

SVENSK STANDARD

SS-EN 17256:2019



Fastställt/Approved: 2019-10-14
Utgåva/Edition: 1
Språk/Language: engelska/English
ICS: 65.120;71.040.40

Djurfoder – Provtagnings- och analysmetoder – Bestämning av ergotalkaloider och tropanalkaloider i foderråvaror och foderblandningar av LC-MS/MS

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

This preview is downloaded from www.sis.se. Buy the entire standard via <https://www.sis.se/std-80017547>

Standarder får världen att fungera

SIS (Swedish Standards Institute) är en fristående ideell förening med medlemmar från både privat och offentlig sektor. Vi är en del av det europeiska och globala nätverk som utarbetar internationella standarder. Standarder är dokumenterad kunskap utvecklad av framstående aktörer inom industri, näringsliv och samhälle och befrämjar handel över gränser, bidrar till att processer och produkter blir säkrare samt effektiviserar din verksamhet.

Delta och påverka

Som medlem i SIS har du möjlighet att påverka framtida standarder inom ditt område på nationell, europeisk och global nivå. Du får samtidigt tillgång till tidig information om utvecklingen inom din bransch.

Ta del av det färdiga arbetet

Vi erbjuder våra kunder allt som rör standarder och deras tillämpning. Hos oss kan du köpa alla publikationer du behöver – allt från enskilda standarder, tekniska rapporter och standardpaket till handböcker och onlinetjänster. Genom vår webbtjänst e-nav får du tillgång till ett lättnavigerat bibliotek där alla standarder som är aktuella för ditt företag finns tillgängliga. Standarder och handböcker är källor till kunskap. Vi säljer dem.

Utveckla din kompetens och lyckas bättre i ditt arbete

Hos SIS kan du gå öppna eller företagsinterna utbildningar kring innehåll och tillämpning av standarder. Genom vår närhet till den internationella utvecklingen och ISO får du rätt kunskap i rätt tid, direkt från källan. Med vår kunskap om standarders möjligheter hjälper vi våra kunder att skapa verklig nytta och lönsamhet i sina verksamheter.

Vill du veta mer om SIS eller hur standarder kan effektivisera din verksamhet är du välkommen in på www.sis.se eller ta kontakt med oss på tel 08-555 523 00.



Standards make the world go round

SIS (Swedish Standards Institute) is an independent non-profit organisation with members from both the private and public sectors. We are part of the European and global network that draws up international standards. Standards consist of documented knowledge developed by prominent actors within the industry, business world and society. They promote cross-border trade, they help to make processes and products safer and they streamline your organisation.

Take part and have influence

As a member of SIS you will have the possibility to participate in standardization activities on national, European and global level. The membership in SIS will give you the opportunity to influence future standards and gain access to early stage information about developments within your field.

Get to know the finished work

We offer our customers everything in connection with standards and their application. You can purchase all the publications you need from us - everything from individual standards, technical reports and standard packages through to manuals and online services. Our web service e-nav gives you access to an easy-to-navigate library where all standards that are relevant to your company are available. Standards and manuals are sources of knowledge. We sell them.

Increase understanding and improve perception

With SIS you can undergo either shared or in-house training in the content and application of standards. Thanks to our proximity to international development and ISO you receive the right knowledge at the right time, direct from the source. With our knowledge about the potential of standards, we assist our customers in creating tangible benefit and profitability in their organisations.

If you want to know more about SIS, or how standards can streamline your organisation, please visit www.sis.se or contact us on phone +46 (0)8-555 523 00



Europastandarden EN 17256:2019 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN 17256:2019.

The European Standard EN 17256:2019 has the status of a Swedish Standard. This document contains the official version of EN 17256:2019.

© Copyright/Upphovsrätten till denna produkt tillhör SIS, Swedish Standards Institute, Stockholm, Sverige. Användningen av denna produkt regleras av slutanvändarlicensen som återfinns i denna produkt, se standardens sista sidor.

© Copyright SIS, Swedish Standards Institute, Stockholm, Sweden. All rights reserved. The use of this product is governed by the end-user licence for this product. You will find the licence in the end of this document.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS som även lämnar allmänna upplysningar om svensk och utländsk standard.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS, who can also provide general information about Swedish and foreign standards.

Denna standard är framtagen av kommittén för Djurfoder, SIS/TK 435/AG 02.

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

EUROPEAN STANDARD

EN 17256

NORME EUROPÉENNE

EUROPÄISCHE NORM

October 2019

ICS 65.120; 71.040.40

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.

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. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

SS-EN 17256:2019 (E)

Contents		Page
European foreword		3
Introduction		4
1	Scope	5
2	Normative references	5
3	Terms and definitions	6
4	Principle	6
5	Reagents	6
6	Apparatus	10
7	Procedure	11
8	Analysis	13
9	Results	14
10	Precision	15
11	Test report	17
Annex A (informative) Precision data		18
Annex B (informative) Example of LC-MS/MS conditions		34
Annex C (informative) Example LC-MS/MS chromatograms of ergot alkaloids and tropane alkaloids in a sample of complementary bovine feed		36
Bibliography		38

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

SS-EN 17256:2019 (E)

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)*

SS-EN 17256:2019 (E)

3 Terms and definitions

No terms and definitions are listed in this document.

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>

4 Principle

The alkaloids are extracted by mixing 4 g of a homogenized, finely ground, sample with 40 ml of 0,4 % formic acid in methanol:water (60:40). The mixture is shaken for 30 min. After centrifugation, a portion of the supernatant is further purified by passing it through a 30 kDa ultrafilter. The filtrate is transferred to a vial and it is analysed with a liquid chromatography – tandem mass spectrometry (LC-MS/MS) system. A reverse-phase column in combination with an aqueous mobile phase with a pH > 7 and an organic modifier is used to separate the analytes. Quantification is performed by one-point standard addition to the sample.

5 Reagents

WARNING: Mycotoxins may be highly hazardous to health. Certain mycotoxins have carcinogenic, mutagenic, toxic, teratogenic and immunotoxic effects. Inhalation or dermal exposure to mycotoxins may occur at workplaces. Depending on the level of exposure, both acute and chronic effects are possible. The tropane alkaloids atropine and scopolamine are acutely toxic, may be fatal if swallowed or if inhaled. In addition, scopolamine may be fatal if in contact with skin.

5.1 Analytical standards

Analytical standards should have a demonstrated purity of at least 90 %, preferably of 95 % or higher.

NOTE 1 Ergotamine and ergometrine are listed as category I drug precursors and for these compounds an official licence would be required and special procedures for storage and management would need to be followed (EC 273/2004) [1]. Atropine is the racemic mixture of L-(-)-hyoscyamine and D-(+)-hyoscyamine. In this method the enantiomers of hyoscyamine are not separated. Both enantiomers produce identical fragmentation spectra. In this method either a standard of atropine or hyoscyamine can be used.

NOTE 2 β -Ergocryptine and β -ergocryptinine are currently not available from commercial providers as analytical standards of sufficient purity and quality. In this document α -ergocryptinine is used for the determination of the sum of α - and β -ergocryptinine. For determination of β -ergocryptine, α -ergocryptine should be used, but this has not been validated in the interlaboratory study. See under Clause 8 for more information on the analysis of these alkaloids.

NOTE 3 Isotopically labelled analogues of ergometrine, ergometrinine, atropine and scopolamine are available from commercial providers. Optionally, these isotopically labelled analogues can be used as internal standards, provided that the mass increment in the molecule by the isotope labels is at least 3.

5.1.1 Ergocornine

5.1.2 Ergocorninine

5.1.3 Ergocristine

5.1.4 Ergocristinine

5.1.5. α -Ergocryptine

5.1.6. α -Ergocryptinine

5.1.7 Ergometrine (maleate)

5.1.8 Ergometrinine

5.1.9 Ergosine

5.1.10 Ergosinine

5.1.11 Ergotamine (tartrate)

5.1.12 Ergotaminine

5.1.13 Atropine or hyoscyamine

5.1.14 Scopolamine (hydrochloride)

5.2 Chemicals

5.2.1. Acetonitrile, LC-MS or HPLC quality

5.2.2. Methanol, LC-MS or HPLC quality

5.2.3. Formic acid, 98 to 100 %

5.2.4. Ammonium carbonate, anhydrous

5.2.5 Ammonia, 25 %

5.2.6 Water

Water of LC-MS grade, double-distilled or water of grade 1 as defined in EN ISO 3696:1995.

5.3 Standard solutions

Accurately weigh (6.1) between 5 mg and 6 mg of each standard (5.1.1 to 5.1.14) into a separate amber-coloured glass bottle of 60 ml (6.12). Add a volume of acetonitrile (5.2.1) to produce a solution with a concentration of 100 μ g/ml. Take into account the weight, the purity and the appearance form of the standard.

Many ergot alkaloid standards are commercially available only in small amounts (5 mg or less). Preferably a standard should be prepared using a quantity of at least 5 mg. However, when the standard is only available in a quantity of 5 mg or less, a smaller quantity can be weighed in, provided an accurate weight measurement can be guaranteed. In principle this is preferred above flushing the contents of the container with several volumes of solvent to dissolve and collect the material. Nevertheless, some ergot standards may only be available as dried down standards that need to be reconstituted in solvent.

Stock solutions are stable for 6 months below -18 °C. However, ergot alkaloid standards are sensitive to light and may epimerise rapidly in the presence of acid or base. Standard solutions should be kept in amber coloured glass bottles and they should be stored at a temperature below -18 °C. Acetonitrile is the preferred solvent because the rate of epimerisation is lowest in this solvent.