

---

---

**Krma - Določanje vsebnosti prostega in skupnega gosipola (prevzet standard ISO 6866:1985 z metodo platnice)**

Animal feeding stuffs - Determination of free and total gossypol

Aliments des animaux - Dosage du gossypol libre et du gossypol total

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST ISO 6866:1995](https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995)

<https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995>

Deskriptorji: kmetijski proizvodi, krmila, kemijska analiza, določanje vsebnosti, gosipol

---

ICS 71.040.40 \* 65.120

Referenčna številka  
SIST ISO 6866:1995 (en)

Nadaljevanje na straneh od II do III in 1 do 3

SIST ISO 6866 : 1995

---

## UVOD

Standard SIST ISO 6866, Krma - Določanje vsebnosti prostega in skupnega gosipola, prva izdaja, 1995, ima status slovenskega standarda in je z metodo platnice prevzet mednarodni standard ISO 6866, Animal feeding stuffs - Determination of free and total gossypol, first edition, 1985-11-15.

## PREDGOVOR

Mednarodni standard ISO 6866:1985 je pripravil tehnični odbor Mednarodne organizacije za standardizacijo ISO/TC 34 Kmetijski pridelki in živilski proizvodi.

Odločitev za prevzem tega standarda po metodi platnice je sprejela delovna skupina WG 10 Analitika krme v okviru tehničnega odbora USM/TC KŽP Kmetijski pridelki in živilski proizvodi.

Ta slovenski standard je dne 1995-05-08 odobril direktor USM.

## ZVEZA S STANDARDI

S prevzemom tega mednarodnega standarda veljajo naslednje zveze:

- SIST ISO 6651:1995 (en) Krma - Določanje vsebnosti aflatoksina B<sub>1</sub>
- SIST ISO 6654:1995 (en) Krma - Določanje vsebnosti sečnine
- SIST ISO 6870:1995 (en) Krma - Določanje vsebnosti zearalenona
- SIST ISO 5498:1995 (en) Kmetijski pridelki in živilski proizvodi - Določanje vsebnosti surove vlaknine - Splošna metoda
- SIST ISO 5983:1995 (en) Krma - Določanje vsebnosti dušika in izračun vsebnosti surovih beljakovin
- SIST ISO 5984:1995 (en) Krma - Določanje surovega pepela
- SIST ISO 5985:1995 (en) Krma - Določanje pepela, netopnega v klorovodikovi kislini
- SIST ISO 6490-1:1995 (en) Krma - Določanje vsebnosti kalcija - 1. del: Titrimetrična metoda
- SIST ISO 6490-2:1995 (en) Krma - Določanje vsebnosti kalcija - 2. del: Metoda atomske absorpcijske spektrometrije
- SIST ISO 6491:1995 (en) Krma - Določanje vsebnosti skupnega fosforja - Spektrofotometrična metoda
- SIST ISO 6495:1995 (en) Krma - Določanje vsebnosti v vodi topnih kloridov
- SIST ISO 6496:1995 (en) Krma - Določanje vsebnosti vlage
- SIST ISO 5506:1995 (en) Sojini proizvodi - Določanje ureazne aktivnosti
- SIST ISO 6541:1995 (en) Kmetijski pridelki in živilski proizvodi - Določanje vsebnosti surove vlaknine - Modificirana Scharrerjeva metoda

## OSNOVA ZA IZDAJO STANDARDA

- Prevzem standarda ISO 6866:1985

**OPOMBI**

- Povsod, kjer se v besedilu standarda uporablja izraz mednarodni standard , to pomeni v SIST ISO 6866:1995 slovenski standard .
- Uvod in predgovor nista sestavni del standarda.

## iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST ISO 6866:1995](https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995)

<https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST ISO 6866:1995](#)

<https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995>

---

International Standard



6866

---

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

---

## Animal feeding stuffs — Determination of free and total gossypol

*Aliments des animaux — Dosage du gossypol libre et du gossypol total*

First edition — 1985-11-15

iteh STANDARD PREVIEW  
(standards.iteh.ai)

[SIST ISO 6866:1995](https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995)

<https://standards.iteh.ai/catalog/standards/sist/ce63d0ac-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995>

---

UDC 636.085.19 : 543.8

Ref. No. ISO 6866-1985 (E)

**Descriptors** : agricultural products, animal feeding products, chemical analysis, determination of content, gossypol.

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6866 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

ITeN STANDARD PREVIEW  
(standards.iteh.ai)

SIST ISO 6866:1995  
<https://standards.iteh.ai/catalog/standards/sist/c663dfbc-4e36-4167-839e-881e93cae61a/sist-iso-6866-1995>

# Animal feeding stuffs – Determination of free and total gossypol

## 1 Scope

This International Standard specifies a method for the determination of the content of free and total gossypol and chemically related substances in animal feeding stuffs.

## 2 Field of application

The method is applicable to cottonseed and cottonseed meals and cakes, and to compound feeding stuffs containing these substances.

The detection limit for free gossypol is 20 mg/kg and that for total gossypol is 50 mg/kg.

## 3 References

ISO 6497, *Animal feeding stuffs — Sampling*.<sup>1)</sup>

ISO 6498, *Animal feeding stuffs — Preparation of test samples*.

## 4 Principle

Extraction of the gossypol in the presence of 3-aminopropan-1-ol, either with a mixture of 2-propanol and hexane for the determination of free gossypol or with dimethylformamide for the determination of total gossypol. Conversion of the gossypol into gossypol-dianiline using aniline. Measurement of the absorbance at the wavelength of maximum absorbance (between 435 and 445 nm).

## 5 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled water or water of at least equivalent purity.

**5.1 2-Propanol/*n*-hexane**, 60 + 40 mixture by volume.

**5.2 Solvent A.**

Place in a 1 000 ml volumetric flask approximately 500 ml of the 2-propanol/hexane mixture (5.1), 2 ml of 3-aminopropan-1-ol, 8 ml of glacial acetic acid and 50 ml of water. Make up to volume with the 2-propanol/hexane mixture.

This reagent is stable for one week only.

**5.3 Solvent B.**

Place in a 100 ml volumetric flask 2 ml of 3-aminopropan-1-ol and 10 ml of glacial acetic acid. Cool to room temperature and make up to volume with *N,N*-dimethylformamide.

This reagent is stable for one week only.

**5.4 Aniline.**

If the absorbance determined in the blank test exceeds 0,022, distil the aniline over zinc dust, discarding the first and last 10 % fractions of distillate. Store in a stoppered brown glass bottle in a refrigerator (0 to 4 °C).

The reagent is stable for several months.

## 6 Apparatus

Usual laboratory apparatus and in particular

**6.1 Tumbler mixer**, having a rotational frequency of approximately 35 min<sup>-1</sup>, or other shaker.

**6.2 Spectrometer**, suitable for making measurements at wavelengths between 435 and 445 nm, with cells of optical path length 10 mm.

**6.3 Volumetric flasks**, of capacities 25 and 50 ml.

**6.4 Conical flasks**, of capacities 50, 100 and 250 ml.

## 7 Sampling

Take the laboratory sample in accordance with ISO 6497.

1) At present at the stage of draft.

## ISO 6866-1985 (E)

## 8 Procedure

## 8.1 Preparation of the test sample

Prepare the test sample in accordance with ISO 6498.

## 8.2 Test portion

The mass of the test portion depends on the expected gossypol content. For the determination of free gossypol in cottonseed and cottonseed meals and cakes the mass of the test portion should not exceed 1 g; for compound feeding stuffs, it may be as much as 5 g. For the determination of total gossypol, the mass of the test portion should be between 0,5 and 5 g.

NOTE — The mass of the test portion should be large enough to give the quantity of free gossypol indicated in 8.3.1.2 or the quantity of total gossypol indicated in 8.3.2.4 in each aliquot portion of the filtrate.

## 8.3 Determination

## 8.3.1 Determination of free gossypol

**8.3.1.1** Transfer the test portion (8.2) to a 250 ml conical flask, the bottom of which has been covered with glass beads. Add, by means of a pipette, 50 ml of solvent A (5.2), stopper the flask and mix, using the tumbler mixer (6.1), for 1 h. Filter through a dry filter paper, collecting the filtrate in a 100 ml conical flask. During filtration, cover the funnel with a watch-glass.

**8.3.1.2** Transfer, by means of a pipette, identical aliquot portions of the filtrate, containing 50 to 100 µg of gossypol, to each of two 25 ml volumetric flasks (6.3) (C and D). If necessary, make up to 10 ml with solvent A (5.2).

**8.3.1.3** Make up the contents of flask C to volume with the 2-propanol/hexane mixture (5.1) and mix.

This solution will be used as the reference solution against which the test solution will be measured.

**8.3.1.4** Transfer, by means of a pipette, 10 ml of solvent A (5.2) into each of two 25 ml volumetric flasks (E and F).

**8.3.1.5** Make up the contents of flask E to volume with the 2-propanol/hexane mixture (5.1) and mix.

This solution will be used as the reference solution against which the blank test solution will be measured.

**8.3.1.6** Add 2,0 ml of the aniline (5.4) to flasks D and F. Heat for 30 min on a boiling water-bath to develop the colour.

**8.3.1.7** Cool to room temperature, make up to volume with the 2-propanol/hexane mixture (5.1), mix and leave for 1 h.

**8.3.1.8** Measure the absorbance of the blank test solution (flask F; 8.3.1.7) against the reference solution (flask E; 8.3.1.5) and the absorbance of the test solution (flask D; 8.3.1.7) against the reference solution (flask C; 8.3.1.3) using the spectrometer (6.2) at the wavelength of maximum absorbance (between 435 and 445 nm).

**8.3.1.9** Subtract the absorbance of the blank test solution from that of the test solution to obtain the corrected absorbance.

## 8.3.2 Determination of total gossypol

**8.3.2.1** Transfer the test portion (8.2) into a 50 ml volumetric flask and add 10 ml of solvent B (5.3).

**8.3.2.2** Transfer 10 ml of solvent B (5.3) to a second 50 ml volumetric flask (6.3).

**8.3.2.3** Heat the two flasks (8.3.2.1 and 8.3.2.2) for 30 min on a boiling water-bath.

Cool to room temperature and make up to volume with the 2-propanol/hexane mixture (5.1). Mix and leave to settle for 10 to 15 min, then filter and collect the filtrates in 50 ml conical flasks (6.4).

**8.3.2.4** Transfer, by means of a pipette, 2 ml of the filtrate obtained from the test portion, containing 40 to 200 µg of gossypol, into each of two 25 ml volumetric flasks and 2 ml of the filtrate obtained from the second flask into each of two other 25 ml volumetric flasks.

Make up the contents of one of the flasks containing the test solution and of one flask from the blank test to volume with the 2-propanol/hexane mixture (5.1).

These solutions will be used as reference solutions.

**8.3.2.5** Add 2,0 ml of the aniline (5.4) to the remaining test and blank test solutions. Heat for 30 min on a boiling water-bath to develop the colour. Cool to room temperature, make up to volume with the 2-propanol/hexane mixture (5.1), mix and leave for 1 h.

**8.3.2.6** Measure the absorbances of the solutions as specified in 8.3.1.8 and calculate the corrected absorbance as specified in 8.3.1.9.

## 8.4 Number of determinations

Carry out two determinations on the same test sample.