SLOVENSKI STANDARD
oSIST prEN 17362:2019

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Krma: metode vzorčenja in analize - Določevanje pentaklorofenola (PCP) v sestavinah krme in krmni mešanici z LC-MS/MS

Animal feeding stuffs: Methods of sampling and analysis - Determination of pentachlorophenol (PCP) in feed materials and compound feed by LC-MS/MS

Futtermittel: Probenahme- und Untersuchungsverfahren - Bestimmung von Pentachlorphenol (PCP) in Futtermittel und Mischfuttermittel mittels LC-MS/MS

Aliments pour animaux - Méthodes d'échantillonnage et d'analyse - Détermination de la teneur en pentachlorophénol (PCP) dans les matières premières pour aliments des animaux et les aliments composés pour animaux par CL-SM/SM

Ta slovenski standard je istoveten z: **prEN 17362**

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Animal feeding stuffs: Methods of sampling and analysis - Determination of pentachlorophenol (PCP) in feed materials and compound feed by LC-MS/MS

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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Contents

Page

European foreword

This document (prEN 17362:2019) 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 has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

1 Scope

This document specifies a liquid chromatographic method with triple-quadrupole mass spectrometry (MS/MS) detection for the determination of pentachlorophenol (PCP) in feed materials and animal feed.

The limit of quantitation (LOQ) for the PCP determination in guar gum, fatty acid distillates (FAD) and animal feed is 10 µg/kg. Individual laboratories are responsible for ensuring that the equipment that they use will achieve this limit of quantification.

The method is validated in an international collaborative trial for pentachlorophenol in compound feed, guar gum and fatty acid distillate in the range between 9 µg/kg and 22 µg/kg.

The results of the collaborative trial, in which 16 laboratories participated, have shown that the method is applicable for the determination of PCP in compound feed, guar gum and FAD at the desired limit of 10 µg/kg. Satisfactory results were obtained for one compound feed sample, guar gum and the two FAD samples (HorRat <2), while for the second compound feed sample a HorRat value of 2,2 was obtained.

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 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
calibration
complete set of operations which estimates under specified conditions the calibration function from observations of the response variable obtained on reference states

[SOURCE: ISO 3534-2:2006, 3.5.13]

3.2
collaborative trial
interlaboratory comparisons
organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions

[SOURCE: EN ISO/IEC 17043:2010, 3.4]

3.3

detection limit

limit of detection

measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

Note 1 to entry: IUPAC recommends default values for α and β equal to 0,05.

Note 2 to entry: The abbreviation LOD is sometimes used.

Note 3 to entry: The term 'sensitivity' is discouraged for ‘detection limit’.

[SOURCE: ISO/IEC Guide 99:2007, 4.18]

3.4

feed material

products of vegetable or animal origin, whose principal purpose is to meet animals’ nutritional needs, in their natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, whether or not containing feed additives, which are intended for use in oral animal-feeding either directly as such, or after processing, or in the preparation of compound feed, or as carrier of premixtures

[SOURCE: Regulation (EC) No 767/2009 (Article 3, 1g)] [1]

3.5

HorRat

ratio of the reproducibility relative standard deviation to that calculated from the Horwitz equation

Note 1 to entry: Predicted relative standard deviation $PRSD_R = 2\ C^{-0,15}$

$$\text{HorRat}_R = RSD_R \ / \ PRSD_R \qquad (1)$$

$$\text{HorRat}_r = RSD_r \ / \ \text{PRSD}_R \qquad (2)$$

Note 2 to entry: If applied to within-laboratory studies, the normal range of HorRat(r) is 0,30 – 1,30.

Note 3 to entry: To check proper calculation of $PRSD_R$, a C of 10−6 should give a $PRSD_R$ of 16 %. C is concentration expressed as a mass fraction (both numerator and denominator expressed in the same units). The HorRat is indicative of method performance for a large majority of methods in chemistry. Normal values lie between 0,50 and 2,00.

[SOURCE: ISO 16577:2016, 3.75]

3.6

precision

closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The ‘specified conditions’ can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1) [2].

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility.

Note 4 to entry: Sometimes 'measurement precision' is erroneously used to mean 'measurement accuracy'.

[SOURCE: ISO IEC Guide 99:2007, 2.15]

3.7
repeatability

precision under repeatability conditions

Note 1 to entry: Repeatability can be expressed quantitatively in terms of the dispersion characteristics of the results.

[SOURCE: ISO 3534-2:2006, 3.3.5]

3.8
repeatability limit

r

repeatability critical difference for a specified probability of 95 %

[SOURCE: ISO 3534-2:2006, 3.3.9]

3.9
repeatability standard deviation

standard deviation of test results or measurement results obtained under repeatability conditions

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under repeatability conditions.

Note 2 to entry: Similarly, “repeatability variance” and “repeatability coefficient of variation” can be defined and used as measures of the dispersion of test or measurement results under repeatability conditions.

[SOURCE: ISO 3534-2:2006, 3.3.7]

3.10
reproducibility

precision under reproducibility conditions

Note 1 to entry: Reproducibility can be expressed quantitatively in terms of the dispersion characteristics of the results.

Note 2 to entry: Results are usually understood to be corrected results.

[SOURCE: ISO 3534-2:2006, 3.3.10]

3.11
reproducibility limit
R
reproducibility critical difference for a specified probability of 95 %

[SOURCE: ISO 3534-2:2006, 3.3.14]

3.12
reproducibility standard deviation
standard deviation of test results or measurement results obtained under reproducibility conditions

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under reproducibility conditions.

Note 2 to entry: Similarly, “reproducibility variance” and “reproducibility coefficient of variation” can be defined and used as measures of the dispersion of test or measurement results under reproducibility conditions.

[SOURCE: ISO 3534-2:2006, 3.3.12]

4 Principle

In order to check for the presence of PCP, a test portion of sample material is fortified with internal standards (13C-PCP). The test portion is extracted using a QuEChERS approach for animal feed and FAD.

A ‘reversed’ QuEChERS approach is used for guar gum. For FAD samples, lipids are removed prior to QuEChERS extraction by the addition of sulfuric acid.

Final extracts from all matrices are analysed by liquid chromatography coupled to triple quadrupole mass spectrometry, operated in negative electrospray ionization mode.

Identification is done on the basis of retention time and mass. Quantification is done using the internal standard method.

5 Reagents and materials

5.1 General

Use only reagents of recognized analytical grade and with a purity suitable for OC residue analysis. Check the purity of the reagents 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.

5.2 Chemicals

5.2.1 Diethylamine (DEA)

5.2.2 Acetonitrile

5.2.3 Methanol

5.2.4 Acetone

5.2.5 n-Hexane

5.2.6 Sulfuric acid

5.2.7 Deionized water

5.2.8 Sodium hydroxide

5.2.8.1 Sodium hydroxide solution (10 M)

Weigh 4 g of sodium hydroxide (5.2.8) and add 10 ml deionized water (5.2.7) to achieve a concentration of 10 M. Store at room temperature in a closed glass bottle.

5.2.9 Magnesium sulphate

5.2.10 Sodium chloride

5.2.11 Formic acid

5.2.12 Ammonium formate

5.2.12.1 Ammonium formate solution (1 M)

Weigh 6,3 g ammonium formate (5.2.12) and add 100 ml deionized water (5.2.7) to achieve a concentration of 1 M. Store the solution at room temperature. The solution is tenable under these conditions during at least 1 month.

5.2.13 Mobile phase A

Take 1 ml ammonium formate solution (1 M) (5.2.12.1) and add 999 ml deionized water (5.2.7) and 20 µL formic acid (5.2.11). Mix well. Store the solution at room temperature. The solution is tenable under these conditions during at least 1 month.

5.2.14 Mobile phase B

Take 1 ml ammonium formate solution (1 M) (5.2.12.1) and add 50 ml deionized water (5.2.7), 949 ml methanol (5.2.3) and 20 µl formic acid (5.2.11) and mix well. Store the solution at room temperature. The solution is tenable under these conditions during at least 1 month.

5.2.15 Pentachlorophenol (PCP sodium salt, 95% purity)

5.2.15.1 PCP Stock solution 1 (2000 µg/ml)

Weigh 25 mg (± 0,01 mg) of PCP (5.2.15) (taking impurities into consideration) and add 12,5 ml deionized water (5.2.7) to achieve a concentration of 2000 µg/ml. Store the solution in a refrigerator at 4 °C (± 3 °C). The solution is tenable under these conditions during at least 12 months.

5.2.15.2 PCP Stock solution 2 (1 ng/µl)

Dilute 50 µl of PCP Stock solution 1 (5.2.15.1) to 100 ml deionized water (5.2.7) in a volumetric flask (5.2.20). Store the solution in a refrigerator at 4 °C (± 3 °C). The solution is tenable under these conditions during at least 12 months.

5.2.15.3 PCP Working solution 1 (0,1 ng/µl)

Take 100 µL of PCP Stock solution 2 (5.2.15.2) and add 900 µl acetonitrile (5.2.2). Prepare this solution daily.

5.2.15.4 PCP Working solution 2 (0,01 ng/µl)

Take 50 µl of PCP Working solution 1 (5.2.15.3). Add450 µl acetonitrile (5.2.2). Prepare this solution daily.

5.2.16 $^{13}C_6$-pentachlorofenol of certified purity (> 99%, 100 ng/µl) as an internal standard

5.2.16.1 $^{13}C_6$-PCP Stock solution (10 ng/µl)

Take 1 ml of $^{13}C_6$-PCP at 100 ng/µl (5.2.16) and dilute with acetone (5.2.4) to 10 ml in a volumetric flask (5.2.20). Mix well. Store the solution in a refrigerator at 4 °C (± 3 °C).

5.2.16.2 $^{13}C_6$-PCP Working solution 1 (1 ng/µl)

Take 100 µl of $^{13}C_6$-PCP Stock solution (5.2.16.1) and add 900 µl acetonitrile (5.2.2). Prepare this solution daily.

5.2.16.3 $^{13}C_6$-PCP Working solution 2 (0,1 ng/µl)

Take 50 µl of $^{13}C_6$-PCP Working solution 1 (5.2.16.2) and add 450 µl acetonitrile (5.2.2). Prepare this solution daily.

5.2.17 Calibration standards

Prepare calibration mixtures according to Table 1 in a final volume of 1,0 ml of acetonitrile. Store the calibration mixtures at 4 °C (± 3 °C).

Table 1 — Calibration mixtures

Level	Concentration [ng/mL]	PCP 0,01 ng/µL (5.2.15.4)	PCP 0,1 ng/µL (5.2.15.3)	PCP 1 ng/µL (5.2.15.2)	Internal standard 0,1 ng/µL (5.2.16.3)	ACN (5.2.1)
1	0				20 µl	980 µl
2	0,1	10 µl			20 µl	970 µl
3	0,5	50 µl			20 µl	930 µl
4	1,0		10 µl		20 µl	970 µl
5	5,0		50 µl		20 µl	930 µl
6	10		100 µl		20 µl	880 µl
7	40			40 µl	20 µl	940 µL

5.2.18 PP screwcap centrifuge tube (Greiner), 50 ml

5.2.19 Glass tube with screw cap, 12 ml

5.2.20 Volumetric flask, 10 ml

5.2.21 Volumetric flask, 100 ml

5.2.22 LC-MS vial, 2 ml