MOA Of Mulberry Leaf Extract 1-deoxynojirimycin HPLC

1 Scope

This document describes the principle, reagents and materials, instruments and equipment, detection method, result calculation and expression, repeatability, precision, and spiked recovery rate for the determination of 1-deoxynojirimycin content in mulberry leaf extract using high performance liquid chromatography.

This document is applicable to the determination of 1-deoxynojirimycin content in mulberry leaf extract.

2 Normative References

The following documents, through normative references in the text, constitute essential provisions 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 6682 Water for Analytical Laboratories – Specifications and Test Methods

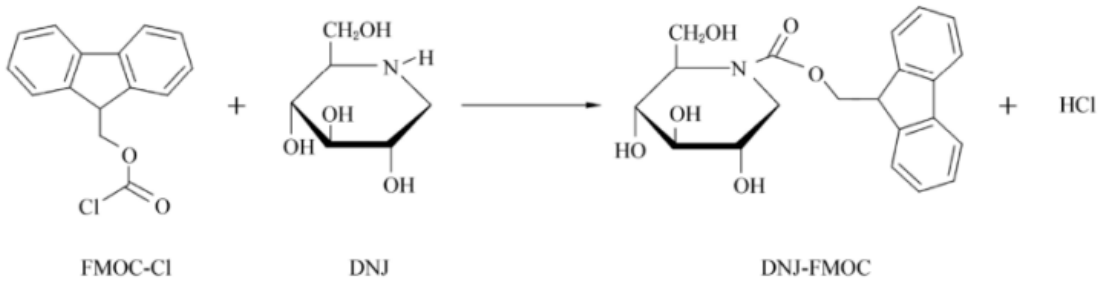
3 Terms and Definitions

This document does not contain any terms and definitions that require definition.

4. Principle

Methoxyyl chloride (FMOC-CD) is an amino acid derivatizing reagent, mainly suitable for the derivatization of primary and secondary amines. The resulting derivatives exhibit strong ultraviolet absorption, with a maximum absorption wavelength of 254 nm. Since 1-deoxynojirimycin (DNJ) contains a secondary amine structure, FMOC-CD can be used as a derivatizing reagent to derivatize DNJ. HPLC is then used for quantitative detection of 1-deoxynojirimycin at a detection wavelength of 254 nm.

The reaction formula of DNJ with oxygen is shown in follow.



FMOC-Cl DNJ DNJ-FMOC

5. Reagents or Materials

Unless otherwise specified, only reagents confirmed to be of analytical grade and water conforming to GB/T 6682 Grade I shall be used in the analysis.

5.1 Reagents

5.1.1 Acetonitrile (C_2H_2N), chromatographic grade.

5.1.2 Glacial acetic acid (CH_3COOH).

5.1.3 Oxygen ($CisH_2ClO_2$).

5.1.4 Glycine (CH_5NO_2).

5.1.5 Potassium borate buffer, pH 8.5.

5.2 Solution Preparation

FMOC-Cl acetonitrile solution: Weigh 0.065 g FMOC-Cl, accurate to 0.001 g, dissolve in acetonitrile, and dilute to 50 mL to obtain a 5 mmol/L FMOC-Cl acetonitrile solution. The FMOC-Cl acetonitrile solution should be prepared fresh for use.

5.3 Standards

1-Deoxynojirimycin standard, purity ≥98%.

5.4 1-Deoxynojirimycin Standard Stock Solution

Weigh 100 mg (accurate to 0.001 g) of 1-deoxynojirimycin standard and place it in a 100 mL volumetric flask. Dissolve and dilute to the mark with 0.01 mol/L hydrochloric acid aqueous solution, and mix well.

5.5 1-Deoxynojirimycin Standard Working Solution

Prepare a series of standard solutions by diluting the 1-deoxynojirimycin standard stock solution with hydrochloric acid aqueous solution. The concentrations of the standard solutions are 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, and 0.10 mg/mL, respectively.

5.6 Materials

Filter membrane, 0.45 μm pore size organic phase filter membrane.

6. Instruments and Equipment

6.1 Analytical Balance: Sensitivity 0.01 g.

6.2 Ultrasonic Oscillator/Ultrasonic Cleaner: Ultrasonic frequency 40 kHz~80 kHz.

6.3 High Performance Liquid Chromatograph (HPLC): Includes UV detector or diode detector and chromatography workstation.

6.4 Constant Temperature Water Bath.

7. Detection Method

7.1 Sample Preparation

Weigh 0.100 g of mulberry leaf extract, accurate to 0.001 g, and dissolve in 0.010 mol/L hydrochloric acid aqueous solution using ultrasonic assistance for 15 min. After cooling to room temperature, bring the volume to 50 mL to obtain the sample solution.

7.2 Derivatization Reaction

Place 100 L of the standard or sample solution into a 1.5 mL centrifuge tube. Add 175 L of 0.4 mol/L potassium borate buffer solution (pH 8.5) and 250 L of 5 mmol/L FMOC-Cl acetonitrile solution sequentially. Mix thoroughly for 30 s, and react in a 25℃ water bath for 25 min. Then add 100 L of 0.1 mol/L glycine solution to neutralize excess FMOC-Cl, and react for 20 min. Next, add 75 L of 1% acetic acid aqueous solution and 300 L of deionized water. After the reaction is complete, filter through a 0.45 μm organic phase filter membrane to obtain the derivatized sample solution. Store the sample solution protected from light for later use.

7.3 Determination Conditions

Run the high-performance liquid chromatograph according to the manufacturer's operating manual. The following chromatographic analysis conditions are for reference only; other conditions should be verified for their suitability. Chromatographic conditions were as follows:

—Column: RP-C18 (250 mm × 4.6 mm id., 5 m);

—Mobile phase: Acetonitrile-0.1% acetic acid aqueous solution (32:68, v/v);

—Flow rate: 1.0 mL/min;

—Column temperature: 30 ℃;

—Injection volume: 10 L;

—Detection wavelength: 254 nm.

7.4 Standard Curve Construction

According to the chromatographic conditions in 7.3, standard working solutions of different concentrations were extracted and derivatized according to 7.2. 10 L of the derivatized sample solution was injected into the HPLC system. The peak area was plotted on the ordinate, and the corresponding sample solution concentration on the abscissa.

Linear regression was performed to obtain the standard curve equation.

The HPLC chromatogram of 1-deoxynojirimycin standard is shown in Appendix A.

7.5 Sample Determination

Inject the derivatized sample solution from step 7.2 under the chromatographic conditions described in 7.3 to obtain the peak area of the sample solution. Qualitative analysis is performed based on the retention time and the chromatogram of the 1-deoxynojirimycin standard. The content of 1-deoxynojirimycin in the sample solution is determined from the standard curve.

The response value of 1-deoxynojirimycin in the sample solution should be within the linear range of the standard curve. If it exceeds the linear range, the sample solution should be diluted and tested again, or the mass of the extract should be increased for retesting.

8 Result Calculation and Expression

8.1 Result Calculation

The content of 1-deoxynojirimycin in the mulberry leaf extract is calculated according to formula (1):

$$X = \frac{c*V}{m} \quad \text{.....(1)}$$

Where:

X——Content of 1-deoxynojirimycin in the sample, in milligrams per gram (mg/g);

c——Concentration of 1-deoxynojirimycin in the sample, in milligrams per milliliter (mg/mL);

V——Volume of the sample after final dilution, in milliliters (L);

m——Mass of the sample, in grams (g).

8.2 Result Representation

The calculated results are expressed as the arithmetic mean of two independent determinations obtained under repeated conditions, rounded to two decimal places.

9 Repeatability

The relative standard deviation of five independent determinations obtained under repeated conditions is less than 5%.

10 Precision

Inject the same sample five times consecutively and analyze its HPLC chromatogram under the chromatographic conditions specified in 7.3. Compare the peak areas of the corresponding chromatographic peak for 1-deoxynojirimycin. The relative standard deviation (RSD) of the peak areas from the five injections should not exceed 5%.

11 Spike Recovery

When the standard concentration is 0.5 to 1.5 times that of the sample, the spike recovery should be between 90% and 110%, and the relative standard deviation should be less than 10%.

Appendix A(Informative)

Chromatogram Examples

The chromatogram examples of 1-deoxynojirimycin standard sample and mulberry leaf extract are shown in Figures A.1 and A.2. Peak 1 is 1-deoxynojirimycin, peak 2 is an oxygen-glycine conjugate, and peak 3 is an oxygen hydrolysate.

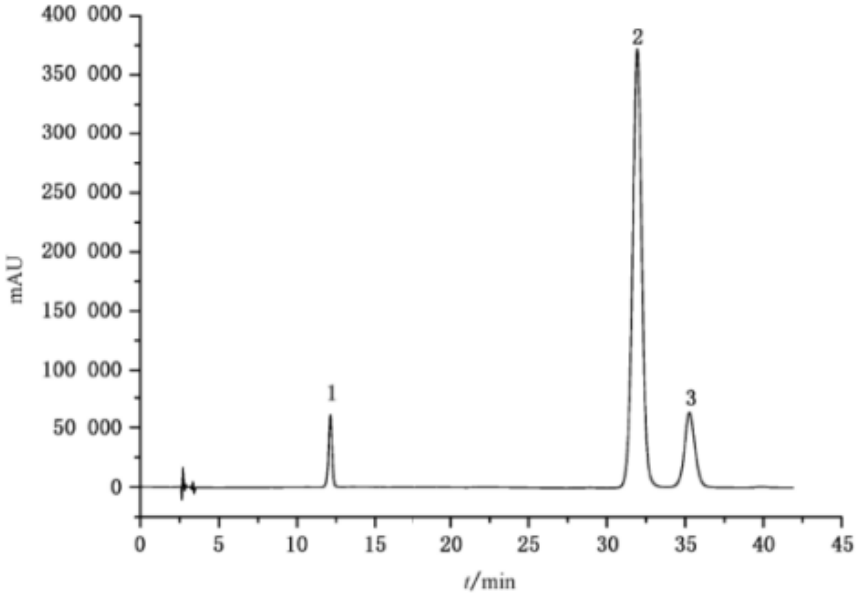


Figure A.1 Chromatogram of 1-deoxynojirimycin standard sample

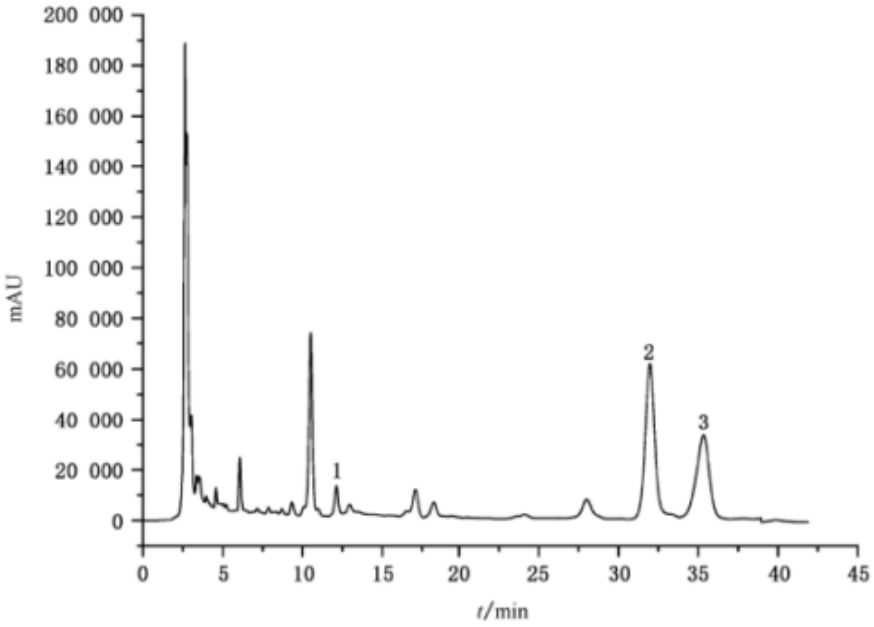


Figure A.2 Chromatogram of mulberry leaf extract