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

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**Determination of merabolites of nitrofurans residues in animal  
origin food for import and export. HPLC-MS/MS**

进出口动物源食品中硝基呋喃类代  
**(English Translation)**

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### Foreword

Appendix A and Appendix B of this standard are informative annexes.

This standard is proposed and managed by the National Certification and Accreditation Administration.

This standard was drafted. Hebei Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

### 1 Scope

This standard specifies the method for the determination of nitrofurantoin metabolite residues in animal-derived foods by liquid chromatography-tandem mass spectrometry.

This standard applies to the determination of nitrofurantoin metabolite residues in shrimp, chicken, honey, and casing.

#### 2.1 Method summary

The protein-bound nitrofurantoin metabolite (see Appendix A) is hydrolyzed and freed in an acidic environment with o-nitrobenzene. After formaldehyde was derivatized, it was extracted with ethyl acetate. After purification and concentration, it was measured by a liquid chromatography mass spectrometer. Quantification by external standard method.

#### 2.2 Reagents and materials

Unless otherwise specified, the reagents used were of analytical grade and the water was ultrapure water.

##### 2.2.1

Acetonitrile. HPLC grade.

**2.2.2**

Methanol. HPLC grade.

**2.2.3**

Ethyl acetate. HPLC grade.

**2.2.4**

o-nitrobenzaldehyde.

**2.2.5**

Dimethyl sulfoxide.

**2.2.6**

anhydrous sodium sulfate. 650 ° C burning for 4h, cooled and stored in a closed container for use.

**2.2.7**

Hydrochloric acid solution. 0.125 mol/L.

**2.2.8**

Sodium hydroxide solution. 1 mol/L.

**2.2.9**

Sodium hydroxide solution. 0.1 mol/L.

**2.2.10**

Sodium chloride solution. 300g/L.

**2.2.11**

Hydrochloric acid solution. 1 mol/L.

**2.2.12**

Derivatization reagent. Weigh 100 mg of o-nitrobenzaldehyde in 12.5 mL of dimethyl sulfoxide, and use it now.

**2.2.13**

Constant volume solution. 0.1% aqueous acetic acid. acetonitrile. methanol 3.3.2 (volume fraction).

**2.2.14**

Nitrofurantoin metabolite standards. AOZ, AMOZ, AHD·HCl, SEM·HCl, purity ≥99.0%.

**2.2.15 Standard stock solution. weigh nitrofurantoin standard AOZ, AMOZ 10.0mg, AHD·HCl**

13.2mg, SEM·HCl 14.9mg (accurate to 0.1mg), dissolved in methanol and transferred to a 20mL volumetric flask, determined with methanol the solution was allowed to reach a mark, and the concentration of the solution was 0.5 mg/mL, and it was stored at 4 ° C or lower.

2.2.16 Standard intermediate solution. separately absorb 1.0 mL of each standard stock solution of four nitrofurantoin metabolites, and dilute to 50 mL with methanol.

The concentration was 10 µg/mL.

2.2.17 Standard working solution. Take 1.0 mL of standard intermediate solution, dilute to 100 mL with methanol, and the concentration is 0.1 µg/mL.

2.2.18 Calibration curve standard working solution. Dilute the standard working solution to the appropriate concentration to make the calibration curve, and prepare it when it is ready.

**2.3 Instruments and equipment**

**2.3.1**

Liquid chromatography mass spectrometer for secondary mass spectrometry analysis.

**2.3.2**

Electronic balance.

**2.3.3**

Ultrasonic cleaner.