



SLOVENSKI STANDARD SIST EN ISO 6867:2001

01-maj-2001

Animal feeding stuffs - Determination of vitamin E content - Method using high-performance liquid chromatography (ISO 6867:2000)

Futtermittel - Bestimmung des Gehalts an Vitamin E -Hochleistungs-flüssigchromatographisches Verfahren (ISO 6867:2000)

Aliments des animaux - Détermination de la teneur en vitamine E - Méthode par chromatographie liquide a haute performance (ISO 6867:2000)

Ta slovenski standard je istoveten z: EN ISO 6867:2000

ICS:

65.120

Krmila

Animal feeding stuffs

SIST EN ISO 6867:2001

en

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 6867

December 2000

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English version

Animal feeding stuffs - Determination of vitamin E content -
Method using high-performance liquid chromatography (ISO
6867:2000)

Aliments des animaux - Détermination de la teneur en
vitamine E - Méthode par chromatographie liquide à haute
performance (ISO 6867:2000)

Futtermittel - Bestimmung des Gehalts an Vitamin E -
Hochleistungs-flüssigchromatographisches Verfahren (ISO
6867:2000)

This European Standard was approved by CEN on 1 December 2000.

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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 Management Centre has the same status as the official versions.

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

Management Centre: rue de Stassart, 36 B-1050 Brussels

Foreword

The text of the International Standard ISO 6867:2000 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis", the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by June 2001, and conflicting national standards shall be withdrawn at the latest by June 2001.

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.

Endorsement notice

The text of the International Standard ISO 6867:2000 was approved by CEN as a European Standard without any modification.

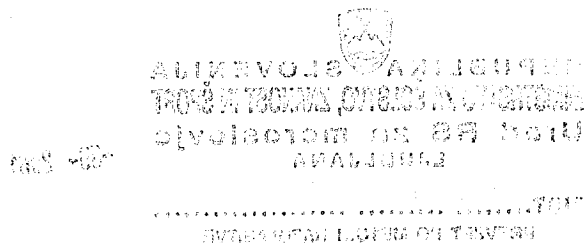
NOTE: Normative references to International Standards are listed in annex ZA (normative).

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Annex ZA
(normative)**Normative references to international publications with their corresponding European publications**

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 (including amendments).

NOTE Where an International Publication has been modified by common modifications, indicated by (mod.), the relevant EN/HD applies.

<u>Publication</u>	<u>Year</u>	<u>Title</u>	<u>EN/HD</u>	<u>Year</u>
ISO 3696	1987	Water for analytical laboratory use - Specification and test methods	EN ISO 3696	1995

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INTERNATIONAL
STANDARD

ISO
6867

First edition
2000-12-01

**Animal feeding stuffs — Determination of
vitamin E content — Method using high-
performance liquid chromatography**

*Aliments des animaux — Détermination de la teneur en vitamine E —
Méthode par chromatographie liquide à haute performance*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6867 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annex A of this International Standard is for information only.

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Animal feeding stuffs — Determination of vitamin E content — Method using high-performance liquid chromatography

1 Scope

This International Standard specifies a method for the determination of the vitamin E (DL- α -tocopherol) content of animal feeding stuffs and pet foods using high performance liquid chromatography.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 6498, *Animal feeding stuffs — Preparation of test samples*.

3 Principle

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A test portion of the sample is saponified with ethanolic potassium hydroxide solution and the vitamin E is extracted into light petroleum. The light petroleum is removed by evaporation and the residue is dissolved in hexane. The vitamin E concentration in the hexane extract is determined by normal-phase liquid chromatography using conditions that separate DL- α -tocopherol from other tocopherols.

4 Reagents

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

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

4.2 Potassium hydroxide solution.

Dissolve 500 g of potassium hydroxide in water (4.1) and dilute to 1 litre.

4.3 Ethanol, $w(\text{C}_2\text{H}_5\text{OH}) = 95\%$ (by volume), or equivalent industrial methylated spirit.

4.4 Hexane, HPLC grade.

4.5 Light petroleum, boiling range 40 °C to 60 °C; the residue on evaporation shall be less than 20 mg/l.

4.6 Vitamin E standard substance: DL- α -tocopherol, minimum purity not less than 96,0 %.

The purity of the standard substance should be checked spectrophotometrically (see 8.5.2).

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- 4.7 **1,4-Dioxan**, HPLC grade.
- 4.8 **Sodium sulfate** (Na_2SO_4), anhydrous.
- 4.9 **Sodium ascorbate solution**, $\rho = 100$ g/l.
- 4.10 **Inert gas**, e.g. nitrogen.
- 4.11 **Mobile phase for liquid chromatography**.
Mix 30 ml 1,4-dioxan (4.7) with 970 ml hexane (4.4).
Filter through a membrane filter (5.5) before use.
- 4.12 **Ethanol**, $w(\text{C}_2\text{H}_5\text{OH}) = 96$ % (by volume).
- 4.13 **Methanol** (CH_3OH), HPLC grade.

5 Apparatus

Using laboratory apparatus and, in particular, the following.

- 5.1 **High-performance liquid chromatograph**, consisting of the following.
- 5.1.1 **Pump**, set to deliver a constant eluent volume flow rate of 1,5 ml/min.
- 5.1.2 **HPLC injection device**.
- 5.1.3 **Column**, length 250 mm, internal diameter 4,6 mm, packed with a stationary phase consisting of silica.
A column with at least 5 000 theoretical plates and a k' value of 0,8 m, both with respect to DL- α -tocopherol, has been found to be satisfactory. The particle size should not be smaller than 5 μm and not greater than 10 μm . Other systems may be used provided that a satisfactory separation of vitamin E from other co-extractives is achieved.
- 5.1.4 **Detector**, allowing the measurement of fluorescence emitted at a wavelength of 326 nm when the column eluent is irradiated with ultraviolet light at a wavelength of 293 nm, with integrator/recorder.
- 5.2 **Boiling water bath**.
- 5.3 **Rotary vacuum evaporator**, with water bath at 40 °C.
- 5.4 **Extraction apparatus** (see Figure 1) consisting of the following:
- a cylinder of 1 litre capacity fitted with a ground glass neck and stopper;
 - a ground glass joint, fitting the cylinder and equipped with an adjustable tube passing through the centre; and
 - a side-arm.
- The adjustable tube should have a U-shaped lower end and a jet at the opposite end so that the upper liquid layer in the cylinder may be transferred to a separating funnel of 1 litre capacity.
- Other extraction equipment such as conical flasks and separating funnels may be used in place of the apparatus shown in Figure 1, provided that satisfactory recoveries of vitamin E are achieved.
- 5.5 **Membrane filter**, 0,45 μm pore size, for filtration of mobile phase (4.11) and sample test solutions.