Claims

1. A method for detecting Andrographis paniculata or a preparation thereof, comprising: establishing a chromatogram of Andrographis paniculata or a preparation thereof utilizing High Performance Liquid Chromatography, and calculating the content of at least one of neoandrographolide, deoxyandrographolide, dehydroandrographolide, and andrograpanin by using relative correction factors of neoandrographolide, deoxy andrographolide, dehydroandrographolide, and andrograpanin respectively with respect to andrographolide and taking andrographolide as an internal standard,

wherein the chromatographic conditions of High Performance Liquid Chromatography are as follows:

Stationary phase: octadecyl bonded silica gel;

Continuous gradient elution with acetonitrile being mobile phase A and water or dilute aqueous acid being mobile phase B; at 0 min, mobile phase A: 10-40%, mobile phase B: 90-60%; at 40-70 mins, mobile phase A: 20-60%, mobile phase B: 80-40%; and after 70 mins, mobile phase A: 60-100%, mobile phase B: 40-0%;

Flow rate of mobile phase: 0.5-2mL/min;

Detection wavelength: 190-400nm;

Column temperature: 0-50 °C.

2. The method according to claim 1, characterized in that the content of neoandrographolide, deoxyandrographolide, dehydroandrographolide or andrograpanin is calculated according to the following equation:

Content (mg/g) = $(C_S/W) \times (A_U/A_S) \times V \times D \times F$

C_S: concentration of reference substance of Andrographis paniculata, in mg/mL;

W: sampling mass of sample, in g;

A_U: peak area of other lactones in the sample;

A_S: peak area of reference substance of andrographolide;

V: volume of extraction solvent, in mL;

D: dilution ratio;

F: relative correction factor;

wherein the relative correction factor F is calculated according to the following equation:

$$F=\frac{f_S}{f_K}=\frac{W_K \times A_S}{W_S \times A_K}$$

in the equation, A_S is the peak area of reference substance of andrographolide, W_S is the concentration or mass of reference substance of andrographolide, A_K is the peak area of a certain component to be detected; W_K is the concentration or mass of a certain component to be detected.

3. The method according to claim 1 or claim 2, characterized in that the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin range from 0.5 to 15, 0.5 to 15, 0.1 to 5 and 0.5 to 15, respectively; preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.30, 0.779 ± 0.30, 0.721 ± 0.30 and 2.41 ± 0.30, respectively; and more preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.15, 0.779 ± 0.15, 0.721 ± 0.15 and 2.41 ± 0.15, respectively.

4. The method according to any one of claims 1 to 3, characterized in that, when detected, Andrographis paniculata or a preparation thereof is soaked in aqueous methanol or ethanol for 0-1 hour, subjected to sonication treatment for 0-1 hour, diluted to a set volume and filtered to obtain a filtrate, and then the filtrate is analyzed by High Performance Liquid Chromatography.

5. The method according to claim 4, characterized in that said aqueous methanol or ethanol is 10-100% v/v aqueous methanol or ethanol.

6. The method according to claim 4, characterized in that said sonication treatment lasts for 0-0.5 hour.

~~7. The method according to any one of claims 1 to 6, characterized in that the chromatographic conditions of High Performance Liquid Chromatography are~~

~~as follows:~~

~~Stationary phase: octadecyl bonded silica gel;~~

~~Continuous gradient elution with acetonitrile being mobile phase A and water or dilute aqueous acid being mobile phase B; at 0 min, mobile phase A: 10-40%, mobile phase B: 90-60%; at 40-70 mins, mobile phase A: 20-60%, mobile phase B: 80-40%; and after 70 mins, mobile phase A: 60-100%, mobile phase B: 40-0%;~~

~~Flow rate of mobile phase: 0.5-2mL/min;~~

~~Detection wavelength: 190-400nm;~~

~~Column temperature: 0-50 °C.~~

~~8~~7. A method for detecting Andrographis paniculata or a preparation thereof, comprising: establishing a chromatogram of Andrographis paniculata or a preparation thereof utilizing High Performance Liquid Chromatography, and calculating the content of at least one of neoandrographolide, deoxyandrographolide, dehydroandrographolide, and andrograpanin by using relative correction factors of neoandrographolide, deoxy andrographolide, dehydroandrographolide, and andrograpanin respectively with respect to andrographolide and taking andrographolide as an internal standard,

wherein~~The method according to any one of claims 1 to 6, characterized in~~ ~~that~~ the chromatographic conditions of High Performance Liquid Chromatography are as follows:

Stationary phase: octadecyl bonded silica gel;

Continuous gradient elution with acetonitrile being mobile phase A and water or dilute aqueous acid being mobile phase B; at 0 min, mobile phase A: 20%, mobile phase B: 80%; at 50-60 mins, mobile phase A: 40%, mobile phase B: 60%; and at 65-75 mins, mobile phase A: 65%, mobile phase B: 35%;

Flow rate of mobile phase: 1mL/min;

Detection wavelength: 200-230nm;

Column temperature: 0-30 °C.

8. The method according to claim 7, characterized in that the content of neoandrographolide, deoxyandrographolide, dehydroandrographolide or

andrograpanin is calculated according to the following equation:

Content (mg/g) = $(C_S/W) \times (A_U/A_S) \times V \times D \times F$

C_S: concentration of reference substance of Andrographis paniculata, in mg/mL;

W: sampling mass of sample, in g;

A_U: peak area of other lactones in the sample;

A_S: peak area of reference substance of andrographolide;

V: volume of extraction solvent, in mL;

D: dilution ratio;

F: relative correction factor;

wherein the relative correction factor F is calculated according to the following equation:

$$F=\frac{f_S}{f_K}=\frac{W_K \times A_S}{W_S \times A_K}$$

in the equation, A_S is the peak area of reference substance of andrographolide, W_S is the concentration or mass of reference substance of andrographolide, A_K is the peak area of a certain component to be detected; W_K is the concentration or mass of a certain component to be detected.

9. The method according to claim 7 or claim 8, characterized in that the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin range from 0.5 to 15, 0.5 to 15, 0.1 to 5 and 0.5 to 15, respectively; preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.30, 0.779 ± 0.30, 0.721 ± 0.30 and 2.41 ± 0.30, respectively; and more preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.15, 0.779 ± 0.15, 0.721 ± 0.15 and 2.41 ± 0.15, respectively.

10. The method according to any one of claims 7 to 9, characterized in that, when detected, Andrographis paniculata or a preparation thereof is soaked in aqueous methanol or ethanol for 0-1 hour, subjected to sonication treatment for

0-1 hour, diluted to a set volume and filtered to obtain a filtrate, and then the filtrate is analyzed by High Performance Liquid Chromatography.

11. The method according to claim 10, characterized in that said aqueous methanol or ethanol is 10-100% v/v aqueous methanol or ethanol.

12. The method according to claim 10, characterized in that said sonication treatment lasts for 0-0.5 hour.

~~9~~13. The method according to claim ~~7~~1 or claim ~~8~~7, characterized in that said dilute aqueous acid of mobile phase B includes aqueous formic acid, aqueous phosphoric acid or aqueous glacial acetic acid; preferably, the concentration of said dilute aqueous acid is 0.05 to 0.5% v/v; and more preferably, the concentration of said aqueous phosphoric acid is 0.2% v/v.

~~10~~14. The method according to any one of claims ~~1~~7 to 12~~-6~~, characterized in that the chromatographic conditions of High Performance Liquid Chromatography are as follows:

Stationary phase: octadecyl bonded silica gel;

Continuous gradient elution with acetonitrile being mobile phase A and water being mobile phase B; at 0 min, mobile phase A: 20%, mobile phase B: 80%; at 55 mins, mobile phase A: 40%, mobile phase B: 60%; at 70 mins, mobile phase A: 65%, mobile phase B: 35%;

Flow rate of mobile phase: 1mL/min;

Detection wavelength: 200-210nm or 220-230nm; preferably, detection wavelength: 205nm or 225nm;

Column temperature: 10 °C.

~~11~~15. The method according to any one of claims 1 to ~~10~~14, characterized in that the preparation includes a solid preparation, a semi-solid preparation and a liquid preparation; and preferably, the preparation is selected from a tablet, a capsule, a pill, a granule, an injection and an ointment.

Claims

1. A method for detecting Andrographis paniculata or a preparation thereof, comprising: establishing a chromatogram of Andrographis paniculata or a preparation thereof utilizing High Performance Liquid Chromatography, and calculating the content of at least one of neoandrographolide, deoxyandrographolide, dehydroandrographolide, and andrograpanin by using relative correction factors of neoandrographolide, deoxy andrographolide, dehydroandrographolide, and andrograpanin respectively with respect to andrographolide and taking andrographolide as an internal standard,

wherein the chromatographic conditions of High Performance Liquid Chromatography are as follows:

Stationary phase: octadecyl bonded silica gel;

Continuous gradient elution with acetonitrile being mobile phase A and water or dilute aqueous acid being mobile phase B; at 0 min, mobile phase A: 10-40%, mobile phase B: 90-60%; at 40-70 mins, mobile phase A: 20-60%, mobile phase B: 80-40%; and after 70 mins, mobile phase A: 60-100%, mobile phase B: 40-0%;

Flow rate of mobile phase: 0.5-2mL/min;

Detection wavelength: 190-400nm;

Column temperature: 0-50 °C.

2. The method according to claim 1, characterized in that the content of neoandrographolide, deoxyandrographolide, dehydroandrographolide or andrograpanin is calculated according to the following equation:

Content (mg/g) = $(C_S/W) \times (A_U/A_S) \times V \times D \times F$

C_S: concentration of reference substance of Andrographis paniculata, in mg/mL;

W: sampling mass of sample, in g;

A_U: peak area of other lactones in the sample;

A_S: peak area of reference substance of andrographolide;

V: volume of extraction solvent, in mL;

D: dilution ratio;

F: relative correction factor;

wherein the relative correction factor F is calculated according to the following equation:

$$F=\frac{f_S}{f_K}=\frac{W_K \times A_S}{W_S \times A_K}$$

in the equation, A_S is the peak area of reference substance of andrographolide, W_S is the concentration or mass of reference substance of andrographolide, A_K is the peak area of a certain component to be detected; W_K is the concentration or mass of a certain component to be detected.

3. The method according to claim 1 or claim 2, characterized in that the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin range from 0.5 to 15, 0.5 to 15, 0.1 to 5 and 0.5 to 15, respectively; preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.30, 0.779 ± 0.30, 0.721 ± 0.30 and 2.41 ± 0.30, respectively; and more preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.15, 0.779 ± 0.15, 0.721 ± 0.15 and 2.41 ± 0.15, respectively.

4. The method according to any one of claims 1 to 3, characterized in that, when detected, Andrographis paniculata or a preparation thereof is soaked in aqueous methanol or ethanol for 0-1 hour, subjected to sonication treatment for 0-1 hour, diluted to a set volume and filtered to obtain a filtrate, and then the filtrate is analyzed by High Performance Liquid Chromatography.

5. The method according to claim 4, characterized in that said aqueous methanol or ethanol is 10-100% v/v aqueous methanol or ethanol.

6. The method according to claim 4, characterized in that said sonication treatment lasts for 0-0.5 hour.

7. A method for detecting Andrographis paniculata or a preparation thereof, comprising: establishing a chromatogram of Andrographis paniculata or a

preparation thereof utilizing High Performance Liquid Chromatography, and calculating the content of at least one of neoandrographolide, deoxyandrographolide, dehydroandrographolide, and andrograpanin by using relative correction factors of neoandrographolide, deoxy andrographolide, dehydroandrographolide, and andrograpanin respectively with respect to andrographolide and taking andrographolide as an internal standard,

wherein the chromatographic conditions of High Performance Liquid Chromatography are as follows:

Stationary phase: octadecyl bonded silica gel;

Continuous gradient elution with acetonitrile being mobile phase A and water or dilute aqueous acid being mobile phase B; at 0 min, mobile phase A: 20%, mobile phase B: 80%; at 50-60 mins, mobile phase A: 40%, mobile phase B: 60%; and at 65-75 mins, mobile phase A: 65%, mobile phase B: 35%;

Flow rate of mobile phase: 1mL/min;

Detection wavelength: 200-230nm;

Column temperature: 0-30 °C.

8. The method according to claim 7, characterized in that the content of neoandrographolide, deoxyandrographolide, dehydroandrographolide or andrograpanin is calculated according to the following equation:

Content (mg/g) = $(C_S/W) \times (A_U/A_S) \times V \times D \times F$

C_S: concentration of reference substance of Andrographis paniculata, in mg/mL;

W: sampling mass of sample, in g;

A_U: peak area of other lactones in the sample;

A_S: peak area of reference substance of andrographolide;

V: volume of extraction solvent, in mL;

D: dilution ratio;

F: relative correction factor;

wherein the relative correction factor F is calculated according to the following equation:

$$F=\frac{f_S}{f_K}=\frac{W_K \times A_S}{W_S \times A_K}$$

in the equation, A_S is the peak area of reference substance of andrographolide, W_S is the concentration or mass of reference substance of andrographolide, A_K is the peak area of a certain component to be detected; W_K is the concentration or mass of a certain component to be detected.

9. The method according to claim 7 or claim 8, characterized in that the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin range from 0.5 to 15, 0.5 to 15, 0.1 to 5 and 0.5 to 15, respectively; preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.30, 0.779 ± 0.30, 0.721 ± 0.30 and 2.41 ± 0.30, respectively; and more preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are 1.150 ± 0.15, 0.779 ± 0.15, 0.721 ± 0.15 and 2.41 ± 0.15, respectively.

10. The method according to any one of claims 7 to 9, characterized in that, when detected, Andrographis paniculata or a preparation thereof is soaked in aqueous methanol or ethanol for 0-1 hour, subjected to sonication treatment for 0-1 hour, diluted to a set volume and filtered to obtain a filtrate, and then the filtrate is analyzed by High Performance Liquid Chromatography.

11. The method according to claim 10, characterized in that said aqueous methanol or ethanol is 10-100% v/v aqueous methanol or ethanol.

12. The method according to claim 10, characterized in that said sonication treatment lasts for 0-0.5 hour.

13. The method according to claim 1 or claim 7, characterized in that said dilute aqueous acid of mobile phase B includes aqueous formic acid, aqueous phosphoric acid or aqueous glacial acetic acid; preferably, the concentration of said dilute aqueous acid is 0.05 to 0.5% v/v; and more preferably, the concentration of said aqueous phosphoric acid is 0.2% v/v.

14. The method according to any one of claims 7 to 12, characterized in that

the chromatographic conditions of High Performance Liquid Chromatography are as follows:

Stationary phase: octadecyl bonded silica gel;

Continuous gradient elution with acetonitrile being mobile phase A and water being mobile phase B; at 0 min, mobile phase A: 20%, mobile phase B: 80%; at 55 mins, mobile phase A: 40%, mobile phase B: 60%; at 70 mins, mobile phase A: 65%, mobile phase B: 35%;

Flow rate of mobile phase: 1mL/min;

Detection wavelength: 200-210nm or 220-230nm; preferably, detection wavelength: 205nm or 225nm;

Column temperature: 10 °C.

15. The method according to any one of claims 1 to 14, characterized in that the preparation includes a solid preparation, a semi-solid preparation and a liquid preparation; and preferably, the preparation is selected from a tablet, a capsule, a pill, a granule, an injection and an ointment.