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

GB/T 13087-2020

Replacing GB 13087-1991

Determination of isothiocyanates in feeds

饲料中异硫氰酸酯的测定方法

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1 Scope

GB/T 13087-2020 is the Chinese national standard on determination of isothiocyanates in feeds, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 21 July 2020 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 February 2021. Classification: ICS 65.120, CCS B46. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This Standard specifies the gas chromatography method for the determination of isothiocyanate in feeds. This Standard is applicable to the determination of isothiocyanate in compound feeds, concentrated feeds, concentrate supplements and feed ingredients. The detection limit of this Standard is 2mg/kg; and the limit of quantification is 5mg/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 5520 Inspection of Grain and Oils - Germination Test of Seeds

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

GB/T 20195 Animal Feeding Stuff Preparation of Test Samples

3 Principle

The glucosinolate in the specimen is in the buffer solution with pH 7.0; under the action of white myrosinase, generate allyl isothiocyanate, butenyl isothiocyanate and pentenyl isothiocyanate. Use the methyl chloride to extract, dehydrate, filter; and then is determined by gas chromatograph; and quantified by internal standard method. The result is calculated as per allyl isothiocyanate.

4 Reagents and Materials

Unless otherwise specified, only use analytically-pure reagents.

4.1 Water: Class-I water specified in GB/T 6682.

4.2 Dichloromethane.

4.3 Petroleum ether (boiling range 30°C ~60°C).

4.4 Anhydrous sodium sulfate.

4.5 Citric acid solution (0.1mol/L): accurately weigh 4.20g of citric acid ($C_6H_8O_7 \cdot H_2O$) and dissolve in water; dilute by water and make constant volume to 200mL; and mix evenly. Prepare for current use.

4.6 Disodium hydrogen phosphate solution (0.2mol/L): accurately weigh 28.39g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) and dissolve in water; dilute by water and make constant volume to 1L; and mix evenly. Prepare for current use.

4.7 Hydrochloric acid solution (0.01mol/L): accurately pipette 0.9mL of concentrated hydrochloric acid; dilute by water and make constant volume of 1L; and mix evenly.

4.8 Sodium hydroxide solution (0.01mol/L): accurately take 0.40g of sodium hydroxide and dissolve in water, dilute with water and dilute to 1L, and mix.

4.9 Buffer solution with pH 7.0: take 176.5 mL of citric acid solution (4.5) in a 1000 mL volumetric flask; dilute by disodium hydrogen phosphate solution (4.6) and make the constant volume to 1000 mL; mix evenly; and use hydrochloric acid solution (4.7) or sodium hydroxide solution (4.8) to adjust the pH to 7.0. Prepare for current use.

4.10 White myrosinase: take 50g of white mustard (*Sinapis alba* L.) seeds (make the germination test in accordance with GB/T 5520; the germination rate shall be greater than 85% within 72h; and the storage period shall not exceed two years); after crushing, place them in a 500mL beaker. Add 100mL of petroleum ether (4.3); stir for 2min; let stand; discard the supernatant liquid; repeat degreasing for 10 times; make the fat content less

than 2%. Put the solvent in a fume hood to evaporate dry; and then pulverize again; 80% passed through the 0.28mm test sieve; pack in an enclosed container; store at -18°C below; valid for 6 months. In order to ensure the activity of white myrosinase, the environment temperature shall be kept below 30°C during the preparation process. Pay attention to a small amount and multiple times during the two crushing to prevent the crusher from overheating.

4.11 Butyl isothiocyanate internal standard solution (0.1mg/mL): accurately take 50mg (accurate to 0.00001g) of butyl isothiocyanate [$\text{CH}_3(\text{CH}_2)_3\text{NCS}$, CAS number: 592-82-5, purity $\geq 99\%$] in a 500mL brown volumetric flask; dissolve and make constant volume by dichloromethane; mix evenly. Store at -18°C below; valid for 3 months.

4.12 Isothiocyanate mixed standard solution (1.0mg/mL): accurately take 10mg (accurate to 0.00001g) of allyl isothiocyanate ($\text{CH}_2 = \text{CHCH}_2\text{NCS}$, CAS number: 57-06-7, purity $\geq 99.7\%$), butenyl isothiocyanate [$\text{CH}_2 = \text{CHCH}_2\text{CH}_2\text{NCS}$, CAS number: 3386-97-8, purity $\geq 95.0\%$], pentenyl isothiocyanate [$\text{CH}_2 = \text{CHCH}_2\text{CH}_2\text{CH}_2\text{NCS}$, CAS number: 18060-79-2, purity $\geq 95.0\%$] in a 10mL brown volumetric flask; dissolve and make constant volume by butyl isothiocyanate internal standard solution (4.11); mix

7.2 Reference conditions for gas chromatography Chromatographic column: polyethylene glycol (FFAP) capillary column, length of 30m, inner diameter of 0.32mm, film thickness of 0.25 μm , or equivalent. Chromatographic column temperature: starting temperature 100°C, keep for 10min; increase to 200°C at 10°C/min; keep for 2min. Inlet temperature: 220°C. Detector temperature: 230°C. Carrier gas: high purity nitrogen. Carrier gas flow rate: 1.0mL/min. Hydrogen flow rate: 35mL/min. Air flow rate: 400mL/min. Makeup flow rate: 20mL/min. Injection volume: 1 μL . Split ratio: 5:1.

7.3 Determination

7.3.1 Determination of standard solution and specimen solution Under the best conditions of the instrument, take the isothiocyanate mixed standard series working solution (4.13) and specimen solution (7.1) on the machine to determine. Refer to Appendix A for the gas chromatogram of each isothiocyanate standard solution.

7.3.2 Qualitative and quantitative Be qualitative by retention time, the retention time of each isothiocyanate in the specimen solution shall be consistent with the retention time of each isothiocyanate in the standard solution; and the relative deviation is within $\pm 2.5\%$. The internal standard method is used for quantification.