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TUNGALTERAALKALOIDIDE JA TROPAANALKALOIDIDE
MÄÄRAMINE SÖÖDATOORAINES NING SEGASÖÖDAS
LC-MS/MS MEETODIL

Animal feeding stuffs: Methods of sampling and analysis
- Determination of ergot alkaloids and tropane
alkaloids in feed materials and compound feeds by
LC-MS/MS

EESTI STANDARDI EESSÕNA

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English Version

**Animal feeding stuffs: Methods of sampling and analysis -
Determination of ergot alkaloids and tropane alkaloids in
feed materials and compound feeds by LC-MS/MS**

Aliments des animaux: Méthodes d'échantillonnage et
d'analyse - Détermination de la teneur en alcaloïdes de
l'ergot et en alcaloïdes tropaniques dans les matières
premières et les aliments composés par CL-SM/SM

Futtermittel: Probenahme- und
Untersuchungsverfahren - Bestimmung der Alkaloide
des Mutterkorns und der Tropanalkaloiden in
Einzelfuttermitteln und Mischfuttermitteln mittels LC-
MS/MS

This European Standard was approved by CEN on 28 July 2019.

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

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European foreword

This document (EN 17256:2019) 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 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of an identical text or by endorsement, at the latest by March 2020, and conflicting national standards shall be withdrawn at the latest by March 2020.

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Introduction

Ergot alkaloids are mycotoxins produced by species of the genus *Claviceps*. In Europe, *Claviceps purpurea* is the most widespread fungal species. The fungus may infest plant species of the Poaceae family (true grasses), producing dark coloured bodies, called sclerotia or (rye) ergot. Economically important cereal grains that may be infected by *C. purpurea* are rye, wheat, triticale, barley, millet and oats. The sclerotia contain a suit of ergot alkaloids, of which twelve have been recognized as major components: ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine. Ergocryptine and ergocryptinine occur as a mixture of α - and β -isomers.

Tropane alkaloids are plant toxins produced by several species within the family of Solanaceae (nightshades). The most relevant are *Datura* (thornapple), *Hyoscyamus* (henbane) and *Atropa* (belladonna, deadly nightshade) species. Seeds and other plant parts contain substantial amounts of atropine (hyoscyamine) and scopolamine, which are the most important toxic principles. *Datura*, *Hyoscyamus* and *Atropa* species can be present as weeds in arable fields and may be co-harvested, resulting in contaminated feed grains and feed products.

This protocol does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health protection measures and to ensure that regulatory and legal requirements are complied with.

1 Scope

This document describes a method for the determination of individual ergot alkaloids and tropane alkaloids in unprocessed cereals and cereal-based compound feeds by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS).

This document has been successfully validated by collaborative trial in the following matrices: rye, barley, wheat, complete feed for bovine, porcine and poultry. Validation in buckwheat produced acceptable results, but the relative standard reproducibility was higher for most analytes in comparison with the other matrices. This may be related to the matrix. The validated range of the method is approximately 10 µg/kg to 250 µg/kg for individual alkaloids. Determination of concentrations above 250 µg/kg is possible by applying a higher spiking level and dilution of the sample extract, but this has not been validated in the collaborative trial.

This document is applicable for the determination, by means of one-point standard addition to the sample, of:

- ergocornine in the tested range of 12 µg/kg to 221 µg/kg;
- ergocorninine in the tested range of 9 µg/kg to 196 µg/kg;
- ergocristine in the tested range of 14 µg/kg to 312 µg/kg;
- ergocristinine in the tested range of 12 µg/kg to 258 µg/kg;
- α-ergocryptine in the tested range of 10 µg/kg to 184 µg/kg;
- the sum of α- and β-ergocryptinine in the tested range of 8 µg/kg to 171 µg/kg;
- ergometrine in the tested range of 12 µg/kg to 174 µg/kg;
- ergometrinine in the tested range of 3 µg/kg to 172 µg/kg;
- ergosine in the tested range of 12 µg/kg to 226 µg/kg;
- ergosinine in the tested range of 9 µg/kg to 273 µg/kg;
- ergotamine in the tested range of 11 µg/kg to 443 µg/kg;
- ergotaminine in the tested range of 10 µg/kg to 273 µg/kg;
- atropine in the tested range of 16 µg/kg to 252 µg/kg;
- scopolamine in the tested range of 15 µg/kg to 246 µg/kg.

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)*