

Cashew Nut Shell Liquid

Proposed revision of Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives.

Appended table 1 (Re: Art. 1)

1. Specifications for ingredients, standards for methods of manufacture, use and storage, and standards for labeling of feeds in general

(1) Specifications for ingredients of feeds in general

- Cashew Nut Shell Liquid as feed additive shall not be used in feeds (including raw materials or ingredients used to manufacture feeds) other than for cattle.
- The content of Cashew Nut Shell Liquid in feed for cattle (except raw materials or ingredients used to manufacture feeds) shall be no more than 0.1 %.

(2) ~ (4) • • •

(5) Standards for labeling of feeds in general

- Feed containing feed additives for which the target animal is specified must be labeled with the type of the target animal.

2. Specifications for feed additives

Active Substance

Compositional specifications

Content: Cashew Nut Shell Liquid is including anacardic acid not less than 59%. Anacardic acid are the sum total of 6-[8(Z)-pentadecenyl]salicylic acid, 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid and 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid.

Physical and chemical properties

- (1) Cashew Nut Shell Liquid is viscous oily liquid which has brown to black brown color.

Confirmation test:

- (1) The peaks of 6-[8(Z)-pentadecenyl]salicylic acid, 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid and 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid in 5 uL of analysis sample have same retention times with the peaks of 6-[8(Z)-pentadecenyl]salicylic acid, 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid and 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid in 5 uL of standard sample, under HPLC analysis procedure and condition described on Assay.

Purity test:

- (1) Lead: Cashew Nut Shell Liquid shall not have more than 4 ug/g as Lead with Method 1 (Atomic Absorption Spectrometry) which weighing amount is 2.5 g (2.45-2.54 g).

(2) Arsenic: Cashew Nut Shell Liquid shall not has stronger color than standard solution with Equipment A, and which sample solution made under Method 3 and its weighing amount is 0.5 g (0.45-0.54 g).

Water content: $\leq 10\%$ (direct titration)

Assay: Sampling approx. 0.15 g of Cashew Nut Shell Liquid by 0.001 g digit and record its actual sampling amount, and then put into 15 mL conical tube. Adding 10 mL of ethyl acetate into the tube with transfer pipet and agitating strongly for 1 min. After leaving 30 mins on room temperature, filtering the organic solution with membrane filter (0.45 μm). Correcting 2 mL of filtered sample solution and dilute by ethyl acetate with 20 mL volumetric flask and call it as analysis sample. 5 μL of analysis sample is analyzed under the HPLC condition below. The peak area of 6-[8(Z)-pentadecenyl]salicylic acid, 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid and 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid are measured by obtained chromatograph and calculate the content with each calibration curves which were made prior to analysis.

$$S_1 = C_1 \times (f_1/100) \times 0.01/W$$

$$S_2 = C_2 \times (f_2/100) \times 0.01/W$$

$$S_3 = C_3 \times (f_3/100) \times 0.01/W$$

The sum total of anacardic acid content (%) = $S_1 + S_2 + S_3$

S_1 : The content of 6-[8(Z)-pentadecenyl]salicylic acid (%)

S_2 : The content of 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid (%)

S_3 : The content of 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid (%)

C_1 : Calculated concentration of 6-[8(Z)-pentadecenyl]salicylic acid ($\mu\text{g/mL}$)

C_2 : Calculated concentration of 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid ($\mu\text{g/mL}$)

C_3 : Calculated concentration of 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid ($\mu\text{g/mL}$)

f_1 : The purity of standard material of 6-[8(Z)-pentadecenyl]salicylic acid (%)

f_2 : The purity of standard material of 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid (%)

f_3 : The purity of standard material of 6-[8(Z),11(Z), 14(Z)-pentadecatrienyl]salicylic acid (%)

W: Sampling amount (g)

HPLC Condition

Detector: UV detector, 280 nm

Column: ODS Column LC18, 75 mm x Φ 4 mm, particle size 3 μ m

Column temperature: Constant temperature around 25°C

Elution solution: Acetonitrile/Water/Acetic acid = 80/20/1 (v/v)

Flow rate: 2.0 mL/min

Column selection criteria: Sampling each 10 mg (9.5-10.4 mg) of 6-[8(Z)-pentadecenyl]salicylic acid standard material and 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid standard material and then dissolve to a suitable amount of ethyl acetate. Then, dilute the solution by ethyl acetate with 10 mL volumetric flask. When 5 μ L of this solution is analyzed by HPLC condition as described above, elution in the order of 6-[8(Z)-pentadecenyl]salicylic acid and 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid, and its separation degree shall be more than 11.

Making Calibration Curves

Sampling 10 mg of 6-[8(Z)-pentadecenyl]salicylic acid standard by 0.1 mg digit and record its actual sampling amount. Dissolve to a suitable amount of ethyl acetate, and then dilute the solution by ethyl acetate with 10 mL volumetric flask and call it as 6-[8(Z)-pentadecenyl]salicylic acid standard stock solution. This standard stock solution is used to make concentration series of standard solution which include 6-[8(Z)-pentadecenyl]salicylic acid accurately 50 μ g, 100 μ g, 250 μ g and 500 μ g/mL for each standard solution. Calibration Curve of 6-[8(Z)-pentadecenyl]salicylic acid is made by HPLC method with 5 μ L of each concentration series and 5 μ L of standard stock solution and its peak area.

Calibration curves of 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid standard and 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid standard are made in the same way.

Manufacturing Method Requirement

Cashew Nut Shell Liquid shall be squeezed with cold-press method without heating and organic solvent extraction.

Storage Requirement

Keep it in shading and airtight package.

Product Formulation

Compositional specifications: Powdery formulation which consists of carriers and water as needed.

Content: Product formulation of Cashew Nut Shell Liquid is including anacardic acid at 90-120% of the amount on the label.

Confirmation test:

- (1) The confirmation test for active substance (Cashew Nut Shell Liquid) shall be applied *mutatis mutandis*.

Assay: The assay for active substance (Cashew Nut Shell Liquid) shall be applied *mutatis mutandis*. In this case, “Sampling approx. 0.15 g of Cashew Nut Shell Liquid by 0.001 g digit” is reinterpreted to “Sampling the amount equivalent to Cashew Nut Shell Liquid approx. 75 mg with 3 significant digits”.

Storage Requirement

Store in a cool dry place, away from direct exposure to sunlight and heat.