

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

EESTI STANDARDI EESSÕNA

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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 Baumwollsamensamen und Futtermitteln mittels LC-
MS/MS

This European Standard was approved by CEN on 10 January 2022.

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Contents	Page
European foreword	3
Introduction	4
1 Scope	5
2 Normative references	5
3 Terms and definitions	5
4 Principle	5
5 Reagents	5
6 Apparatus	7
7 Procedure	8
7.1 General	8
7.2 Sample pre-treatment	8
7.3 Test portion	9
7.4 Extraction	9
8 LC-MS/MS analysis	10
8.1 General	10
8.2 Analysis sequence	11
9 Results	11
9.1 Identification	11
9.2 Quantification	12
10 Precision	14
10.1 General	14
10.2 Repeatability	14
10.3 Reproducibility	14
11 Test report	15
Annex A (informative) Precision data	16
Annex B (informative) Example of LC-MS/MS conditions	18
B.1 General	18
B.2 HPLC conditions	18
B.3 MS conditions	19
Annex C (informative) Example chromatogram gossypol in compound feed	20
Bibliography	21

European foreword

This document (EN 17504:2022) 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of an identical text or by endorsement, at the latest by September 2022, and conflicting national standards shall be withdrawn at the latest by September 2022.

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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 might 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 specifies a method for the determination of free gossypol, extractable by acidified acetonitrile/water, in cottonseed, cottonseed products and compound feeds by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

The method described in 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 compound feeds for bovine, porcine and poultry.

NOTE It is possible to reach quantification limits of approximately 5 mg/kg in compound feeds. The method might be applicable at lower and at higher concentrations than the concentration range validated in the collaborative trial. However, this needs to be assessed by in-house validation.

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, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

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:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

4 Principle

Free gossypol is extracted by mixing an homogenized sample of 0,4 g of cottonseed or 1,0 g of cottonseed product or compound feed with 40 ml of 0,1 % phosphoric acid in acetonitrile:water (80:20) (V:V). 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 can 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 %, as gossypol base

Gossypol is a racemic mixture of (+)- and (-)-gossypol. In this method the enantiomers of gossypol are not separated. A standard containing a mixture of both enantiomers may be used.

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 may be used.

NOTE Gossypol is sensitive to light.