



**SLOVENSKI STANDARD**  
**SIST EN 13191-2:2001**

**01-februar-2001**

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Non-fatty food - Determination of bromide residues - Part 2: Determination of inorganic bromide

Fettarme Lebensmittel - Bestimmung von Bromidrückständen - Teil 2: Bestimmung von anorganischem Bromid

Aliments non gras - Détermination des résidus de bromures - Partie 2: Détermination des bromures inorganiques

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**Ta slovenski standard je istoveten z: EN 13191-2:2000**

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**ICS:**

67.050

Splošne preskusne in  
analizne metode za živilske  
proizvode

General methods of tests and  
analysis for food products

**SIST EN 13191-2:2001**

**en**

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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

EN 13191-2

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ICS 67.050

English version

Non-fatty food - Determination of bromide residues - Part 2:  
Determination of inorganic bromide

Aliments non gras - Détermination des résidus de bromures  
- Partie 2: Détermination des bromures inorganiques

Fettarme Lebensmittel - Bestimmung von  
Bromidrückständen - Teil 2: Bestimmung von  
anorganischem Bromid

This European Standard was approved by CEN on 8 April 2000.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

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**Foreword**

## iTeh STANDARD PREVIEW (standards.iteh.ai)

This European Standard has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN. [EN 13191-2:2001](https://standards.iteh.ai/catalog/standards/sist/8b381c8c-ae3e-4287-8b76-)

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This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2000, and conflicting national standards shall be withdrawn at the latest by November 2000.

This European Standard "Non-fatty foods - Determination of bromide residues" consists of two parts:

Part 1: Determination of total bromide as inorganic bromide

Part 2: Determination of inorganic bromide

The Annexes A and B are informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

## 1 Scope

This European Standard specifies a gas chromatographic method for the determination of inorganic bromide residues in non-fatty foods.

Generally, the maximum residue levels are expressed in terms of bromide ion from all sources but not including covalently bound bromine.

The method is applicable to cereals, dried fruit, dried vegetables, dried mushrooms, fresh fruit and vegetables. It has been validated in interlaboratory studies on maize flour, carrot flakes, lettuce, potatoes, cereal flour and hazelnuts [1], [2].

## 2 Normative reference

This European Standard incorporates, by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN ISO 3696            Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)

## 3 Principle

The test portion is suspended in an aqueous solution of propylene oxide acidified with sulfuric acid whereupon inorganic bromide is extracted simultaneously and converted to a mixture of 1-bromo-2-propanol and 2-bromo-1-propanol [3] (derivatization A). The derivatives are partitioned into ethyl acetate and determined by gas chromatography with electron-capture detection [4], [5].

As an alternative, ethylene oxide which is more difficult to handle and which is somewhat more toxic, can be used instead of propylene oxide. In this case (derivatization B) inorganic bromide is converted to 2-bromoethanol [4], [5].

## 4 Reagents

### 4.1 General and safety aspects

Unless otherwise specified, use reagents of recognized analytical grade, preferably for pesticide residue analysis, and only water of grade 2 according to EN ISO 3696.

Take every precaution to avoid possible contamination of water, solvents, inorganic salts, etc. by plastics and rubber materials. Use only glass containers for storage and handling of all water and reagents.

**WARNING:** Ethylene oxide and propylene oxide are highly reactive and cancerogenic. Work always in a well-ventilated fume hood. Consult the safety data sheet of the manufacturer for information and follow local instructions.

To destroy excess ethylene and propylene oxide solutions, add a surplus of sodium chloride solution, shake several times and allow the mixture to stand for some hours.

**4.2 Propylene oxide**,  $\Phi$  ( $C_3H_6O$ ) of at least 99,5 % volume fraction. Store at approximately 4 °C.

### 4.3 Propylene oxide solution

In a well-ventilated fume hood, pour 95 ml of ice-cold water into a 100 ml volumetric flask, and add propylene oxide (4.2) dropwise to the mark and mix well. Store in a refrigerator at 4 °C. Prepare fresh daily.

**4.4 Ethylene oxide (optional)**,  $\Phi$  ( $C_2H_4O$ ) of at least 99,5 % volume fraction, in pressurized can fitted with valve. Store at approximately - 20 °C.

### 4.5 Ethylene oxide solution (optional)

In a well-ventilated fume hood, pour 96 ml of ice-cold water into a 100 ml volumetric flask, and add ethylene oxide (4.4) dropwise to the mark from the completely inverted, ice-cold pressurized can and mix well. Store at approximately 4 °C. Prepare fresh daily.

**4.6 Sulfuric acid**,  $\alpha(H_2SO_4) = 3$  mol/l.

#### 4.7 Ethyl acetate

Before use, check each newly opened bottle by injecting the same volume into the gas chromatograph as used in 6.3. If interfering peaks are observed, purify the ethyl acetate by distillation.

**WARNING:** Ethyl acetate is flammable and is irritating to eyes and respiratory tract.

#### 4.8 Ammonium sulfate

**4.9 Sodium sulfate**, anhydrous. Heat for 5 h at 500 °C. Cool in a desiccator.

**4.10 2-Bromoethanol standard solution** in ethyl acetate,  $\rho$  (C<sub>2</sub>H<sub>5</sub>BrO) = 1 mg/l.

**4.11 Potassium bromide**. Heat for 1 h at 130 °C.

**4.12 Bromide stock solution**,  $\rho$  (Br<sup>-</sup>) = 50 mg/l.

Dissolve 149 mg of potassium bromide (4.11) in 100 ml of water, and dilute 5 ml of this solution with water to a volume of 100 ml.

#### 4.13 Bromide standard solutions

Prepare appropriate dilutions of the bromide stock solution (4.12) which contain 2 µg, 5 µg, 10 µg, 25 µg and 50 µg of bromide per millilitre.

### 5 Apparatus

Usual laboratory apparatus and, in particular, the following:

**5.1 Homogenizer or high speed blender**, fitted with glass jar.

**5.2 Test tubes**, e.g. 20 ml, with ground joint.

**5.3 Conical flask**, e.g. 200 ml, with ground joint.

**5.4 Gas chromatograph**, equipped with an electron-capture detector.

### 6 Procedure

#### 6.1 Extraction and derivatization

##### 6.1.1 Method with propylene oxide (Derivatization A)

For cereals and dried materials, weigh 1,0 g of the finely ground powder (test portion) into a conical flask (5.3), slurry in 10 ml of water, add 10 ml propylene oxide solution (4.3) and add 2 ml of sulfuric acid (4.6).

For fresh fruit and vegetables, homogenize the material in a blender (5.1), weigh 10,0 g of the homogenate (test portion) into a conical flask, and add 10 ml of propylene oxide solution and 2 ml of sulfuric acid.

In both cases, stopper the flask and allow to stand for 1 h at room temperature.

##### 6.1.2 Method with ethylene oxide (Derivatization B)

For cereals and dried materials, weigh 1,0 g of the finely ground powder (test portion) into a conical flask (5.3), slurry in 10 ml of ethylene oxide solution (4.5) and add 1 ml of sulfuric acid (4.6).

For fresh fruit and vegetables, homogenize the test sample in a blender (5.1), weigh 5,0 g of the homogenate (test portion) into a conical flask, and add 5 ml of ethylene oxide solution and 1 ml of sulfuric acid.

In both cases, stopper the flask and allow to stand for 30 min at room temperature.

#### 6.2 Partition

Add 50 ml of ethyl acetate (4.7) and 4,0 g of ammonium sulfate (4.8). Stopper the flask, shake vigorously for 1 min, and then allow to stand for approximately 20 min with occasional shaking. Decant approximately 10 ml of the upper organic phase into a test tube (5.2). Add 0,5 g of sodium sulfate (4.9), stopper the test tube and shake vigorously (sample test solution).

#### 6.3 Gas chromatography

Inject equal volumes of the sample test solution derived from 6.2 and either of the solutions obtained in 6.5 for the calibration curve (derivatization A) or of dilutions of the 2-bromoethanol standard solution (4.10) (derivatization B) into the gas chromatograph (1).

Make sure that the gas chromatographic conditions (column length, stationary phase type, injector, detector and column temperatures, gas flow rates, etc.) are such that the separation of the bromine derivatives from possible interfering peaks originating from the samples is as complete as possible.

Typical gas chromatographic operating conditions are given in annex A.

NOTE 1: Due to their higher separation efficiency, capillary columns are preferably used.

NOTE 2: Two chloropropanols (derivatization A) or 2-chloroethanol (derivatization B), respectively, are formed from chloride ions naturally occurring in the sample material. Their peaks, however, have shorter retention times than the corresponding bromo derivatives and do not interfere with the determination.

#### 6.4 Test for interferences and recoveries

Prepare reagent blanks and carry out spiked recovery tests at levels appropriate to the maximum residue level.

The chromatogram of the reagent blank should not show a peak at the retention times of the bromopropanols (derivatization with propylene oxide) or of 2-bromoethanol (derivatization with ethylene oxide), respectively.

NOTE: Occasionally, a tailing peak with a longer retention time appears which is due to propylene glycol or ethylene glycol, respectively, and does not interfere with the determination.

#### 6.5 Standard solutions

Prepare appropriate dilutions of the bromide stock solution (4.12) which contain 2 µg, 5 µg, 10 µg, 25 µg and 50 µg of bromide per millilitre. To 1,00 ml each of the bromide standard solutions (4.13), add 9 ml of water, 10 ml of propylene oxide solution (4.3) and 2 ml of sulfuric acid. Allow to stand for 1 h at room temperature and follow the procedure in 6.2 and 6.3. Plot a calibration curve from the peak areas (or peak heights) obtained for the higher one of the two bromopropanol peaks against the mass of bromide added.

### 7 Evaluation of results

#### 7.1 Calculation for propylene oxide derivatization A

Read off the mass of bromide present in the injection volume from the calibration curve. Calculate the mass fraction  $w$  of bromide, in milligrams per kilogram, using equation (1):

$$w = \frac{x}{m_s} \quad (1)$$

where:

$x$  is the mass of bromide read off from the calibration curve, in micrograms;

$m_s$  is the mass of the test portion, in grams.

If the results indicate that the amount of residue approaches or exceeds the maximum residue level, examine at least two further test portions.

#### 7.2 Calculation for ethylene oxide derivatization B

Measure the peak height (or peak area) obtained from the sample test solution and compare it with the peak heights (or peak areas) obtained from appropriate dilutions of the 2-bromoethanol standard solution (4.10).

Calculate the mass fraction  $w$  of bromide, in milligrams per kilogram, using equation (2):

$$w = \frac{F_A \times V_{end} \times m_{St}}{F_{St} \times V_i \times m_s} \times 0,639 \quad (2)$$

where:

$m_s$  is the mass of the test portion, in grams;

$V_{end}$  is the volume of ethyl acetate used for partition in 6.2 (here: 50), in millilitres;

$V_i$  is the injection volume of the sample test solution, in microlitres;

- $m_{St}$  is the mass of 2-bromoethanol injected with standard solution, in nanograms;  
 $F_A$  is the peak area or peak height, obtained for the injected sample test solution;  
 $F_{St}$  is the peak area or peak height, obtained for  $m_{St}$  in the injected standard solution;  
 0,639 is the factor for conversion of 2-bromoethanol to bromide.

If the results indicate that the amount of residue approaches or exceeds the maximum residue level, examine at least two further test portions.

## 8 Confirmatory tests

The bromide content can be confirmed by using the alternative extraction and derivatization method (6.1.1 or 6.1.2), respectively.

## 9 Precision

### 9.1 General

Details of the interlaboratory tests of the precision of the method are summarized in Annex B. The values derived from the interlaboratory tests according to ISO 5725 : 1986 [6] may not be applicable to analyte concentration ranges and matrices other than given in Annex B.

### 9.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit  $r$  in not more than 5 % of the cases.

The values for derivatization with propylene oxide are:

Lettuce	$\bar{x}$	=	49,2 mg/kg	$r$	=	7,4 mg/kg
Potatoes	$\bar{x}$	=	4,3 mg/kg	$r$	=	0,83 mg/kg
cereal flour	$\bar{x}$	=	23,5 mg/kg	$r$	=	2,83 mg/kg
hazelnuts	$\bar{x}$	=	120,0 mg/kg	$r$	=	16,1 mg/kg

The values for derivatization with ethylene oxide are:

maize flour	$\bar{x}$	=	42,0 mg/kg	$r$	=	4,5 mg/kg
carrot flakes	$\bar{x}$	=	26,1 mg/kg	$r$	=	3,1 mg/kg

### 9.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit  $R$  in not more than 5 % of the cases.

The values for derivatization with propylene oxide are:

Lettuce	$\bar{x}$	=	49,2 mg/kg	$R$	=	19,5 mg/kg
Potatoes	$\bar{x}$	=	4,3 mg/kg	$R$	=	1,79 mg/kg
cereal flour	$\bar{x}$	=	23,5 mg/kg	$R$	=	7,13 mg/kg
hazelnuts	$\bar{x}$	=	120,0 mg/kg	$R$	=	45,9 mg/kg

The values for derivatization with ethylene oxide are:

maize flour	$\bar{x}$	=	42,0 mg/kg	$R$	=	7,0 mg/kg
carrot flakes	$\bar{x}$	=	26,1 mg/kg	$R$	=	9,4 mg/kg



## 10 Test report

The test report shall contain at least the following information:

- all information necessary for the identification of the sample;
- a reference to this European Standard or to the method used;
- the results and the units in which the results have been expressed;
- date and type of sampling procedure (if known);
- date of receipt of sample in the laboratory;
- date of test;
- any particular points observed in the course of the test;
- any operations not specified in the method or regarded as optional which might have affected the results.

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