

Animal feeding stuffs: Methods of sampling and analysis - Determination of vitamin A, E and D content - Method using solid phase extraction (SPE) clean-up and high-performance liquid chromatography (HPLC)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN 17547:2021 sisaldab Euroopa standardi EN 17547:2021 ingliskeelset teksti.	This Estonian standard EVS-EN 17547:2021 consists of the English text of the European standard EN 17547:2021.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 10.11.2021.	Date of Availability of the European standard is 10.11.2021.
Standard on kättesaadav Eesti Standardimis- ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 65.120

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardimis- ja Akrediteerimiskeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardimis- ja Akrediteerimiskeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autoriõiguse kaitse kohta, võtke palun ühendust Eesti Standardimis- ja Akrediteerimiskeskusega: Koduleht www.evs.ee; telefon 605 5050; e-post info@evs.ee

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation and Accreditation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation and Accreditation.

If you have any questions about standards copyright protection, please contact the Estonian Centre for Standardisation and Accreditation: Homepage www.evs.ee; phone +372 605 5050; e-mail info@evs.ee

English Version

Animal feeding stuffs: Methods of sampling and analysis -
Determination of vitamin A, E and D content - Method
using solid phase extraction (SPE) clean-up and high-
performance liquid chromatography (HPLC)

Aliments des animaux - Méthodes d'échantillonnage et
d'analyse - Détermination de la teneur en vitamines A,
E et D - Méthode utilisant la purification par extraction
en phase solide (SPE) et la chromatographie liquide à
haute performance (CLHP)

Futtermittel - Probenahme- und
Untersuchungsverfahren - Bestimmung des Gehalts an
Vitamin A, E und D - Verfahren mittels Reinigung durch
Festphasenextraktion und Hochleistungs-
Flüssigchromatographie

This European Standard was approved by CEN on 27 September 2021.

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 CEN-CENELEC Management Centre 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 CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Contents	Page
European foreword	3
Introduction	4
1 Scope.....	5
2 Normative references.....	5
3 Terms and definitions.....	5
4 Principle	6
5 Reagents and materials	8
6 Apparatus	9
7 Sampling.....	10
8 Sample preparation	11
9 Procedure	11
10 Expression of results	21
11 Observations.....	24
12 Precision	25
13 Test report.....	26
Annex A (informative) Examples of combinations of weighing, aliquot and dilution to reach concentrations within the calibration curve	27
Annex B (informative) Preparation of stock standard solution of vitamin E (α-tocopherol) from α-tocopherol acetate.....	30
B.1 General.....	30
B.2 Reagents.....	30
B.3 Preparation of stock standard	30
B.4 Standardization of the vitamin E (α-tocopherol) stock standard solution in cyclohexane.....	30
B.5 Calibration solutions and plotting of calibration graph for vitamins A (retinol) and E (α-tocopherol).....	31
Annex C (informative) Results of the interlaboratory study.....	32
Bibliography	37

European foreword

This document (EN 17547:2021) has been prepared by 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 May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.

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

This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

WARNING — The method described in this document implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this document to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

1 Scope

This document specifies a method for the determination of the content of the total vitamin A (retinol), vitamin E (α -tocopherol) and vitamin D₃ (cholecalciferol) in animal feed using solid phase extraction (SPE) clean-up and high-performance liquid chromatography (HPLC).

NOTE The procedure also enables determination of vitamin D₂ but with the use of another internal standard. The method is fully validated only for vitamin D₃.

The method has been successfully tested in collaborative trial for complete feed for broilers, pigs, and turkey, for premixture for broilers and piglets, for complementary feed for cows and mineral feed within the following ranges:

- vitamin A: 4 365 IU/kg – 4 118 352 IU/kg;
- vitamin E: 22 mg/kg – 13 800 mg/kg;
- vitamin D₃: 1 668 IU/kg – 1 638 150 IU/kg.

The limits of quantification were not determined within the validation study. Quantification limits of 1 100 IU for vitamin A/kg (using UV-detection), 4 mg for vitamin E/kg (using UV-detection), 2 mg for vitamin E/kg (using fluorescence detection) and 2 000 IU for vitamin D/kg (using UV-detection) should be normally achieved. Lower limits are possible provided they are validated by the user.

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.

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

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:

- IEC Electropedia: available at <https://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

3.1

vitamin A content

retinol

content of all-trans- and cis-isomers of retinol determined in accordance with this document

Note 1 to entry: The vitamin A (retinol) content is expressed in International Units per kilogram (IU/kg).

Note 2 to entry: 1 IU of vitamin A (retinol) is equal to 0,300 μ g of all-trans-retinol or 0,344 μ g all-trans-retinol acetate or 0,546 μ g all-trans-retinol palmitate or 0,359 μ g all-trans-retinol propionate.