

## **Method of Test for Total Glycoalkaloids in Food**

### **1. Scope**

This method is applicable to the determination of total glycoalkaloids including  $\alpha$ -solanine and  $\alpha$ -chaconine in potatoes.

### **2. Method**

After extraction, glycoalkaloids 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: electrospray ionization, ESI.

2.1.1.2. Column: BEH C18, 1.7  $\mu$ m, 2.1 mm i.d.  $\times$  10 cm, or an equivalent product.

2.1.2. Blender.

2.1.3. Centrifuge: centrifugal force  $> 3000 \times g$ , temperature control  $< 5^{\circ}\text{C}$ .

2.1.4. Ultrasonicator.

#### **2.2. Chemicals**

Acetic acid, residue grade;

Formic acid, residue grade;

Acetonitrile, HPLC grade;

Deionized water, resistivity  $\geq 18 \text{ M}\Omega\cdot\text{cm}$  (at  $25^{\circ}\text{C}$ );

$\alpha$ -Solanine and  $\alpha$ -chaconine, 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: 0.22  $\mu$ m, PVDF.

#### **2.4. 5% Acetic acid**

Dilute 5 mL of acetic acid with deionized water to 100 mL.

#### **2.5. 2% Acetic acid**

Dilute 20 mL of acetic acid with deionized water to 1000 mL.

#### **2.6. 0.1% Formic acid**

Dilute 0.5 mL of formic acid with deionized water to 500 mL.

#### **2.7. Mobile phase**

Mix 0.1% formic acid and acetonitrile at the ratio of 7: 3 (v/v), filter with a membrane filter, and take the filtrate as the mobile

phase.

## 2.8. Standard solution preparation

Transfer about 10 mg of  $\alpha$ -solanine and  $\alpha$ -chaconine reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with 5% acetic acid to volume as the standard stock solutions. Store under refrigeration. When to use, mix appropriate volume of each standard stock solution, and dilute with the mobile phase to 0.025-10  $\mu\text{g/mL}$  as the standard solutions.

## 2.9. Sample solution preparation

Transfer about 5 g of the fine-cut and homogenized sample accurately weighed into a 50-mL centrifuge tube, add 30 mL of 2% acetic acid, and ultrasonicate for 15 min. Centrifuge at  $3000 \times g$  for 5 min at  $5^\circ\text{C}$ , and collect the supernatant. Add 30 mL of 2% acetic acid to the residue, and repeat the above extraction process twice. Combine the supernatants, dilute with 2% acetic acid to 100 mL, and filter with a membrane filter. Take the filtrate as the sample solution.

## 2.10. Identification and quantification

Accurately inject 10  $\mu\text{L}$  of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Identify glycoalkaloids based on the retention time and the relative ion intensities<sup>(note</sup>

1). Calculate the amount of total glycoalkaloids in the sample by the following formula:

The amount of total glycoalkaloids in the sample ( $\text{mg/kg}$ ) =

$$\frac{\sum C \times V}{M}$$

Where,

C: the concentration of  $\alpha$ -solanine and  $\alpha$ -chaconine in sample solution calculated by the standard curve ( $\mu\text{g/mL}$ )

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

M: the weight of the sample (g)

LC-MS/MS operating conditions<sup>(note 2)</sup>

Column: BEH C18, 1.7  $\mu$ m, 2.1 mm i.d.  $\times$  10 cm.

Mobile phase: prepared as section 2.7.

Flow rate: 0.25 mL/min.

Injection volume: 10  $\mu$ L.

Capillary voltage: 3.2 kV.

Ionization mode: ESI<sup>+</sup>

Ion source temperature: 150°C.

Desolvation temperature: 400°C.

Detection mode: multiple reaction monitoring (MRM).

Detection ion pair, cone voltage and collision energy are shown as follows:

Analyte	Precursor ion ( <i>m/z</i> ) > product ion ( <i>m/z</i> )	Cone voltage (V)	Collision energy (eV)
$\alpha$ -Solanine	868.5 > 398*	54	80
	868.5 > 722.5	54	80
$\alpha$ -Chaconine	852.5 > 706.5*	44	76
	852.5 > 398	44	75

\*The quantitative ion.

Note: 1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions ( $\leq$  100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	$\pm$ 20
> 20 ~ 50	$\pm$ 25
> 10 ~ 20	$\pm$ 30
$\leq$ 10	$\pm$ 50

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

**Remark**

1. Limits of quantification (LOQs) for  $\alpha$ -solanine and  $\alpha$ -chaconine

are 0.5 mg/kg.

2. Further validation should be performed when interfering compounds are found in the samples.

### **Reference**

Sánchez Maldonado, A. F., Mudge, E., Gänzle, M. G. and Schieber, A. 2014. Extraction and fractionation of phenolic acids and glycoalkaloids from potato peels using acidified water/ethanol-based solvents. Food Res. Int. 65: 27-34.

## Reference chromatogram

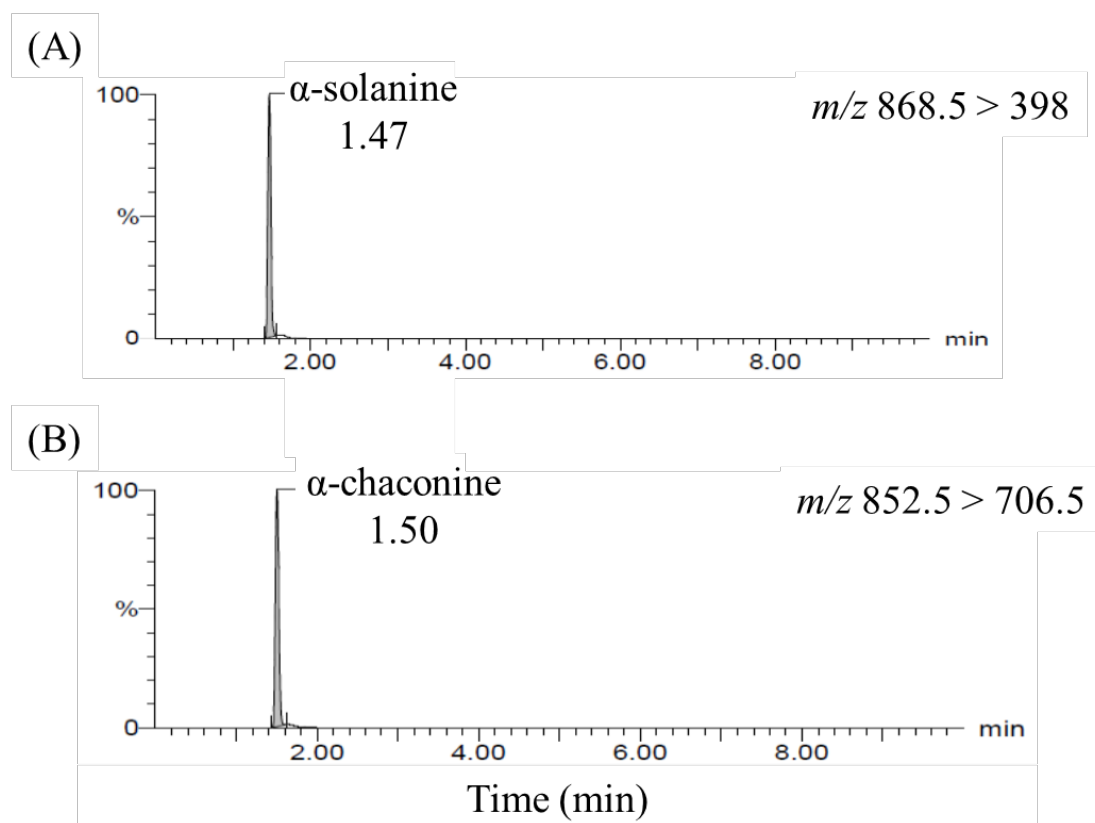


Figure. MRM chromatograms of  $\alpha$ -solanine (A) and  $\alpha$ -chaconine (B) standards analyzed by LC-MS/MS.