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Determination of penicillins residues in foodstuffs of animal origin - LC-MS/MS method

动物源性食品中青霉素族抗生素残留量检测方法

液相色谱-质谱/质谱法

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Determination of penicillins residues in foodstuffs of animal origin - LC-MS/MS method

1 Scope

This Standard specifies the determination and confirmation of LC-MS/MS method for penicillins residues in foodstuffs of animal origin.

This Standard applies to the determination of 11 penicillins residues of amoxicyllin, ampicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin, gpenicillin, penicillin, azlocillin, methicillin, phenethicillin in pig muscles, pig liver, pig kidneys, milk, and eggs.

2 Normative references

The following documents contain provisions which, through reference in this Standard, constitute provisions of this Standard. For the dated references, their subsequent amendments (excluding corrections) or revisions do not apply to this Standard. However, the parties who enter into agreement based on this Standard are encouraged to investigate whether the latest editions of these documents are applicable. For undated reference documents, the latest editions apply to this Standard.

GB/T 6682-1992 Water for analytical laboratory use - Specification and test methods (neq ISO 3696:1987)

3 Principles

The penicillins residues in the sample are extracted using acetonitrile-water solution. After the extract is concentrated, USE a buffer solution to dissolve and a solid-phase extraction cartridge to purify. After the eluent is blow-dried by nitrogen, USE liquid chromatography-mass spectrometry/mass spectrometry to determine; USE external standard method to quantify.

4 Reagents and materials

Unless otherwise stated, the reagents used are of analytical pure; and the water is the Grade 1 water specified in GB/T 6682-1992.

WEIGH about 5 g (accurate to 0.01 g) of sample in a 50 mL centrifuge tube; ADD 15 mL of acetonitrile-water solution (4.9); homogenize for 30 s; CENTRIFUGE at 4000 r/min for 5 min; TRANSFER the supernatant to a 50 mL centrifuge tube. TAKE another centrifuge tube; ADD 10 mL of acetonitrile-water solution (4.9); WASH the homogenizer head. USE a glass rod to mash the precipitate in the centrifuge tube; ADD the above-mentioned washing homogenizer head solution; oscillate on a vortex mixer for 1 min; CENTRIFUGE at 4000 r/min for 5 min. The supernatant is combined into a 50 mL centrifuge tube. The head is washed repeatedly using 10 mL of acetonitrile-water solution (4.9) and extracted once. The supernatant is combined into a 50 mL centrifuge tube. USE acetonitrile-water solution (4.9) to dilute to 40 mL. Accurately PIPETTE 20 mL into a 100 mL heart-shaped bottle.

7.1.2 Milk sample

WEIGH 10 g (accurate to 0.01 g) of sample in a 50 mL centrifuge tube; ADD 20 mL of acetonitrile (4.9); homogeneous extraction for 30 s; CENTRIFUGE at 4000 r/min for 5 min; TRANSFER the supernatant to a 50 mL centrifuge tube. TAKE another centrifuge tube; ADD 10 mL of acetonitrile-water solution (4.9); WASH the homogenizer head. USE a glass rod to mash the precipitate in the centrifuge tube; ADD the above-mentioned washing homogenizer head solution; oscillate on a vortex mixer for 1 min; CENTRIFUGE at 4000 r/min for 5 min. The supernatant is combined into a 50 mL centrifuge tube. The head is washed repeatedly using 10 mL of acetonitrile-water solution (4.9) and extracted once. The supernatant is combined into a 50 mL centrifuge tube. USE acetonitrile-water solution (4.9) to dilute to 50 mL. Accurately PIPETTE 25 mL into a 100 mL heart-shaped bottle.

The heart-shaped bottle is evaporated on a rotary evaporator (37 °C water bath) to remove acetonitrile (Easy-to-foam sample can be added with 4 mL of saturated sodium chloride solution).

7.2 Purification

Immediately ADD 25 mL of phosphate buffer solution (4.11) to the heart-shaped bottle from which acetonitrile has been removed; vortex-mix for 1 min; USE 0.1 mol/L sodium hydroxide to adjust the pH to 8.5. At a rate of 1 mL/min, PASS the pretreated solid-phase extraction cartridge; first USE 2 mL of phosphate buffer solution (4.11) to rinse twice; then USE 1 mL of ultrapure water to rinse; and USE 3 mL of acetonitrile to elute (The speed is controlled at 1 mL/min). The eluent, at 45 °C, is blown dry with nitrogen. USE 0.025 mol/L phosphate buffer solution (4.12) to dilute to 1 mL. After passing through a 0.45 μm filter membrane, immediately USE liquid chromatograph-mass spectrometer/mass spectrometer to determine.

reference retention time of 11 penicillins is approximately: amoxicyllin 8.5 min, ampicillin 12.2 min, azlocillin 16.5 min, methicillin 16.8 min, gpenicillin 18.1 min, penicillin 19.4 min, oxacillin 20.3 min, phenethicillin 20.5 min, cloxacillin 21.5 min, nafcillin 22.3 min, dicloxacillin 23.5 min. The reconstructed ion chromatogram of the quantitative ion pair of penicillins standard solutions is shown in Figure B.1.

7.3.4 Qualitative determination

According to the above conditions, the sample is determined; and a standard working curve is established. If the retention time of the compound mass chromatographic peak in the sample, compared with the standard solution, is within the allowable deviation of ±2.5%; the signal-to-noise ratio of the reconstructed ion chromatographic peak of the qualitative ion pair of the compound under test is greater than or equal to 3 (S/N≥3); the signal-to-noise ratio of the reconstructed ion chromatographic peak of the quantitative ion pair is greater than or equal to 10 (S/N≥10); and the relative abundance of the qualitative ion pair, compared with the standard solution of the equivalent concentration, has a deviation not exceeding the requirements of Table 2; then it can judged that there is the corresponding target compound in the sample.

Table 2 -- Maximum allowable deviation of relative ion abundance during qualitative confirmation

Relative ion abundance	>50%	>20%~50%	>10%~20%	≤10%
Allowable relative deviation	±20%	±25%	±30%	±50%

7.3.5 Quantitative determination

According to the external standard method, USE the standard working curve to perform quantitative determination.

7.3.6 Blank test

Except that no sample is added, all the operation procedures mentioned above are followed.

8 Calculation and expression of results

USE chromatographic data processor or formula (1) to calculate the penicillins residues in the sample. The calculation result needs to deduct the blank value:

$$X = \frac{c \times V \times 1\,000}{m \times 1\,000} \qquad \cdots\cdots(1)$$

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