SLOVENSKI STANDARD
SIST ISO 6463:1998

01-december-1998

Ģivalske in rastlinske maģļ obe in olja - Doloļ evanje butilhidroksianizola (BHA) in butilhidroksitoluena (BHT) - Plinsko-tekoļ inska kromatografska metoda

Animal and vegetable fats and oils -- Determination of butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) -- Gas-liquid chromatographic method

Corps gras d'origines animale et végétale -- Dosage du butylhydroxyanisol (BHA) et du butylhydroxytoluène (BHT) -- Méthode par chromatographie en phase gazeuse

Ta slovenski standard je istoveten z: **ISO 6463:1982**

ICS:

67.200.10	Rastlinske in ģivalske maģļ obe in olja	Animal and vegetable fats and oils

SIST ISO 6463:1998 **en**

International Standard 6463

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Animal and vegetable fats and oils — Determination of butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) — Gas-liquid chromatographic method

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First edition — 1982-09-01

UDC 664.3 : 543.544.45 : 547.53 **Ref. No. ISO 6463-1982 (E)**

Descriptors : agricultural products, animal fats, vegetable fats, determination, antioxidants, gas chromatographic analysis.

Price based on 3 pages

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 6463 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1981.

It has been approved by the member bodies of the following countries :

Australia	India	Romania
Austria	Iran	South Africa, Rep. of
Canada	Iraq	Sri Lanka
Chile	Israel	Tanzania
Czechoslovakia	Italy	Thailand
Dominican Republic	Kenya	United Kingdom
Egypt, Arab Rep. of	Korea, Rep. of	USA
Ethiopia	Mexico	USSR
France	Netherlands	Yugoslavia
Germany, F. R.	New Zealand	
Hungary	Portugal	

No member body expressed disapproval of the document.

This International Standard has also been approved by the International Union of Pure and Applied Chemistry (IUPAC).

Printed in Switzerland

Animal and vegetable fats and oils — Determination of butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) — Gas-liquid chromatographic method

1 Scope and field of application

This International Standard specifies a gas-liquid chromatographic method for the determination of butylhydroxyanisole (*tert*-butyl-4-methoxyphenol) (BHA) and butylhydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol) (BHT), used as antioxidants, in animal and vegetable fats and oils.

NOTE — The method also permits quantitative determination of tertiobutylhydroquinone (TBHQ).

2 Reference

ISO 5558, *Animal and vegetable fats and oils — Detection and identification of antioxidants — Thin-layer chromatographic method.*

3 Principle

Dissolution of the fat or oil in a suitable solvent, direct injection into a gas chromatograph, and use of the internal standard method of calibration.

4 Reagents

4.1 Carrier gas : an inert gas (such as nitrogen, helium or argon), carefully dried and containing less than 10 mg of oxygen per kilogram.

4.2 Auxiliary gases :

— hydrogen, minimum purity 99,9 %, free from organic compounds;

— air or oxygen, free from organic compounds.

4.3 Dichloromethane or, failing this, **carbon disulphide**, containing no impurities which could interfere with the determination of BHA or BHT by gas chromatography.

WARNING — Dichloromethane and carbon disulphide are toxic. In addition, carbon disulphide is very volatile and explosive and particular care must be exercised in using it.

4.4 Methyl undecanoate, minimum purity 99 %.

4.5 Butylhydroxyanisole, minimum purity 98 %.

4.6 Butylhydroxytoluene, minimum purity 98 %.

5 Apparatus

Usual laboratory equipment, and in particular :

5.1 Gas chromatograph, with a **flame ionization detector** and **recorder**, comprising :

5.1.1 Injection device, together with one of the following systems to retain the non-volatile fats and oils :

a) a pre-column packed with siliconized glass wool or glass beads;

b) a sleeve lined with siliconized glass wool placed in the injector (only in the case of a horizontal injector).

5.1.2 Column, made of stainless steel or glass, permitting good separation of BHA and BHT, of length about 2 m and 2 to 4 mm in internal diameter, packed, for example, with 10 % methylpolysiloxanes[1] on acid-washed, silylated brick dust.[2]

1) DC 200 [of kinematic viscosity 1,25 m^2/s (12 500 cSt)] is suitable.

2) Gas/Chrom Q, of particle size 150 to 180 µm (80 to 100 mesh) is suitable.

5.2 Volumetric flasks, of capacities 10, 20 and 100 ml.

5.3 Graduated pipettes, of capacities 1 and 2 ml.

5.4 Analytical balance.

6 Detection

See ISO 5558.

7 Procedure

7.1 Setting up the apparatus

7.1.1 Injection device

— Temperature : 250 °C

The sleeve or pre-column (5.1.1) shall be removed after each day of analysis and conditioned overnight at the test temperature.

NOTE — Verify the proper condition of the sleeve or pre-column by passing, from time to time, a fat or oil of known composition through the chromatograph.

7.1.2 Oven and column

— Temperature under isothermal conditions : 160 °C

— Flow rate of carrier gas : optimum value to be established by the operator.

Before first use, condition the filled columns for 24 h at 220 °C with the carrier gas flowing.

7.1.3 Detector

— Temperature : 250 °C

— Flow rate of auxiliary gases :

hydrogen : approximately 20 ml/min

air or oxygen : according to the manufacturer's instructions.

7.2 Calibration

7.2.1 Method

Use the internal calibration method, in which a known quantity of a known compound, the peak corresponding to which does not interfere with other peaks, is added to the sample and the measurements of the peaks of the various constituents, corrected using their respective calibration coefficients, are compared with the measurement of the peak of the known compound.

7.2.2 Standard mixtures

7.2.2.1 Internal standard

Use as the internal standard a 30 µg/ml methyl undecanoate solution, prepared as follows.

Weigh, to the nearest 0,1 mg, 30 mg of the methyl undecanoate (4.4) into a 100 ml volumetric flask (5.2). Dilute to the mark with the solvent (4.3). Transfer 2 ml of this solution, by means of a pipette (5.3), to a 20 ml volumetric flask (5.2) and dilute to the mark with the solvent.

7.2.2.2 Standard antioxidant solutions

Weigh, to the nearest 0,1 mg, exactly 100 mg of antioxidant (BHA or BHT) (4.5 or 4.6) into a 100 ml volumetric flask (5.2). Dilute to the mark with the solvent (4.3). Transfer 1 ml of this solution, by means of a pipette (5.3), to a 10 ml volumetric flask (5.2) and dilute to the mark with the solvent.

Into a series of five 10 ml volumetric flasks (5.2), introduce, by means of a pipette (5.3), 0,2 — 0,5 — 0,8 — 1 and 1,2 ml of the antioxidant solution. Add, by means of a pipette, to each flask, 2 ml of the internal standard (7.2.2.1) and dilute to the mark with the solvent.

These five solutions contain, respectively, 2, 5, 8, 10 and 12 µg of antioxidant per millilitre.

NOTE — Verify, by means of a blank test, that there is no interference with the methyl undecanoate. If there is, use methyl myristate as the internal standard.

7.2.3 Determination of the calibration coefficient and plotting the calibration graph

Inject each solution (7.2.2.2) into the chromatograph and calculate the proportionality coefficient K from the formula

$$K = \frac{A_a}{A_s} \times \frac{m_s}{m_a}$$

where

A_a is the area of the peak corresponding to the antioxidant;

A_s is the area of the peak corresponding to the internal standard;

m_a is the mass, in grams, of antioxidant in the standard solution;

m_s is the mass, in grams, of internal standard added.

If required, plot a graph having the ratios of the area of the peaks corresponding to the antioxidant to the area of the peak corresponding to the internal standard as ordinates and the concentrations of antioxidant in the solutions injected as abscissae.

7.3 Determination

Weigh, to the nearest 1 mg, 1 g of the fat or oil and transfer it to a 10 ml volumetric flask (5.2). Add 2 ml of the internal standard (7.2.2.1) and dilute to the mark with the solvent (4.3).

Ensure that the volumetric flask is stoppered each time.

Inject 1 to 7 µl of the mixture into the chromatograph.

8 Expression of results

The BHA or BHT content, expressed in milligrams per kilogram (ppm) of product, is equal to

$$\frac{m_s \times A_a}{m \times A_s \times K}$$

where

m is the mass, in grams, of the test portion (7.3);

m_s is the mass, in micrograms, of internal standard added (approximately 60 µg);

A_a is the area of the peak corresponding to the antioxidant;

A_s is the area of the peak corresponding to the internal standard;

K is the proportionality coefficient for the antioxidant with respect to the internal standard.

9 Test report

The test report shall show the method used and the results obtained. It shall also mention any operating conditions not specified in this International Standard or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.