SLOVENSKI STANDARD
SIST EN 17362:2020

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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

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 17362:2020) 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 shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2020, and conflicting national standards shall be withdrawn at the latest by October 2020.

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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 compound feed.

The limit of quantitation (LOQ) for the PCP determination in guar gum, fatty acid distillates (FAD) and compound 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 https://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 [1]]

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 [2]]

3.3
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(2)(g)) [3]]

3.4
HorRat
ratio of the reproducibility relative standard deviation to that calculated from the Horwitz equation

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

$$HorRat_R = RSD_R / PRSD_R \quad (1)$$

$$HorRat_r = RSD_r / PRSD_r \quad (2)$$

Note 2 to entry: If applied to within-laboratory studies, the normal range of HorRat(r) is 0,30 to 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 [4]]

3.5
limit of quantitation
measured quantity value, obtained by a given measurement procedure, which is the lowest concentration of a measurand that can be determined with an acceptable level of repeatability precision and trueness

Note 1 to entry: The "limit of quantitation" is not a concept defined in ISO/IEC Guide 99:2007 [5] but has been defined as described above.

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

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 [6]).

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 [5]]

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 [1]]

3.8
repeatability limit
r
repeatability critical difference for a specified probability of 95 %

[SOURCE: ISO 3534-2:2006, 3.3.9 [1]]

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 [1]]

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 [1]]

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

[SOURCE: ISO 3534-2:2006, 3.3.14 [1]]

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 [1]]

4 Principle

In order to check for the presence of PCP, a test portion of sample material is fortified with internal standards (^{13}C-PCP). The test portion is extracted using a QuEChERS approach for compound 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-to-charge ratio. 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 organochlorine 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 (ACN)

5.2.3 Methanol

5.2.4 Acetone

5.2.5 n-Hexane

5.2.6 Sulphuric acid (concentrated, 95 % – 98 %)

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 with a plastic cap or stopper.

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 stable under these conditions for 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 stable under these conditions for 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 stable under these conditions for at least 1 month.

5.2.15 Pentachlorophenol (PCP sodium salt, 95 % purity)

5.2.15.1 PCP Stock solution 1 (2 000 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 2 000 µg/ml. Store the solution in a refrigerator at 4 °C (± 3 °C). The solution is stable under these conditions for 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 stable under these conditions for 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 $^{13}C_6$-pentachlorophenol of certified purity (>99 %, 100 ng/µl in nonane) 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). The solution is stable under these conditions for at least 12 months.

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). The solution is stable under these conditions for at least 12 months.

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. The range of concentrations can be adapted depending on the expected concentrations in the sample, as long as the range is linear. Store the calibration mixtures at 4 °C (± 3 °C). Prepare these solutions daily.

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 (5.2.2)
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 Polypropylene (PP) screwcap centrifuge tube, 50 ml

5.2.19 Glass tube with screw cap (polytetrafluoroethylene (PTFE) inlay), 12 ml

5.2.20 Volumetric flask, 10 ml

5.2.21 Volumetric flask, 100 ml

5.2.22 LC-MS vial, 2 ml

5.2.23 pH paper, range 0-14, or another suitable testing range