

Method of Test for Total Amount of Maleic Acid and Maleic Anhydride in Foods

1. Scope

This method is applicable for the determination of total amount of maleic acid and maleic anhydride in maleic anhydride esterified starch and its products.

2. Method

After extraction and basic hydrolysis, maleic acid is determined by high performance liquid chromatograph (HPLC).

2.1. Equipment

2.1.1. High Performance Liquid chromatograph.

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: GL Sciences InertSustain C18, 5 μm , 4.6 mm i.d. \times 25 cm, or an equivalent product.

2.1.2. Shaker.

2.1.3. pH meter.

2.2. Chemicals

Methanol, HPLC grade;

Phosphoric acid, 85%, AR grade;

Hydrochloric acid, AR grade;

Potassium hydroxide, AR grade;

Mili-Q water, resistivity $\geq 18 \text{ M}\Omega \cdot \text{cm}$ (at 25°C);

Maleic acid, reference standard.

2.3. Apparatus

2.3.1. Volumetric flask: 50 mL and 100 mL.

2.3.2. Centrifuge tube: 50 mL, PP.

2.3.3. Membrane filter: 0.22 μm , PVDF.

2.4. Reagents

2.4.1. 50% methanol

Dilute 250 mL of methanol with Mili-Q water to 500 mL.

2.4.2. 5N hydrochloric acid

Cautiously add 42 mL of hydrochloric acid to 50 mL of Mili-Q water, then cool to room temperature, and dilute with Mili-Q water to 100 mL.

2.4.3. 0.5N potassium hydroxide

Dissolve 14 g of potassium hydroxide in Mili-Q water and dilute with Mili-Q water to

500 mL.

2.4.4. 0.1% phosphoric acid

Dilute 1.2 mL of phosphoric acid with Mili-Q water to 1000 mL.

2.4.5. Mobile phase solution

0.1% phosphoric acid : methanol (98:2, v/v).

2.5. Standard solution preparation

Accurately weigh 100 mg of maleic acid reference standard to 100-mL volumetric flask, dissolve and dilute to volume with Mili-Q water as standard stock solution. When to use, dilute standard stock solution with Mili-Q water to 0.02 - 1.0 µg/mL as working standard solution.

2.6. Sample solution preparation

Transfer about 1 g of the sample accurately weighed to a centrifuge tube, add 25 mL of 50% methanol, shake for 30 min. add 20 mL of 0.5N potassium hydroxide and mix. After standing for 2 hrs, acidify with about 3 mL of 5N hydrochloric acid, dilute with Mili-Q water to 50 mL, and stand for several minutes. Dilute 100 µL (a) of the upper solution with Mili-Q water to 1000 µL (b), then mix and filtrate with membrane filter as the sample solution.

2.7. Identification and quantification

Accurately inject 20 µL of the sample solution and standard solution into HPLC respectively according to below HPLC operating conditions. Identify and quantify maleic acid against the retention time and absorbance spectrum. Calculate the total amount of maleic acid and maleic anhydride in the sample by the following formula:

$$\text{The total amount of maleic acid and maleic anhydride in sample (ppm)} = \frac{C \times V \times F}{M}$$

where

C: the concentration of maleic acid in the sample solution calculated by the standard curve (µg/mL)

V: the make up volume of sample (mL)

M: the weight of sample (g)

F: the dilution factor, b/a

Note: The total amount of maleic acid and maleic anhydride is expressed "as maleic acid".

HPLC operating conditions:

Photodiode array detector: UV 214 nm

Mobile phase: 0.1% phosphoric acid : methanol (98:2, v/v)

Column: GL Sciences InertSustain C18, 5 µm, 4.6 mm i.d. × 25 cm

Injection volume: 20 µL

Flow rate: 1 mL/min

Note:

1. The column used should effectively separate maleic acid with other acids, such as fumaric acid, acetic acid, malic acid and succinic acid, etc.

2. All the parameters can be adjusted according to the equipment used if above conditions are not applicable.

Remarks:

1. The limit of quantification (LOQ) for maleic acid is 10 ppm.
2. Further validation shall be done when interference compounds appeared in samples.
3. As verify by liquid chromatograph/tandem mass spectrometer (LC/MS/MS), use 0.1% formic acid: methanol (98:2, v/v) as the mobile phase, and the parameters of multiple reaction monitoring (MRM) are shown as follows:

Analyte	Ionization mode	Detection ion (m/z)	Declustering potential (V)	Collision energy (eV)
		precursor ion > product ion		
Maleic acid	ESI ⁻	115 > 71	-15	-11

4. Trace amounts of maleic acid may be present in foods, therefore, to determine the illegal addition of maleic anhydride esterified starch, further inspection and interpretation of raw materials shall be conducted.