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Responsible committee: AW/10 Animal feeding stuffs

Interested committees:

Title: Draft BS EN 17504 Animal feeding stuffs: Methods of sampling and analysis - Determination of gossypol in cotton seed and feeding stuff by LC-MS/MS.

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Introduction

This draft standard is based on national and international discussions. Your comments on this draft are invited and will assist in the preparation of the consequent standard.

For international standards, comments will be reviewed by the relevant UK national committee before sending the consensus UK vote and comments to the international committee, which will then decide appropriate action. If the international standard is approved, it is usual for the text to be published as a British Standard.

For national standards, comments will be reviewed by the relevant UK national committee and the resulting standards published as a British Standard.

UK Vote

Please indicate whether you consider the UK should submit a negative (with supporting technical reasons) or positive vote on this draft. Please indicate if you are aware of any reason why this draft standard should not be published as a British Standard.

Submission of Comments

- **Annotated drafts are not acceptable and will be rejected.**
- All comments should be submitted online at <https://standardsdevelopment.bsigroup.com/>. You will need to register in order to comment.

Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/Figure/ Table/Note	Type of comment	Commend (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	

April 2020

ICS 65.120

English Version

Animal feeding stuffs: Methods of sampling and analysis -
Determination of gossypol in cotton seed and feeding stuff
by LC-MS/MS

Aliments des animaux - Méthodes d'échantillonnage et
d'analyse - Dosage du gossypol dans les graines de
coton et les aliments pour animaux par CL-SM/SM

Futtermittel - Probenahme- und
Untersuchungsverfahren - Bestimmung von Gossypol
in Baumwollsaamen und Futtermitteln mittels LC-
MS/MS

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 327.

If this draft becomes a European Standard, 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.

This draft European Standard was established by CEN 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.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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European foreword

This document (prEN 17504:2020) 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 is currently submitted to the CEN Enquiry.

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

Introduction

Gossypol is a polyphenolic plant toxin produced by species of the genus *Gossypium* (cotton plant). The plants are primarily grown for fibre and oil production. The seeds and processed seed materials are also used as animal feeding stuffs. Gossypol is present in the seeds in two forms: free gossypol and bound gossypol (mostly to proteins). The measured content of free gossypol depends on the method of extraction and the specificity of the subsequent method of analysis used ([1], [2]). Consequently, this method may not be directly comparable to existing standards based on spectrophotometric measurement (e.g. [3], [4]).

WARNING — 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 free gossypol, extractable by acidified acetonitrile/water, in cottonseed, cottonseed products and complete feed by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

This document has been successfully validated in the range of 69 mg/kg to 5 950 mg/kg by collaborative trial in the following matrices: cottonseed, cottonseed products (cake/meal, hulls) and complete feed for bovine, porcine and poultry.

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)*

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 <http://www.iso.org/obp>

4 Principle

Free gossypol is extracted by mixing a sample of 0,4 g of cottonseed or 1,0 g of cottonseed product or compound feed with 40 ml acetonitrile:water:phosphoric acid (80:20:0,1). The mixture is shaken for 1 h. After centrifugation a portion of the supernatant is diluted with extraction solvent in a vial. The final extract is analysed by reversed phase liquid chromatography with tandem mass spectrometry (LC-MS/MS). Quantification of gossypol in cottonseed and cottonseed products is based on multi-level calibration using standards in extraction solvent. Quantification of gossypol in compound feeds is based on multi-level calibration using standards in extraction solvent and corrected for the apparent recovery.

5 Reagents

WARNING — Gossypol may be hazardous to health. It has a strong toxic effect on the reproductive organs. Depending on the level of exposure, both acute and chronic effects are possible.

5.1 Gossypol, ≥ 95 %, HPLC grade

NOTE 1 Gossypol is a racemic mixture of (+)- and (-)-gossypol. In this method the enantiomers of gossypol are not separated.

NOTE 2 Gossypol is also available in the form of a 1:1 complex with acetic acid. In this method either a standard of gossypol or gossypol-acetic acid can be used.

NOTE 3 Gossypol is sensitive to light.

5.2 Acetonitrile, LC-MS or HPLC quality

5.3 Phosphoric acid 85 %