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Determination of vitamin K_3 in feeds - High performance liquid chromatography

饲料中维生素 K_3 的测定 高效液相色谱法

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Determination of vitamin K_3 in feeds - High performance liquid chromatography

1 Scope

This Standard specifies the high performance liquid chromatography for the determination of vitamin K_3 (menadione sodium bisulfite, nicotinamide bisulfite menadione, dimethylpyrimidinol menadione sulfite, as menadione) in feeds.

This Standard applies to the determination of vitamin K_3 in compound feeds, concentrated feeds, additive premixed feeds and concentrate supplements. The limit of quantification of the method is 0.4mg/kg.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 603, *Chemical reagent - Preparations of reagent solutions for use in test methods*

GB/T 6682, *Water for analytical laboratory use - Specification and test methods*

GB/T 14699.1, *Feeding Stuffs - Sampling*

GB/T 20195, *Animal feeding stuffs - Preparation of test samples*

3 Principle

The specimen is extracted with chloroform and sodium carbonate solution and converted into free menadione. It is separated by reversed-phase C_{18} column, detected by UV detector, and quantified by external standard method.

4 Reagents and materials

Unless otherwise stated, all reagents in this Standard are analytically pure. The test water is in line with the requirements for grade three water in GB/T 6682. Chromatographic water is in line with the requirements for grade one water in GB/T 6682. The solution is prepared according to GB/T 603.

6 Sampling and specimen preparation

Take representative feed samples according to GB/T 14699.1. Conduct down-sampling by quartering.

Prepare specimens according to GB/T 20195. Grind. All pass through the 0.25mm hole sieve. Mix well. Pack in an airtight container. Store at low temperature away from light.

7 Analysis steps

WARNING -- Because vitamin K_3 is sensitive to air and ultraviolet light, and the chloroform solution used as an extractant has certain toxicity, all operations should be performed in a fume hood away from light.

7.1 Preparation of specimen solutions

Weigh 0.25g~0.5g of vitamin premix feed (accurate to 0.0001g) or 1g of compound premix feed or 5g of concentrated feed (accurate to 0.001g), 5g~10g of compound feed and concentrate supplement (accurate to 0.001g). Put into a 100mL conical flask with stopper. Accurately add 50mL of chloroform (4.1) and place it on a rotary shaker (5.5) for 2 min. Add 5mL of sodium carbonate solution (4.4) and rotate for 3 min. Add 5g of diatomaceous earth and anhydrous sodium sulfate mixture (4.7). Shake on a rotating shaker for 30 min. Then use medium-speed filter paper to filter (or transfer into a centrifuge tube and centrifuge at 5000r/min for 10min).

Determine the aliquot amount according to the expected amount of the sample, the weighing amount and the amount of the extract (see Table A.1). Accurately draw an appropriate amount of chloroform extract (V_2). Blow dry with nitrogen (or evaporate to dryness at 40°C under reduced pressure). Use methanol (4.2) to dissolve. Set volume (V_3). Make the concentration of the specimen solution be 0.1μg~5μg of menadione per ml. Filter through a 0.45μm organic filter membrane for analysis by high performance liquid chromatography.

7.2 Determination

7.2.1 Reference chromatographic conditions

Chromatographic column: C_{18} type column; length is 150mm, inner diameter is 4.6mm, particle size is 5μm, or analytical column with similar performance;

Mobile phase: methanol + water (75+25);

Flow rate: 1.0mL/min;

Column temperature: room temperature;

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