8.1.4](#))

Sample 3: Premixed feed stuff ([8.1.2](#), [8.1.4](#))

Sample 6: Finished mixed feed stuff ([8.1.4](#))

## Bibliography

- [1] ISO 5725:1986,<sup>1)</sup>*Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*
- [2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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1) This document has been withdrawn. [Replaced by ISO 5725 (all parts).]







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