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Method of Test for Aloin in Foods

1. Scope

This method is applicable to the determination of aloin of aloin A and aloin B in foods.

2. Method

After extraction, analytes are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: negative ion electrospray ionization (ESI^-).

2.1.1.2. Column: Acquity UPLC® BEH C18, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Vortex mixer

2.1.3. Ultrasonicator

2.1.4. Centrifuge: centrifugal force > 4500 ×g.

2.2. Chemicals

Acetonitrile, HPLC grade;
Methanol, HPLC grade;
Acetic acid, AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);
Aloin A and aloin B, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL.

2.3.2. Centrifuge tube: 50 mL, PP.

2.3.3. Membrane filter: 0.2 μm, PTFE.

2.4. Reagents

2.4.1. Methanol: deionized water (1:1, v/v)

Mix methanol and deionized water at the ratio of 1:1 (v/v).

2.4.2. Acetonitrile: deionized water (4:6, v/v)

Mix acetonitrile and deionized water at the ratio of 4:6 (v/v).

2.5. Mobile phase

2.5.1. Solvent A

Mix 1 mL of acetic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Mix 1 mL of acetic acid with methanol to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 5 mg of aloin A and aloin B reference standards accurately weighed to each 10-mL volumetric flask. Dissolve and dilute to volume with methanol: deionized water (1:1, v/v) as the standard stock solutions. Store at -20℃ in the dark. When to use, mix appropriate volume of each standard stock solution, and dilute with acetonitrile: deionized water (4:6, v/v) to 1 μg/mL as the standard solution(note).

Note: As aloin would decay easily, check the amount of aloin in the stock standard solution before use.

2.7. Sample solution preparation

2.7.1. Food in capsule and tablet form

Transfer about 1 g of the sample accurately weighed into a 10-mL volumetric flask. Add 8 mL of acetonitrile: deionized water (4:6, v/v), vortex for 1 min, ultrasonicate for 10 min, and vortex for 1 min again. Dilute to 10 mL with acetonitrile: deionized water (4:6, v/v), and centrifuge at 4500 ×g for 10 min. Take the supernatant as the sample stock solution. Take 500 μL (a) of the sample stock solution, and dilute with acetonitrile: deionized water (4:6, v/v) to 1000 μL (b). Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.2. Other foods:

Transfer about 5 g of the sample accurately weighed into a 10-mL volumetric flask. Add 5 mL of acetonitrile: deionized water (4:6, v/v), vortex for 1 min, ultrasonicate for 10 min, and vortex for 1 min again. Dilute to 10 mL with acetonitrile: deionized water (4:6, v/v), and centrifuge at 4500 ×g for 10 min. Take the supernatant as the sample stock solution. Take 500 μL (a) of the sample stock solution, and dilute with acetonitrile: deionized water (4:6, v/v) to 1000 μL (b). Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Matrix-matched calibration curve

Take a blank sample, and follow the procedure described in section 2.7 to obtain the blank sample stock solution. Take several 500 μL (a) of the blank sample stock solution, add 5~500 μL of the standard solution, and dilute with

acetonitrile: deionized water (4:6, v/v) to 1000 μL (b) as the matrix-matched stand solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curves of aloin A and aloin B by the peak areas of aloin A and aloin B vs. the added concentrations (0.005~0.5 μg/mL).

LC-MS/MS operating conditions(note):

Column: Acquity UPLC® BEH C18, 1.7 μm, 2.1 mm × 10 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0.0 → 0.4	70 → 70	30 → 30
0.4 → 5.0	70 → 15	30 → 85
5.0 → 6.0	15 → 15	85 → 85
6.0 → 7.0	15 → 0	85 → 100
7.0 → 8.0	0 → 0	100 → 100
8.0 → 9.0	0 → 70	100 → 30
9.0 → 15.0	70 → 70	30 → 30

Flow rate: 0.3 mL/min.

Sample rack temperature: 10℃.

Injection volume: 5 μL.

Ionization mode: ESI^-.

Capillary voltage: 1.5 kV.

Ion source temperature: 150℃.

Desolvation temperature: 450℃.

Cone gas flow rate: 30 L/hr.

Desolvation flow rate: 900 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are shown as follows:

Analyte	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Aloin A	417 > 297*	31	22
	417 > 268	31	33
Aloin B	417 > 297*	31	22
	417 > 268	31	33

*The quantitative ion.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification:

Accurately inject 5 μL of the sample solution and the matrix-matched standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.8. Identify aloin based on the retention time and the relative ion intensities(note). Calculate the amount of aloin in the sample by the following formula:

$$\text{The amount of the aloin in the sample (mg/kg)} = \frac{\sum C \times V \times F}{M}$$

Where,

C：the concentration of aloin A or aloin B in the sample solution calculated by the matrix-matched calibration curve (μg/mL)

V：the final make-up volume of the sample (mL)

M：the weight of the sample (g)

F：dilution factor, b/a

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Remark

1. Limits of quantification (LOQs) are 0.1 mg/kg for foods in capsule and tablet form, and 0.02 mg/kg for other foods.
2. Further validation should be performed when interfering compounds appear in samples.

Reference

1. Wang, P. G., Zhou, W., Wamer, W. G., Krynitsky, A. J. and Rader, J. I. 2012. Simultaneous determination of aloin A and aloe emodin in products containing *Aloe vera* by ultra-performance liquid chromatography with tandem mass

spectrometry. Anal. Methods 4: 3612-3619.
2. Brown, P. N., Yu, R., Kuan, C. H., Finley, J., Mudge, E. M. and Dentali, S. 2014. Determination of aloin A and aloin B in *Aloe vera* raw materials and finished products by high-performance liquid chromatography: single-laboratory validation. J. AOAC Int. 97: 1323-1328.
3. Ding, W. J., Wu, X. F., Zhong, J. S. and Wan, J. Z. 2014. Effects of temperature, pH and light on the stability of aloin A and characterisation of its major degradation products. Int. J. Food Sci. Technol. 49: 1773-1779.

Reference chromatogram

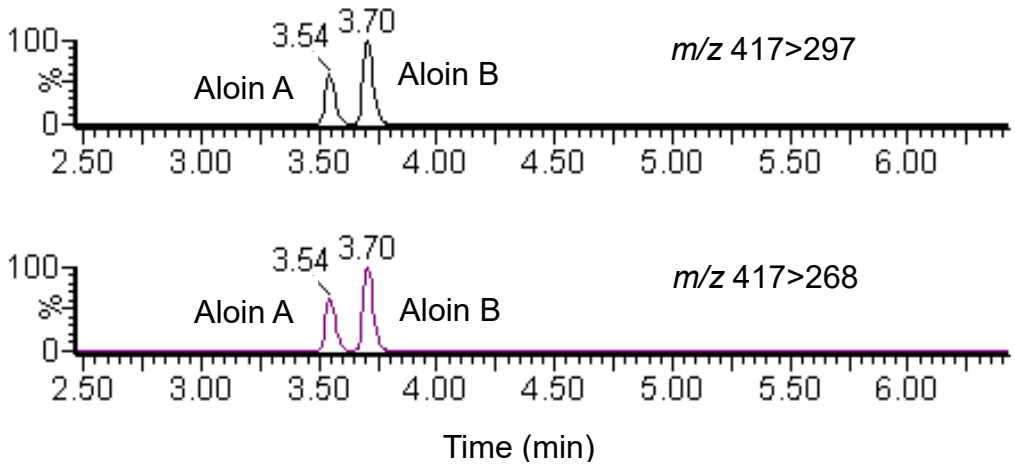

Figure. MRM chromatograms of aloin A and aloin B standards analyzed by LC-MS/MS.