



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 13086-2020

Method for determination of free gossypol in feeds

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Foreword

This Standard was drafted as per the rules specified in GB/T 1.1-2009.

This Standard replaced GB/T 13086-1991 Method for Determination of Free Gossypol in Feeds.

Compared with GB/T 13086-1991, this Standard has the major technical changes as follows besides the editorial modifications:

- Modify the scope of application (see Clause 1 of this Edition; Clause 1 of 1991 Edition);
- Increase the limit of quantification (see Clause 1 of this Edition);
- Delete reagents in the commercially available form that were only used for preparation (see Clause 3 of 1991 Edition);
- Increase the validity period of aniline and solvent A (see 4.2 of this Edition);
- Modify the constant temperature water bath in the apparatus into a water bath (see Clause 5 of this Edition; Clause 5 of 1991 Edition);
- Add preparation method for the cottonseed sample (see Clause 6 of this Edition);
- Modify the sample-weighing amount (see 7.1 of this Edition; 6.1 of 1991 Edition);
- Add the calculation formula for correcting absorbance (see 8.1 of this Edition);

--- Modify the calculation formula of free gossypol content in the sample (see 8.2 of this Edition; 7.1 of 1991 Edition);

--- Add the calculation formula for the results of free gossypol in cottonseed (see

8.2 of this Edition);

--- Modify the significant figures of measurement results (see Clause 8 of this Edition;

7.2 of 1991 Edition);

--- Modify the precision provisions (see Clause 9 of this Edition; 7.3 of 1991 Edition);

--- Add the informative Appendix A (see Appendix A of this Edition).

This Standard uses the redrafting method to modify and adopt the international standard ISO 6866:1985 Animal Feed Stuffs - Determination of Free and Total Gossypol.

Compared with ISO 6866:1985, this Standard has major adjustments in structure.

Appendix A lists the clause number comparison between this Standard and ISO 6866:1985.

The technical differences and causes between this Standard and ISO 6866:1985 are as follows:

--- Regarding the normative references, this Standard adjusts the technical differences to adapt to China's technical conditions. The adjusted conditions and technologies are reflected in Clause 2 Normative Reference. The specific adjustments are as follows:

? Delete the reference document ISO 6497 in ISO 6866:1985;

? Replace ISO 6498 by GB/T 20195 which is equivalent to adopting international standard;

? Add reference of GB/T 6682.

--- The measurement wavelength of the absorbance value is changed from "the wavelength of maximum absorption (435nm~445nm)" to "the wavelength of 440nm" (see Clause 3, 7.3 of this Edition; 8.3.1.8 of ISO 6866:1985);

--- Delete the content related to the determination of total gossypol (see 8.3.2 of

ISO 6866:1985);

--- Add the calculation formula of corrected absorbance [see Formula (1) of this Edition];

--- Delete the related content of total gossypol calculation (see 9.1 of ISO 6866:1985);

--- Modify the calculation formula of free gossypol content in the sample [see Formula (2) of this Edition; 9.1 of ISO 6866:1985];

--- Add the calculation formula for the results of free gossypol in cottonseed [see Formula (3) of this Edition];

--- Delete the content of the test report (see Clause 10 of ISO 6866:1985).

This Standard also makes the following editorial modifications:

--- Modify the name of the standard; and delete the determination of total gossypol, which is suitable for the purpose of sampling in China;

Please note that certain contents of this document may involve patents. The issuing agency of this document is not responsible for identifying these patents.

Method of Determination of Free Gossypol in Feeds

1 Scope

This Standard specifies the spectrophotometric method for the determination of free gossypol in feeds.

This Standard is applicable to the determination of free gossypol in compound feeds, concentrated feeds, concentrate supplements, cottonseed, cottonseed meal, and other feed materials (except cottonseed oil) containing cottonseed and cottonseed meal.

The limit of quantification of this Standard is 20mg/kg.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the

amendments) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

(GB/T 6682-2008, ISO 3696:1987, MOD)

GB/T 20195 Animal Feeding Stuffs - Preparation of Test Samples (GB/T 20195-

2006, ISO 6498:1998, IDT)

3 Principle

Extract the free gossypol in the sample by a mixture of isopropanol-n-hexane containing 3-Aminopropanol; and use the aniline to convert the gossypol into aniline gossypol with aniline. Measure the absorbance value at a wavelength of 440nm.

4 Reagents and Materials

Unless otherwise specified, only the analytically-pure reagents shall be used.

7.1.1 Sample weighing

Do two tests in parallel. The sample weights for different tests are as follows:

--- Cottonseed: weigh 1g of each cottonseed and cottonseed kernel shell, accurate to 0.0001g;

---Cottonseed meal: weigh 1g, accurate to 0.0001g;

--- Other samples: weigh 5g, accurate to 0.01g.

7.1.2 Extraction of free gossypol

The sample was placed in a 250mL triangular flask with stopper; add 20 pieces of glass beads; accurately add 50mL of solvent A; and the mixture is shaken and extracted at room temperature at 120 times/min for 1h. Filter by filter paper; and cover the funnel by a watch glass during filtering. Discard 5mL of the initial filtrate; and collect all the remaining filtrate into a 100mL triangular flask with stopper for later-use. For cottonseed samples, cottonseed and cottonseed kernel shells shall be placed in different triangular flasks; and the other operations are the same as above.

7.2.1 Use a pipette to accurately pipette two aliquots of filtrate (7.1.2) of 5mL~10mL