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NY

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

NY/T 1975-2010

**Water-soluble fertilizers - Determination of amino-acids
contents**

水溶肥料 游离氨基酸含量的测定

(English Translation)

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1 Scope

This standard specifies the automatic amino acid analyzer method and pre-column derivatization-liquid chromatography method for the determination of free amino acids in water-soluble fertilizers.

This standard applies to the determination of free amino acid content in liquid or solid water-soluble fertilizers.

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) are applicable to this standard.

GB/T 8170 Rules of rounding off for numerical values & expression and judgement of limiting values

HG/T 2843 Chemical fertilizer products - Standard volumetric, standard, reagent and indicator solutions for chemical analysis

NY/T 887 Density testing of liquid fertilizer

3 Determination of free amino acid content - Amino

acid automatic analyzer method (arbitration method)

3.1 Principle

After precipitating the protein from the specimen by the use of sulfosalicylic acid, the EDTA complexes metal elements are used to release amino acids. The amino acids are separated by a separation column, to develop color with ninhydrin, to determine the contents of totally 17 amino acids: aspartic acid, threonine, serine, glutamic acid, Proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine. The sum of these amino acids is the content of free amino acids.

3.2 Reagents and materials

The preparation of reagents, water and solutions used in this standard shall comply with the provisions of HG/T 2843 unless otherwise specified for the specifications and preparation methods.

3.2.1

Sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$).

3.2.2

Hydrochloric acid.

3.2.3

Hydrochloric acid solution

$C(\text{HCl}) = 0.06 \text{ mol/L}$.

3.2.4

Sodium hydroxide solution

$\text{Rho}(\text{NaOH}) = 500 \text{ g/L}$.

3.2.5

Sulfosalicylic acid solution

$\text{Rho}(\text{C}_6\text{H}_6\text{O}_6\text{S} \cdot 2\text{H}_2\text{O}) = 50 \text{ g/L}$.

3.2.6

Disodium edetate solution

$\text{Rho}(\text{EDTA-Na}) = 10 \text{ g/L}$.

3.2.7

Sodium citrate buffer

PH 2.2. Weigh 19.6 g of sodium citrate (3.2.1).

Dissolve it. Pipette it to a 1000 mL volumetric flask. Add 16.5 mL of hydrochloric acid (3.2.2). Add water to the mark. Mix it uniformly. If necessary, it may use hydrochloric acid (3.2.2) and sodium hydroxide solution (3.2.4) to adjust the pH to 2.2.

3.2.8 Mixed amino acid standard solution, chromatographically pure. It contains

total 17 amino acids: aspartic acid, threonine, serine, glutamic acid, Proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine.

3.3 Instruments

3.3.1

Commonly used laboratory instruments.

3.3.2

Automatic amino acid analyzer.

3.3.3 Centrifuge (above 10000 r/min) or 0.45 µm microporous membrane, filter

and syringe.

3.3.4 Steam drying device: Test tube concentrator or other concentration device.

3.4.1 Preparation of specimens

After the solid sample has been reduced several times, take out about 100 g.

Grind it quickly to make all pass through a 0.50 mm aperture sieve (if the sample is wet, it may pass a 1.00 mm sieve). Mix it evenly. Put it in a clean, dry container.

After multiple shaking of the liquid sample, quickly take about 200 mL and place

After precipitating the protein of specimen by sulfosalicylic acid, use the EDTA to make the metal element release the amino acid. The amino acid is chemically derivatized and separated by liquid chromatography to determine the contents of totally 17 amino acids: aspartic acid, threonine, serine, glutamic acid, Proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine. The sum of these amino acids is the content of free amino acids in the fertilizer.

4.2 Reagents and materials

The preparation of reagents, water and solutions used in this standard shall comply with the provisions of HG/T 2843 unless otherwise the specifications and preparation methods are indicated.

4.2.1

Sulfosalicylic acid solution

Rho ($C_7H_6O_6S \cdot 2H_2O$) = 50 g/L.

4.2.3 Mixed amino acid standard solution, chromatographically pure. It contains

total 17 amino acids: aspartic acid, threonine, serine, glutamic acid, Proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine.

4.2.4 Reagents required by the selected derivatization method.

4.3 Instruments

4.3.1

Commonly used laboratory instruments.

4.3.2 Centrifuge (above 10000 r/ min) or 0.45 µm microporous membrane and filter, syringe.

4.3.3 Liquid chromatograph, equipped with UV detector.

4.3.4 The equipment required for the selected derivative method.

4.4.1 Preparation of specimen

After the solid sample has been reduced several times, take about 100 g and quickly grind it to pass through a 0.50 mm aperture sieve (if the sample is wet, it can pass through a 1.00 mm sieve). Mix it evenly. Place it in a clean, dry container. After shaking the liquid sample many times, quickly take about 100 mL and place it in a clean, dry container.

4.4.2 Preparation of sample solution

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