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Analysis Method of Carnosic acid

1,Reagents and materials

Water:First grade water in accordance with GB/T6682
Acetone: HPLC
Acetonitrile: HPLC
Carnosic acid reference standard: purity ≥98%
Phosphoric Acid solution: 0.1ml Phosphoric Acid added into 100ml water

2,Instruments, Equipment and Reference Chromatographic Condition

HPLC: equipped with UV-detector or diode-array detector
Column: C18 reverse column
Mobile phase A: Methanol: Water + Phosphoric acid solution
Mobile phase B: Methanol: Acetonitrile + Phosphoric acid solution
Flow Rate: 1 ml/min
Column temperature: 30 ℃
Injection volume: 10UL
Detection wavelength: 280nm
Retention time: 30min
Gradient elution conditions: see Table A.1.

Table A.1 Gradient elution condition

Time/min	Mobile phase A/	Mobile phase B/	Flow Rate /(mL/min)
0.0	77	23	1.0
1.0	77	23	1.0
25.0	0	100	1.0
30.0	0	100	1.0
30.5	77	23	1.0
35.0	77	23	1.0

3,Analysis Procedures

①,Preparation of sample solution

Accurately weigh 140 mg~180 mg of sample (accurate to 0.0001g), dissolve in 20 mL Acetone in a 25 mL volumetric flask, mix well and filtrate through a 0.22 m microporous membrane, obtain the sample solution.
Dissolve in 20 mL Acetone in a 25 mL volumetric flask, mix well and filtrate through a 0.22 m microporous membrane.

②,Drawing of standard curve

The standard solution of mixed gradient was prepared by dissolving the standard substance of carnosic acid with acetone, and the concentration gradient of carnosic acid was 0.010 mg/mL~1.000 mg/mL.Under a.2.4 reference chromatographic conditions, the standard solution was determined and the sample was repeated once.According to the concentration and peak area of the standard solution, draw the standard curve.The linear relationship should be R2≥0.99.Record the linear formula for the standard curve Y= A × C + B.Where, C is the concentration of carnosic acid,Y is the peak area corresponding to this concentration, and A and B are the slope and intercept of the standard curve respectively.

4,Determination

$$C1 = \frac{Y1 - b}{a} \times 100\%$$

C1-The concentration % of Carnosic acid, unit: mg/mL
Y1-The peak area of Carnosic acid in sample solution
b-The intercept of standard curve formula of Carnosic acid;
a-The intercept of standard curve formula of Carnosic acid