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Determination of Levodopa in Plant Derived Products - High Performance Liquid Chromatography

植物源产品中左旋多巴的测定

高效液相色谱法

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Table of Contents

Determination of Levodopa in Plant Derived Products - High Performance Liquid Chromatography

1 Scope

This Standard describes the determination of levodopa content in plant derived products through the high performance liquid chromatography.

This Standard is applicable to the determination of levodopa content in plant derived dry samples, such as: *Vicia faba* L., *Stizolobium cochinchinensis* (Lour.) Tang et Wang and *Mucuna sempervirens* Hemsl., etc.

2 Normative References

The content of the following documents constitutes indispensable clauses of this document through normative references in the text. In terms of references with a specified date, only versions with a specified date are applicable to this document. In terms of references without a specified date, the latest version (including all the modifications) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

3 Terms and Definitions

There are no terms and definitions that need to be defined in this document.

4 Principle

After the specimen is ultrasonically extracted with 2% (volume fraction) hydrochloric acid solution, it is separated through reversed-phase chromatographic column and detected by ultraviolet (or diode array) detector. It is qualitatively determined by retention time and quantitatively determined by the external standard method.

5 Reagents and Materials

5.1 Reagents

Unless it is otherwise specified, the reagents used by this Method shall be analytically pure. The water shall be Grade-1 water specified in GB/T 6682.

Microporous membrane (aqueous phase): with a pore size of 0.45 μm.

6 Instruments and Equipment

6.1 High performance liquid chromatograph: equipped with ultraviolet detector (UV) or diode array detector (DAD).

6.2 Analytical balance: with a division value of 0.01 mg and 0.0001 g.

6.3 Ultrasonic generator: with a power of 250 W and a working frequency of 40 kHz.

6.4 Centrifuge: with a rotating speed of not less than 8,000 *g*.

6.5 Acidity meter: with an accuracy of 0.01 pH unit.

6.6 Vortex mixer.

6.7 Electric pulverizer.

7 Sample

Take about 250 g samples of *Vicia faba* L., *Stizolobium cochinchinensis* (Lour.) Tang et Wang and *Mucuna sempervirens* Hemsl.; use the electric pulverizer to smash them; let them pass through a sieve with an inner diameter of 355 μm ± 13 μm. Mix the prepared specimen and equally divide it into two parts. Put it into a clean specimen container; seal and label it. Store it at room temperature. Keep it sealed and protected from light.

8 Test Procedures

8.1 Specimen Processing

Weigh-take 1 g of specimen (accurate to 0.001 g) into a 50 mL centrifuge tube with stopper. Add 10 mL of 2% (volume fraction) hydrochloric acid solution. After vortex mixing, ultrasonically extract it for 30 min; every 15 min, shake it once. Then, at 8,000 (× *g*), centrifuge for 5 min; transfer the supernatant to a 25 mL volumetric flask. Use 10 mL of 2% (volume fraction) hydrochloric acid solution to re-extract the residue. Combine the two extracts. Then, use 2% (volume fraction) hydrochloric acid solution to reach a constant volume; shake it well. Let it pass through a 0.45 μm microporous membrane; reserve it for later test.

8.2 Determination

8.2.1 Liquid chromatography reference conditions

The liquid chromatography reference conditions are shown below:

a) Chromatographic column: C_{18} chromatographic column, 4.6 mm × 250 mm, particle size 5 μm, or others with equivalent performance;

b) Mobile phase: methanol-10 mmol/L dipotassium hydrogen phosphate solution = 5:95;

c) Flow rate: 0.7 mL/min;

d) Column temperature: 20 °C;

e) Injection volume: 10 μL;

f) Detection wavelength: 280 nm.

8.2.2 Drawing of standard working curve

In accordance with the levodopa content in the specimen, select the standard working solution with an appropriate range of mass concentration. From low concentration to high concentration, successively inject specimen for analysis. Take the chromatographic peak area of levodopa as the y-coordinate; take the mass concentration of the solution as the x-coordinate; draw the standard working curve. The chromatogram of the standard working solution is shown in Figure A.1 of Appendix A.

8.2.3 Sample determination

Inject the specimen solution into the high performance liquid chromatograph. Conduct qualitative determination by the retention time; record the peak area. In accordance with the standard working curve, obtain the mass concentration of levodopa in the specimen solution. The mass concentration of levodopa in the specimen solution shall be within the range of mass concentration of the standard working curve. Samples that exceed the upper limit of the mass concentration of the standard working curve shall be diluted and injected for analysis. The chromatogram of a typical specimen solution is shown in Figure A.2.

8.3 Parallel Test

In accordance with the stipulations of 8.1 and 8.2, conduct two parallel determinations on the same specimen.

9 Test Data Processing

The levodopa content in the specimen shall be calculated in accordance with Formula (1):

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