



陕西绿清生物工程有限公司

Shaanxi Green Bio-Engineering Co.,Ltd

## Determination of Diindolylmethane content

A.1 Method summary After the sample is extracted by ultrasonic, it is tested by high performance liquid chromatography.

A.2 Test instruments and appliances

A.2.1 Analytical balance, with an accuracy of one hundred thousandths

A.2.2 Ultrasonic cleaner: 250W, 20kHz

A.2.3 High performance liquid chromatograph

A.3 Reagents and solutions

A.3.1 Acetonitrile, analytical grade

A.3.2 Methanol, analytically pure

A.3.3 Water, double distilled water

A.3.4 Diindolylmethane reference substance

A.3.5 Mobile phase: methanol-acetonitrile-0.07% phosphoric acid solution=12:20:68

Column temperature: 25°C Flow rate: 1ml/min

A.3.6 Detector and detection wavelength: UV spectrophotometer, detection wavelength 260nm

A.4 Operation method

A.4.1 Preparation of reference substance solution: Accurately weigh the diindolylmethane reference substance (accurate to 0.01mg), add methanol to make a solution containing 70 $\mu$ g per 1mL, as the reference substance solution.

A.4.2 Preparation of test solution: Take about 10 mg of diindolylmethane sample, accurately weigh it, and dissolve it with methanol ultrasonically, and use methanol as the test solution.

A.4.3 Determination method

Precisely draw 10 µL of the reference solution and the test solution respectively, and inject them into the liquid chromatograph for determination.

#### A.5 Result calculation

The content of diindolylmethane is calculated according to the formula (B.1):

$$\text{Diindolylmethane (\%)} = \frac{S_1 \times C \times A}{S_0 \times (M - M \times B)} \times 100\% \quad \dots\dots\dots (B. 1)$$

S1---The peak area value of the test solution;

S0---The peak area value of the reference substance solution;

C---Concentration of reference substance solution (mg/ml);

A---Reference substance content (%);

B---Moisture content in the sample (%);

M---The concentration of the test solution (mg/ml).