SLOVENSKI STANDARD
oSIST prEN 16215:2018

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Krma: metode vzorčenja in analize - Določevanje dioksinov in dioksinu podobnih PCB z GC/HRMS in indikatorjev PCB z GC/HRMS

Animal feeding stuffs: Methods of sampling and analysis - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung von Dioxinen und dioxin-ähnlichen PCB mittels GC/HRMS und von Indikator-PCB mittels GC/HRMS

Aliments des animaux : Méthodes d'échantillonnage et d'analyse - Dosage des dioxines, des PCB de type dioxine et des PCB indicateurs par GC/HRMS

Ta slovenski standard je istoveten z: **prEN 16215**

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Animal feeding stuffs: Methods of sampling and analysis - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 327.

If this draft becomes a European Standard, 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.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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Contents

Page

European foreword

This document (prEN 16215:2018) 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 document is currently submitted to the CEN Enquiry.

This document will supersede EN 16215:2012.

In comparison with the previous edition, the following technical modifications have been made:

— incorrect technical details have been corrected,

— references have been updated,

— inconsistencies were removed, and

— editorial adaptations have been made.

Introduction

The previous version of this document was developed in response to Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. The document provides analytical laboratories active in the field of feed analysis with guidance for the analysis of dioxins and PCBs and meets criteria as set in Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed.

In this updated version, obvious mistakes were corrected.

WARNING — The use of this document can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This document is applicable to the determination of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), (together termed 'dioxins' (PCDD/Fs)) and dioxin-like PCBs and non-dioxin-like PCBs (dl-PCBs and ndl-PCBs) in animal feeding stuffs. Collaborative studies have been carried out. The method is suitable for the determination of dioxins, dl-PCBs and ndl-PCBs at the appropriate MRL in compound feed and ingredients e.g. oil, mineral clay. The method is applicable to samples containing trace level amounts of one or more dioxins, dioxin-like PCBs and non-dioxin-likePCBs. The limit of quantification (LOQ) is

— 0,05 pg/g (OCDD/F = 0,1 pg/g) for the relevant individual congeners of dioxins/furans,

— 0,05 pg/g for non-ortho PCBs,

— 10 pg/g for mono-ortho PCBs, and

— 100 pg/g for non-dioxin-like-PCBs.

For determination of dioxins and dioxin-like PCBs, the procedure can be used as confirmatory method as defined by Commission Regulation (EC) No 152/2009 for dioxins and dl-PCB in feed [1]. Confirmatory methods as described in this standard are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. If only the analysis of non-dioxin-like PCBs is required, a GC-LRMS method can be used (e.g. EN 15741 [2]) provided that appropriate analytical performance criteria are met in the relevant range for the matrix of interest.

This document is split into four modules. Each module describes a part of the whole procedure (see Figure 1 and Figure 2) to be followed:

a) Module A: Description of standards which might be used;

b) Module B: Description of extraction procedures;

c) Module C: Description of clean-up procedures;

d) Module D: GC/HRMS determination.

Each module describes a part of the whole method as well as, when applicable, alternatives which should be equivalent. Each module has to be regarded as an example. Combining modules and/or alternatives gives a highly flexible, "performance based" procedure. It is permitted to modify the method if all performance criteria laid down in Commission Regulation (EC) No 152/2009 [1] are met.

Any deviation of the described method, combination of modules needs to be recorded as part of the QA/QC procedures of accredited laboratories and should be available on request.

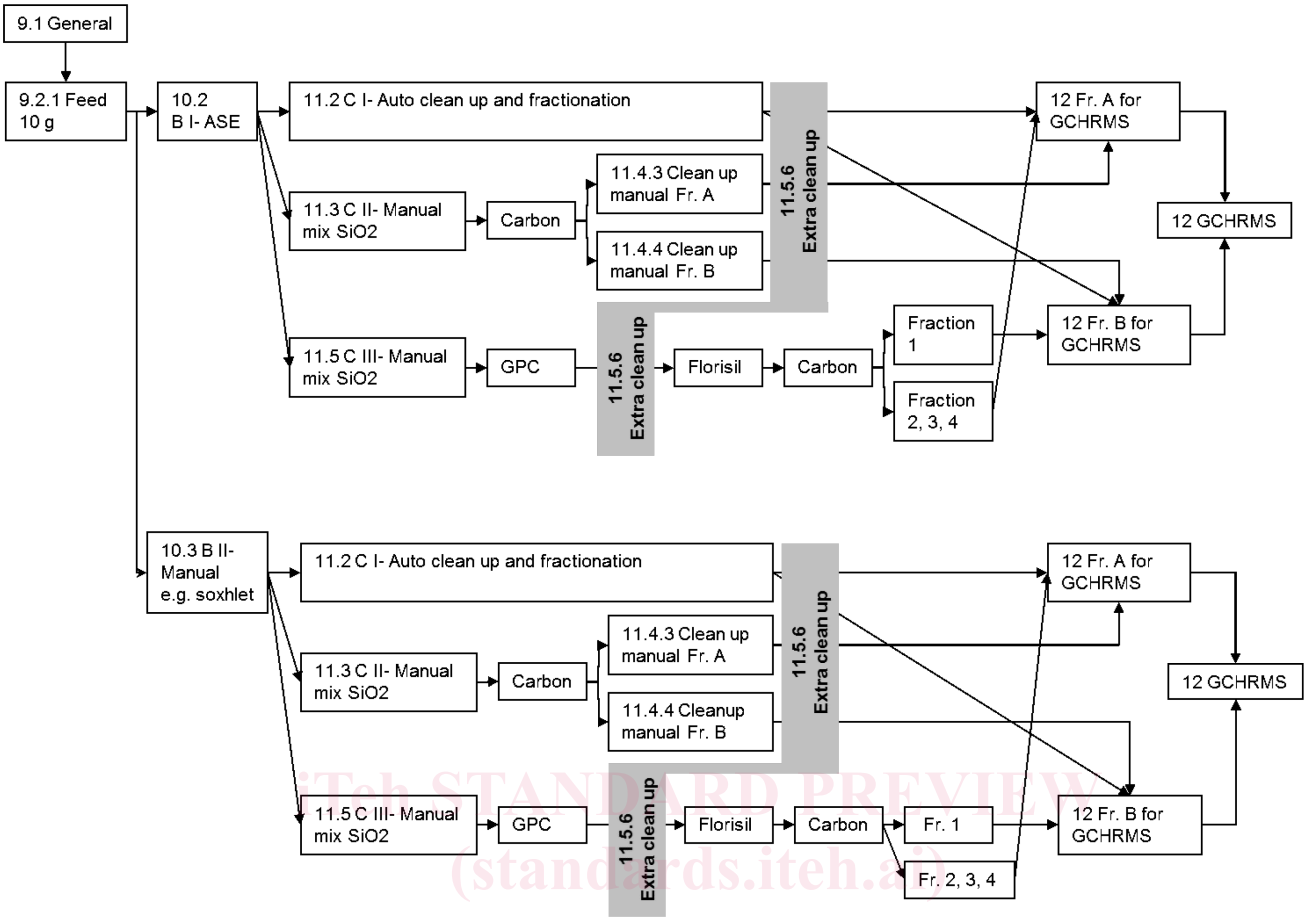

Figure 1 — Flow scheme for the determination of dioxins, dl-PCBs and non-dioxin-like-PCBs in feed

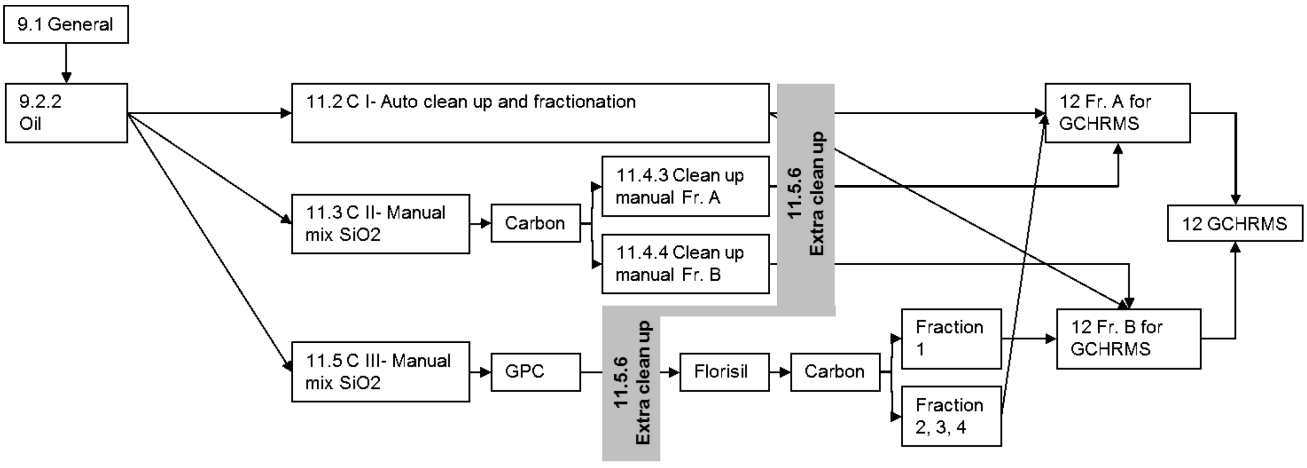

Figure 2 — Flow scheme for the determination of dioxins, dl-PCBs and non-dioxin-like-PCBs in oil and fat

2 Normative references

The following referenced documents are indispensable for the application 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 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO 6498)*

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 http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
limit of detection
smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty

Note 1 to entry: The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations (n > 10).

3.2
limit of quantification
lowest content of the analyte that can be measured with reasonable statistical certainty

Note 1 to entry: If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to six times the standard deviation of the mean of blank determinations (n > 10).

Note 2 to entry: For dl-PCBs and PCDD/F: Use correct definition for limit of quantification for each congener as in Commission Regulation (EC) No 152/2009 [1].

Note 3 to entry: Limit of quantification should be in the range of about one fifth of the level of interest.

3.3
feed additives
substances that comply with the definition of feed additives given in the Commission Regulation (EC) No 1831/2003 [3]

3.4
upper, middle and lower bound concentrations for WHO-PCDD/F-TEQ and WHO-PCB-TEQ
concentrations calculated assuming that all values of the different congeners less than the limit of quantification are equal to the limit of quantification

Note 1 to entry: For lower bound concentrations LOQ = 0 is used. For middle bound concentrations ½ LOQ is used.

4 Principle

A test portion of animal feeding stuff or ingredient is fortified with ^{13}C labelled internal standards (dioxins, furans, dioxin-like PCBs and non-dioxin-like PCBs) and extracted using a manual or an automated method.

After automated or manual clean-up an aliquot of the extract is concentrated and injected into a GC-HRMS using a split less injector (an alternative here is PTV injection (Programmed Temperature Vaporizer injection), see the NOTE below.

Quantification is based on isotope dilution.

Preconditions of combining modules for extraction and clean-up are:

a) for each extraction module an equal sample intake of 10 g for feed or feed ingredients with a fat content ≤ 25 % or 2,5 g fat or oil is required;

b) in order to achieve the required LOQ for dioxins a final volume of 10 µl in combination with an injection volume of 2 µl is required. If a different injection volume is applied, the final volume has to be adjusted directly proportional.

NOTE In case more sensitivity is necessary or less volume reduction is wanted, injection of a larger volume by PTV (an example is described in Annex A) or higher sample intake is possible (see also 0 NOTE).

5 Reagents

5.1 General

Use only reagents of recognized analytical grade and with purity suitable for dioxin and PCB residue analysis. Check the purity of the reagents and reference materials (e.g. standard solutions) by performing a blank test under the same conditions as used in the method. The chromatogram should not show any interfering impurity at the retention time of compounds of interest.

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5.2 Dioxins, furans, non-ortho PCBs, mono-ortho PCBs and non-dioxin-like PCBs and their labelled analogues

— ^{13}C-spiking solution for PCDD/F (internal standard);

— ^{13}C-spiking solution for PCB (internal standard);

— calibration solutions PCDD/F;

— calibration solutions PCB;

— recovery standard PCDD/F;

— recovery standard PCB.

See Annex B for a description of standards and concentrations of the standard solutions.

6 Principle

All technical descriptions are examples of possible system setups and parameters and should be scaled or adopted to the user's equipment.

6.1 Evaporator, suitable for volumes up to 200 ml and inlet for nitrogen gas.

6.2 Evaporator tubes, endpoint about 0,5 ml.

6.3 Homogeniser.

6.4 Pasteur pipette, borosilicate glass, 150 mm.

6.5 Vortex mixer.

6.6 Measuring cylinder, borosilicate glass, 100 ml, 2 ml graduations with a precision of ± 0,5 ml.

6.7 Measuring cylinder, borosilicate glass, glass-stoppered, 25 ml, 1 ml graduation with a precision of ± 0,5 ml graduation and 50 ml, 2 ml graduation with a precision of ± 0,5 ml graduation.

7 Sampling

The sample should be truly representative and not damaged or changed during transport or storage. Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497 [4].

8 Preparation of test sample

Prepare the test sample in accordance with EN ISO 6498.

Dry or low moisture products such as cereals and cereal products, mixed feeds, and hay should be ground carefully so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

High moisture products such as grasses and silages and liquid feed should be (freeze-)dried and after that ground carefully so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

Oil / fat are directly dissolved in n-hexane.

9 Procedure

9.1 General

Analyse the following samples in each series:

— procedure blank (n = 1);

— (certified) reference material at appropriate level or a home-made reference sample;

— all samples (maximum 20).

The procedure blank should be free of contaminants at or above the limits of quantification.

9.2 Animal feed stuff sample and oil/fat sample

9.2.1 10 g animal feed stuff sample (12 % moisture content)

Weigh an appropriate amount, e.g. 10,0 g (± 0,10 g) of the prepared test sample into a 100 ml glass vial. Sample amount is based on 12 % moisture content. If extracting by Pressurized Fluid Extraction (PFE), add 3 g diatomaceous earth and mix thoroughly. Fortify the sample with 500 µl ^{13}C-DIOXNOP-2 (Annex B, B.2.31) and 500 µl ^{13}C-MOPIP-2 (Annex B, B.2.33) and incubate until solvent has been evaporated and continue at 10.2 (module BI) or 10.3 (module BII). For samples with more than 25 % fat, the sample intake should be reduced proportionally.

9.2.2 2,5 g oil/fat sample

Weigh an appropriate amount, e.g. 2,5 g (± 0,10 g) of the oil/fat sample into graduated cylinder of 25 ml (6.7). Fortify the sample with 500 µl ^{13}C-DIOXNOP-2 (Annex B, B.2.31) and 500 µl ^{13}C-MOPIP-2 (Annex B, B.2.33). Fill the graduated cylinder to 25 ml with n-hexane. Close the graduated cylinder with a glass stopper and mix thoroughly. Continue sample clean-up procedure at paragraph 11.2 (module CI) or 11.3 (module CII) or 11.5 (module CIII).

NOTE The calculation in 13.4 is based on sample intake of 10 g for feed with fat content of ≤ 25 % and 2,5 g for fat and oil. Deviations of sample intake should be considered in the formulas in 13.4 (M = sample intake in gram).

10 Extraction

10.1 General

The sample amount used for extraction may vary from 5 g to 50 g depending on the expected level of contamination. However, the calculation in 13.4 is based on sample intake of 10 g for feed with fat content of ≤ 25 % and 2,5 g for fat and oil. Deviations of sample intake should be considered in the formula's in 13.4 (M = sample intake in gram).

The internal standard consisting of ^{13}C-labelled congeners listed in Table B.1 shall be added directly onto the sample before extraction, or onto the oil sample before clean-up.

The extraction procedure is carried out using Pressurized Fluid Extraction (PFE) with consecutively toluene and a mixture of toluene/ethanol (module BI) or Soxhlet extraction (module BII). Duration of extraction should be adjusted according to kind and amount of sample used. The minimum requirement for Soxhlet extraction is 50 extraction cycles.

Other extraction techniques like microwave assisted extraction can also be used but shall be of proven equal performance.

10.2 Module BI Extraction using automated Pressurized Fluid Extraction (PFE) system

10.2.1 Reagents and materials

10.2.1.1 Diatomaceous earth.

10.2.1.2 n-Hexane, for dioxin and PCB analysis.

10.2.1.3 Toluene, for dioxin and PCB analysis.

10.2.1.4 Ethanol, for dioxin and PCB analysis.

10.2.1.5 Toluene/ethanol, in volume portions of 9/1.

Mix 900 ml toluene (10.2.1.2) with 100 ml ethanol (10.2.1.3) thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 1 year.

10.2.1.6 Anhydrous sodium sulphate, heated at 160 °C for at least 24 h.

10.2.1.7 Nitrogen.

10.2.1.8 Silanised glass wool.

10.2.1.9 Apparatus.

10.2.1.10 Pressurized Fluid Extraction apparatus.

The apparatus shall be able to extract the samples at 100 °C and 10 MPa.

10.2.1.11 Pressurized Fluid Extraction cell, e.g. 30-40 ml.

10.2.1.12 Measuring cylinder, borosilicate glass, 25 ml, 1 ml graduation with a precision of ± 0,5 ml.

10.2.1.13 Funnel.

10.2.1.14 Evaporator, suitable for volumes up to 200 ml and inlet for nitrogen gas.

10.2.1.15 Evaporator tubes, 0,5 ml endpoint.

10.2.2 Procedure

Put the sample (9.2.1) in a Pressurized Fluid Extraction cell (10.2.1.11) and fill with diatomaceous earth (10.2.1.1) and place the extraction cell into the Pressurized Fluid Extraction apparatus (10.2.1.10). For the extraction the following parameters might be used:

— temperature 100 °C;

— pressure 10 MPa;

— preheat 0 min;

— heat 5 min;

— static 15 min;

— flush 40 vol. % of extraction cell, e.g. for a 33 ml extraction cell = 13,2 ml;

— purge 300 s;

— cycles 3;

— solvent cycle 1 toluene (10.2.1.3);

— solvent cycles 2 and 3 toluene/ethanol in volume portions of 9/1 (10.2.1.4).

Combine solvent obtained with each cycle and filter over a funnel (10.2.1.13) equipped with a glass wool plug (10.2.1.8) and 5 g pre-dried sodium sulphate (10.2.1.6). Evaporate the filtrate using an evaporator (10.2.1.14) until an end volume of 0,5 ml. Take the sample extract from the evaporator tube and place in a glass-stoppered graduated 25 ml cylinder (6.7) and wash the evaporator tube 5 times with 4 ml n-hexane each time (i.e. 20 ml total) (10.2.1.2). The n-hexane is added to graduated cylinder containing the