SLOVENSKI STANDARD
SIST EN 16215:2012

01-september-2012

Krma - Določevanje dioksinov in dioksinu podobnih PCB z GC/HRMS in indikatorjev PCB z GC/HRMS

Animal feed - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

Futtermittel - Bestimmung von Dioxinen und dioxin-ähnlichen PCBs mittels GC/HRMS und von Indikator-PCBs mittels GC/HRMS

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English Version

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

Aliments des animaux - Dosage des dioxines, des PCB de type dioxine et des PCB indicateurs par GC/HRMS

Futtermittel - Bestimmung von Dioxinen und dioxinähnlichen PCBs mittels GC/HRMS und von Indikator-PCBs mittels GC/HRMS

This European Standard was approved by CEN on 9 March 2012.

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

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Foreword

This document (EN 16215:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, 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of an identical text or by endorsement, at the latest by October 2012, and conflicting national standards shall be withdrawn at the latest by October 2012.

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1 Scope

This European Standard 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 residues of one or more of the following dioxins, dioxin-like PCBs and indicator PCBs. The limit of quantification (LOQ) for the relevant individual congeners of dioxins/furans is 0,05 pg/g (OCDD/F = 0,1 pg/g), of non-ortho PCBs 0,05 pg/g, of mono-ortho PCBs 10 pg/g and of indicator PCBs 100 pg/g.

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 [6]. Confirmatory methods are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. If only the analysis of indicator PCBs is required, a GC-LRMS method can be used (e.g. EN 15741 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/MS [1] and EN 15742 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/ECD [2]) provided that appropriate analytical performance criteria are met in the relevant range for the matrix of interest.

This European Standard is split into four modules each describing 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 procedure which is "performance based". It is permitted to modify the method if all performance criteria laid down in Commission Regulation (EC) No 152/2009 [6] 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.

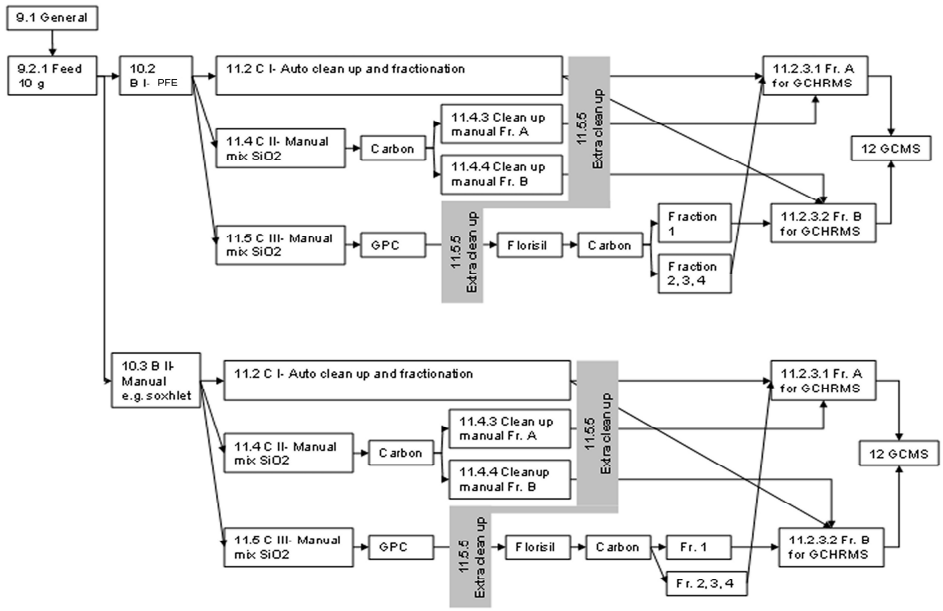

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Figure 1 — Flow scheme for the determination of Dioxins, dl-PCBs and Indicator PCBs in feed

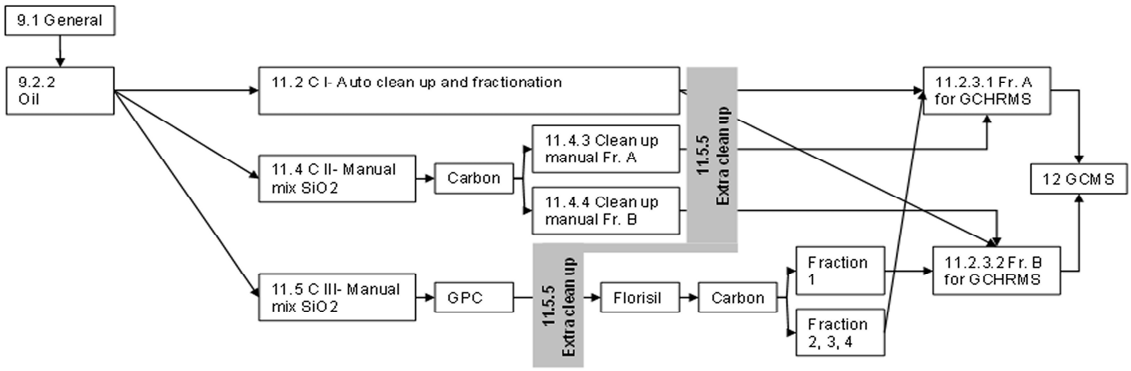

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Figure 2 — Flow scheme for the determination of Dioxins, dl-PCBs and Indicator PCBs in oil / fat

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

prEN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO/DIS 6498)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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 [6].

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 [7]

3.4
upper, middle and lower bound
upper, middle and lower bound results for WHO-PCDD/F-TEQ and WHO-PCB-TEQ are defined as follows: upper bound concentrations are calculated assuming that all values of the different congeners less than the limit of quantification are equal to the limit of quantification; for lower bound: LOQ = 0 and middle bound: 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 indicator 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 NOTE.

Quantification is based on isotope dilution. If only indicator PCBs are required, they can be determined with GC-LRMS (e.g. according to EN 15741 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/MS [1] and EN 15742 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/ECD [2])

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 9.2.2 NOTE).

5 Reagents

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.

WARNING — The use of this European Standard 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 to determine the applicability of regulatory limitations prior to use.

5.1 Dioxins, furans, non-ortho PCBs, mono-ortho PCBs and indicator 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: Description of standards and concentration of the standard solutions.

6 Apparatus

All technical descriptions are examples of possible system setups and parameters and have to 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 Vortexmixer

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 [3].

8 Preparation of test sample

Prepare the test sample in accordance with prEN 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 has to 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 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 taken into account 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 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 taken into account in the formula's in 13.4 (M = sample intake in gram).

The internal standard consisting of ^{13}C-labelled congeners listed in Annex B, 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 (10.2.1.3) with 100 ml ethanol (10.2.1.4) 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

10.2.2 Apparatus

10.2.2.1 Pressurized Fluid Extraction apparatus

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

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

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

10.2.2.4 Funnel

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

10.2.2.6 Evaporator tubes, 0,5 ml endpoint.

10.2.3 Procedure

Put the sample (9.2.1) in a Pressurized Fluid Extraction cell (10.2.2.2) and fill with diatomaceous earth (10.2.1.1) and place the extraction cell into the Pressurized Fluid Extraction apparatus (10.2.2.1). 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 cycle 2 and 3 toluene/ethanol in volume portions of 9/1 (10.2.1.5).

Combine solvent obtained with each cycle and filter over a funnel (10.2.2.4) 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 (10.2.2.5)

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 sample extract. Bring the volume to 25 ml with n-hexane (10.2.1.2), close the graduated cylinder with the glass stopper and mix thoroughly. Continue sample clean up procedure using the automated procedure (module CI, 11.2) or at paragraph using the manual method (module CII, 11.3 or module CIII, 11.5).

NOTE Comparable techniques in combination with appropriate parameters can be used provided that Commision Regulation (EC) No 152/2009 [6] is obeyed.

10.3 Module BII: Manual extraction procedure

10.3.1 Reagents and materials.

10.3.1.1 DCM, dichloromethane, for dioxin and PCB analysis.

10.3.1.2 Toluene, for dioxin and PCB analysis.

10.3.1.3 Ethanol, for dioxin and PCB analysis.

10.3.1.4 n-Hexane, for dioxin and PCB analysis.

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

Mix 900 ml toluene (10.2.1.3) with 100 ml ethanol (10.2.1.4) thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 1 year.

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

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

10.3.1.8 Silanised glass wool

10.3.2 Apparatus

10.3.2.1 Extraction thimbles, cleaned by extracting in soxhlet extractor for 2 h with DCM (10.3.1.1).

10.3.2.2 Recirculating cooler

10.3.2.3 Soxhlet extractor

10.3.2.4 Heating apparatus, e.g. mantle.

10.3.2.5 Anti-bumping granules

10.3.2.6 Round bottom flask (RBF), borosilicate glass, 500 ml.

10.3.2.7 Funnel, standard, 150 mm, 80 mm diameter, borosilicate glass.

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

10.3.2.9 Evaporator tubes, 0,5 ml endpoint.

10.3.3 Procedure

Wash the soxhlet extractor (10.3.2.3) including round bottom flasks (RBF) (10.3.2.6) subsequently with toluene (10.3.1.2) and dichloromethane (DCM) (10.3.1.1). Switch on the refrigerated recirculator (10.3.2.2) and leave to cool. Put sample (9.2.1) in the prepared extraction thimble (10.3.2.1) and place thimbles (10.3.2.1) plugged with silanised glass wool (10.3.1.8) into soxhlet extractor (10.3.2.3). Add 200 ml toluene (10.3.1.2) and 3 - 6 anti-bumping granules (10.3.2.5) to the RBF (10.3.2.6) and place into heating mantle (10.3.2.4). Connect all the soxhlet extractor together including the condenser and check all seals are tight. Set the soxhlets running at a rate of 5 cycles per hour to 7 cycles per hour. Leave the soxhlets extracting for 4 h.

Switch off heating mantles and allow apparatus to cool and remove the RBF containing toluene from the soxhlet apparatus. Add 200 ml toluene/ethanol (10.3.1.5) and 3 anti-bumping granules to 6 anti-bumping granules (10.3.2.5) to a new RBF (10.3.2.6) and place into heating mantle (10.3.2.4). Connect all the soxhlet equipment together including the condenser and check all seals are tight. Set the soxhlets running at a rate of 5 cycles per hour to 7 cycles per hour. Leave the soxhlets extracting for 16 h until 20 h or overnight.

Switch off heating mantles and allow apparatus to cool and combine the toluene extract and the toluene/ethanol extract. Filter the soxhlet-extract through a funnel equipped with a silanised glass wool plug (10.3.1.8) and 5 g -anhydrous sodium sulphate (10.3.1.6). Evaporate the filtrate using an evaporator (6.1) until the solvent has evaporated (approximately 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) (10.3.1.4). The n-hexane is added to graduated cylinder containing the sample extract. Bring the volume to 25 ml with n-hexane (10.3.1.4), close the graduated cylinder with the glass stopper and mix thoroughly. Continue sample clean up procedure using the automated procedure (module CI, 11.2) or at paragraph using the manual method (module CII, 11.3 or module CIII, 11.5).

NOTE Comparable techniques, e.g. twisselmann (hot extraction) in combination with appropriate parameters can be used provided that Commision Regulation (EC) No 152/2009 [6] is obeyed. In addition, the azeotropic mixture of toluene/ethanol in volume portions of 3/7 can be used.

11 Clean up

11.1 General

Clean up methods shall prepare the sample extract in an appropriate manner for the subsequent quantitative determination. Clean up procedures have to concentrate PCDDs/PCDFs and dioxin-like PCBs in the extracts and to remove interfering matrix components present in the raw extract.

Below principles of frequently used clean up techniques are briefly described.

— Gel permeation chromatography

  The interesting molecular weight range for PCDDs/PCDFs and dioxin-like PCBs of 200 g/mol to 500 g/mol can be isolated from larger molecules such as fat / oil and polymers which might overload other clean-up methods.

— Multi-layer column

  Multi-layer column liquid chromatography using silica with different activity grades and surface modifications. Compounds with different chemical properties than PCDDs/PCDFs and dioxin-like PCBs can be removed.

— Sulphuric acid treatment

  A direct treatment of the sample extract for removal of oxidizable coextractives with sulphuric acid is possible but is not recommended due to safety reasons. Furthermore, this has to be carried out very carefully to avoid losses of PCDDs/PCDFs and dioxin-like PCBs on the formed carboniferous surfaces.