

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

SN/T 5116-2019

**Import and export of food animals, feed malachite green, crystal
violet determination by liquid chromatography-mass
spectrometry/mass spectrometry**

进出口食用动物、饲料孔雀石绿、结晶紫测定 液相色谱-质谱/质谱法

(English Translation)

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1 Scope

This standard specifies the malachite green, crystal violet and metabolite malachite green, cryptic crystal violet in food animals and feed for import and export.

Liquid chromatography-mass spectrometry/mass spectrometry.

This standard applies to fish, shrimp, crab, shellfish, fish meal, plant-based feed, concentrated feed, compound feed, feed additives, concentrate supplements Determination of malachite green, leucomalachite green, crystal violet, leuco crystal violet.

2 Normative references

The following documents are essential for the application of text files. For dated references, only the dated version applies to this article Pieces. For undated references, the latest version (including all amendments) applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods.

3 Method summary

The malachite green, leuco malachite green, crystal violet, and leuco crystal violet residues in the sample were extracted with acetonitrile, neutral alumina and cation exchange.

Purification by solid-phase extraction column, detection by liquid chromatography-mass spectrometry/mass spectrometry, and quantification by internal standard method.

4 Reagent materials

Unless otherwise specified, the reagents used are of analytical grade, and the water is deionized water, which meets the requirements of GB/T 6682.

4.1

Acetonitrile. Chromatographic grade.

4.2

Methanol. chromatographic grade.

4.3

Dichloromethane. chromatographic grade.

4.4

Formic acid. chromatographic grade.

4.5

Ammonium acetate. chromatographic grade.

4.6

Hydroxylamine hydrochloride.

4.7

p-Toluenesulfonic acid.

4.8

Ammonia water. 25 to 28%.

4.9

Neutral alumina column. 1g/3mL, activated with 5mL acetonitrile before use.

4.10

Cation exchange column. 60mg/3mL. Before use, activate with 3mL methanol and 3mL 2% formic acid solution.

4.11. 25g/mL hydroxylamine hydrochloride solution. Weigh 25g hydroxylamine hydrochloride, dissolve in water and make up to 100mL.

4.12 1mol/L p-toluenesulfonic acid solution. Weigh 17.2g of p-toluenesulfonic acid, dissolve in water and make up to 100mL.

4.13 5mol/L ammonium acetate solution. Accurately weigh 0.435g of ammonium acetate (accurate to 0.01g), dissolve in water and make up to 1L.

4.14 2% formic acid solution. accurately transfer 2mL of formic acid, and make up to 100mL with water.

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4.15

Methanol solution containing 5% ammonia water. Accurately remove 5mL of ammonia water and make up to 100mL with methanol.

4.16 5mol/L ammonium acetate solution, containing 0.1% formic acid. accurately weigh 0.435g of ammonium acetate (accurate to 0.01g), add 1mL of formic acid, Dissolve in water and make up to 1L.

4.17

Acetonitrile containing 0.1% formic acid; accurately transfer 1mL of formic acid and make up to 1L with acetonitrile.

4.18

Standards. Malachite Green (MalachiteGreen CAS Number. 2437-29-8), D5-Malachite Green (D5-Malachite Green), Hidden Color Malachite Green (LeucomalachiteGreenCAS No .. 129-73-7), D6-Leucomalachite Green (D6-LeucomalachiteGreen), Crystal Violet (CAS Number. 548-62-9), D6-Crystal Violet (D6-Crystal Violet), Leuco Crystal Staple Violette CAS number. 603-48-5), D6-leucocrystal Violet, with a purity greater than 98%.

4.19

Preparation of standard working solution. Weigh 0.01g (accurate to 0.0001g) malachite green, leucomalachite green, crystal violet, cryptic respectively Color crystal violet standard, dissolved with acetonitrile and made up to 10mL, each standard stock solution concentration is 1mg/mL; standard stock solution Store in a refrigerator at -18 ° C, protected from light, with a validity of 6 months. Take appropriate amount of the above standard stock solution, dilute with acetonitrile solution step by step, prepare it becomes a standard working solution of 100ng/mL and is stored in a refrigerator at 4 ° C, protected from light, with a validity of one month.

4.20

Preparation of internal standard working solution. Weigh 0.01g (accurate to 0.0001g) D5-malachite green, D6-cryptocachite green, D6-Crystal Violet and D6-Crystalline Violet Crystal Violet Standards were dissolved in acetonitrile and made up to 10 mL.

1mg/mL; The standard stock solution is stored in a refrigerator at -18 ° C, protected from light, and has a validity of 6 months. Transfer the appropriate amount of the internal standard storage it is ready to dissolve. It is diluted with acetonitrile solution step by step to prepare an internal standard working solution of 100ng/mL. It is stored in a refrigerator at 4 ° C, protected from light.

1 month.

4.21

Drawing of standard curve. Absorb a certain amount of standard working solution and internal standard working solution as needed, and prepare with blank solution it becomes a standard curve, and it is equipped immediately before use.

4.22

Filter. 0.22 µm organic phase filter.

5 Instruments and equipment

5.1

Liquid chromatography-mass spectrometry/mass spectrometer. equipped with electrospray ionization source (ESI).

5.2

Analytical balance. accurate to 0.0001 g and 0.01 g.

5.3

Homogenizer.

5.4

Centrifuge. The speed can reach 10,000r/min...

5.5

Ultrasonic generator.

5.6

Rotary evaporator.

5.7

Solid-phase extraction device.