

A METHOD FOR DETECTING ANDROGRAPHIS PANICULATA OR PREPARATIONS THEREOF

5 **Technical Field**

The present invention relates to a method for detecting traditional Chinese medicine (TCM), and relates to a convenient, quick method for detecting Andrographis paniculata or preparations thereof.

10 **Background Art**

Currently, a method most commonly used in quality evaluation study of Andrographis paniculata is to control the quality of traditional Chinese medicine by means of a single index or major index components. However, the complexity of the components in Andrographis paniculata results in that
15 neither the method could represent the quality control of Andrographis paniculata, nor guarantee the quality thereof (Wang Hong, Zhang Xuedong, Discussion of quality standard of Andrographis paniculata and Andrographis paniculata tablet [J], Northwest Pharmaceutical Journal, 2002, 17(2):63-64). The TCM fingerprints can comprehensively reflect the type and quantity of
20 substance groups contained in TCM, and can provide more information related to the intrinsic quality of TCM. But what should be seen is that factors caused by fingerprints such as being unclear and unquantifiable also limit the scientificity of this quality standard, and there are still many key problems (Jiang Zhenou, Rao Weiyan. HPLC fingerprint studies of Andrographis
25 paniculata [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2008, 14(3):6-7). Multiple index quantitative control modes necessitate plenty of TCM chemical reference substances that are difficult to be separated and purified, unstable or expensive, which together with the imperfect market
30 mechanism result in a severe supply shortage of TCM chemical reference substances. Therefore, this mode is currently limited to research to a large extent, and is difficult to be applied into practical quality monitor and evaluation.

The basic idea of designing Quantitative Assay of Multi-components by Single-marker (QAMS) is to achieve simultaneous monitor of multiple

components (reference substances of which are not available or difficult to be supplied) by only determine one component (reference substance of which is available) through intrinsic functional relationships and proportional relationships present between the active components of TCM. According to the literature survey, QAMS mode has achieved good application in simultaneously detecting of multiple components in a variety of herbs, such as *Caulis Akebiae*, *Panax Ginseng*, *Coptis Chinensis*, *Herba Epimedii*, *Rheum Officinale*, *Scutellaria Baicalensis* and so on.

10 **Description of the Invention**

In view of the problem of supply shortages of reference substances in multiple index quantitative control, the object of present invention is to achieve the simultaneous determination of the contents of andrographolide, neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin with only the reference substance of andrographolide by means of quality evaluation mode of "QAMS".

In the absence of reference substances of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin, the method for detecting *Andrographis paniculata* or a preparation thereof according to the present invention could be used in TCM quality evaluation as a method for detecting multiple-index content.

Unless otherwise indicated, in the present invention, the percentages of extraction solvents and the percentages of mobile phases in chromatographic conditions are volume percentage.

The present invention provide 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 (HPLC), 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.

Preferably, in the method for detecting *Andrographis paniculata* or a preparation thereof, the content of neoandrographolide, deoxyandrographolide, dehydroandrographolide or andrograpanin is calculated according to the following equation:

5 $\text{Content (mg/g)} = (C_S/W) \times (A_U/A_S) \times V \times D \times F$

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;

10 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
15 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
20 the concentration or mass of a certain component to be detected.

Preferably, in the method for detecting *Andrographis paniculata* or a preparation thereof, 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.

25 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.

Particularly preferably, the relative correction factors of neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin are $1.150 \pm$
30 0.15 , 0.779 ± 0.15 , 0.721 ± 0.15 and 2.41 ± 0.15 , respectively.

Preferably, in the method for detecting *Andrographis paniculata* or a preparation thereof, 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
5 filtrate, and then the filtrate is analyzed by HPLC.

Preferably, said aqueous methanol or ethanol is 10-100% v/v aqueous methanol or ethanol.

Preferably, said sonication treatment lasts for 0-0.5 hour.

Preferably, the chromatographic conditions of HPLC are as follows:

10 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
15 phase B: 40-0%;

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

Detection wavelength: 190-400nm;

Column temperature: 0-50 °C.

Preferably, the chromatographic conditions of HPLC are as follows:

20 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%;

25 Flow rate of mobile phase: 1mL/min;

Detection wavelength: 200-230nm;

Column temperature: 0-30 °C.

Preferably, said dilute aqueous acid of mobile phase B includes aqueous formic acid, aqueous phosphoric acid and aqueous glacial acetic acid; preferably,
30 the concentration of said dilute aqueous acid is 0.05 to 0.5% v/v; more preferably, the concentration of said aqueous phosphoric acid is 0.2% v/v.

Particularly preferably, the chromatographic conditions of HPLC 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;

Column temperature: 10 °C;

More preferably, the above-described detection wavelength is 205nm or 225nm.

Preferably, in the method for detecting *Andrographis paniculata* or preparation thereof, the preparation includes a solid preparation, a semi-solid preparation and a liquid preparation.

Preferably, the preparation is selected from a tablet, a capsule, a pill, a granule, an injection, and an ointment.

The invention only uses reference substance of andrographolide to achieve simultaneous determination of the contents of andrographolide, neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin, with high repeatability, good stability, easy and convenient operation and having scientific foundation, which could effectively control the quality of *Andrographis paniculata* and a preparation thereof. The method for detecting *Andrographis paniculata* or a preparation thereof according to the present invention can provide a low-cost method of quality control with high operability for multiple-components quality evaluation of *Andrographis paniculata* and a preparation thereof, guarantee a reliable and stable quality of *Andrographis paniculata* and a preparation thereof, reduce test cost and improve test efficiency, etc.

Brief Description of the Drawings

In the following, embodiments of the present invention are described in detail with reference to the drawings, in which:

Figure 1 is a HPLC chromatogram of reference substance of *Andrographis paniculata*;

Figure 2 is a HPLC chromatogram of *Andrographis paniculata* (1: andrographolide; 2: neoandrographolide; 3: deoxyandrographolide; 4: dehydroandrographolide; 5: andrograpanin);

Figure 3 is a HPLC chromatogram of a Xiaoyanlidan tablet;

Figure 4 is a HPLC chromatogram of an *Andrographis paniculata* capsule;

Figure 5 is a HPLC chromatogram of an *Andrographis paniculata* pill;

Figure 6 is a HPLC chromatogram of an *Andrographis paniculata* injection.

10

Best Mode for Carrying Out the Invention

The present invention is now further described in detail in conjunction with following examples.

Instruments: High Performance Liquid Chromatograph (DIONEX SUMMIT P680 HPLC, Dionex Corporation, USA), octadecyl bonded silica gel column (250 mm x 4.6 mm, 5 μ m); neutral alumina column (200~300 mesh, 5g, inner diameter of 1.5cm).

Samples: reference substances of andrographolide and dehydroandrographolide (purchased from National Institutes for Food and Drug Control); reference substances of neoandrographolide and deoxyandrographolide (purchased from Nanjing TCM Institute of Chinese Materia Medica, purity $\geq$ 98%); reference substance of andrograpanin (purchased from ChromaDexInc., purity $\geq$ 98%); *Andrographis paniculata* (Hutchison Whampoa Guangzhou Baiyunshan Chinese Medicine Co., Ltd.); Xiaoyanlidan tablet (Hutchison Whampoa Guangzhou Baiyunshan Chinese Medicine Co., Ltd.); *Andrographis paniculata* capsules (Tianjin Meilun Medical Co., Ltd); *Andrographis paniculata* pills (Guangxi Yulin Pharmaceutical Co., Ltd.); *Andrographis paniculata* injections (Hainan Pharmaceutical Factory Co., Ltd.).

Unless otherwise indicated, in the following examples, the percentages of extraction solvents and the percentages of mobile phases in chromatographic conditions are volume percentages.

Example 1

About 0.5g of *Andrographis paniculata* powder (sieved through sieve No. IV) was taken, accurately weighed, and placed in a stoppered conical flask. 50mL of 40% methanol was added into the conical flask precisely, and the mixture was weighed. The *Andrographis paniculata* powder was soaked for 1 hour, followed by ultrasonic treatment for 30 minutes. Then the mixture was allowed to be cool, weighed again and complemented weight loss with 40% methanol, shook up and filtered, and the filtrate was filtered through 0.45µm filter membrane. After that, 10µL of the filtrate was taken for sample injection.

Appropriate amount of reference substance of andrographolide was taken, accurately weighed, and placed in a volumetric flask. Adding methanol into the volumetric flask to prepare a reference solution with 0.1 mg of the reference substance contained in per 1 mL of the solution. The reference solution was filtered through 0.45µm filter membrane, and 10µL of the subsequent filtrate was taken for sample injection.

The chromatographic conditions of HPLC are as follows: employing octadecyl bonded silica gel as stationary phase and using continuous gradient elution, with acetonitrile being mobile phase A and water being mobile phase B; continuous gradient elution procedure is as follows:

At 0 min, mobile phase A: 20%, mobile phase B: 80%;

At 55 mins, mobile phase A: 40%, mobile phase B: 60%; and

At 70 mins, mobile phase A: 65%, mobile phase B: 35%;

Flow rate of mobile phase: 1mL/min; detection wavelength: 205nm; column temperature: 10 °C. The peak areas of andrographolide, neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin were obtained respectively.

The contents of andrographolide, neoandrographolide, deoxyandrographolide, dehydroandrographolide and andrograpanin in *Andrographis paniculata* are calculated according to the following equation:

$$\text{Equation: Content (mg/g)} = (C_S/W) \times (A_U/A_S) \times 50F$$

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;

5 F: relative correction factor (1 for andrographolide, 1.150 for neoandrographolide, 0.779 for deoxyandrographolide, 0.721 for dehydroandrographolide, and 2.41 for andrograpanin).

The HPLC results of mixed reference substances for *Andrographis paniculata* and *Andrographis paniculata* are shown in Figure 1 and 2.

10 10 batches of *Andrographis paniculata* samples were detected for 10 times respectively and the results are shown in Table 1, errors are in the range of 10% or less.

Table 1. Comparison of contents measurement results of 10 batches of *Andrographis paniculata*

N o.	Androgr apholide	Neoandrographolide			Deoxyandrographolide			Dehydroandrographolid e			Andrograpanin		
	Content mg/g	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %
1	12.23	2.40	2.47	3.1	0.83	0.82	-1.2	2.78	2.72	-2.1	0.42	0.40	5.7
2	18.36	2.98	3.09	3.5	3.80	4.00	5.1	3.25	3.20	-1.5	0.70	0.69	2.1
3	4.36	5.10	5.40	5.9	2.46	2.60	5.5	5.85	5.96	1.8	0.55	0.50	8.8
4	9.61	3.62	3.78	4.3	3.00	3.15	5.0	4.48	4.49	0.1	0.46	0.44	5.1
5	8.37	7.14	7.52	5.4	1.61	1.66	3.1	5.69	5.74	0.9	0.52	0.53	-1.8
6	13.65	3.78	3.94	4.1	5.80	6.14	5.9	1.53	1.44	-6.2	0.54	0.57	-4.6
7	10.33	8.32	8.77	5.3	2.02	2.10	3.8	6.98	7.06	1.2	0.68	0.71	-4.4
8	9.75	8.29	8.73	5.4	1.94	2.01	3.7	6.63	6.71	1.1	0.77	0.75	2.1
9	8.57	7.64	8.05	5.4	1.79	1.85	3.6	5.47	5.52	0.8	0.79	0.83	-5.2
10	11.44	3.55	3.70	4.1	4.39	4.64	5.6	3.41	3.38	-1.1	1.14	1.13	1.2

15

Example 2

10 of Xiaoyanlidan tablets was taken with coating removed, accurately weighed and ground into fine powder. About 0.4g of the powder was taken and accurately weighed, and placed in a 10mL volumetric flask. 70% methanol was
20 added precisely to volume, and the mixture was sealed with stopper, weighed, followed by ultrasonic treatment for 30 minutes, then allowed to be cool, weighed again and complemented weight loss with 70% methanol, shook up and

filtered, and the filtrate was filtered through 0.45µm filter membrane. After that, 10µL of the filtrate was taken for sample injection.

Appropriate amount of reference substance of andrographolide was taken, accurately weighed, and placed in a volumetric flask. Adding methanol into the volumetric flask to prepare a reference solution with 0.1 mg of the reference substance contained in per 1 mL of the solution. The reference solution was filtered through 0.45µm filter membrane, and 10µL of the subsequent filtrate was taken for sample injection.

The chromatographic conditions of HPLC are as follows: employing octadecyl bonded silica gel as stationary phase and using continuous gradient elution, with acetonitrile being mobile phase A and 0.2%v/v phosphoric acid solution being mobile phase B; continuous gradient elution procedure is as follows:

At 0 min, mobile phase A: 20%, mobile phase B: 80%;

At 55 mins, mobile phase A: 40%, mobile phase B: 60%; and

At 70 mins, mobile phase A: 85%, mobile phase B: 15%;

Flow rate of mobile phase: 1mL/min; detection wavelength: 205nm; column temperature: room temperature. The peak areas of andrographolide, neoandrographolide, deoxyandrographolide, and dehydroandrographolide were obtained respectively.

The contents of andrographolide, neoandrographolide, deoxyandrographolide and dehydroandrographolide in *Andrographis paniculata* are calculated according to the following equation:

$$\text{Equation: Content (mg/g)} = (C_S/W) \times (A_U/A_S) \times 10F$$

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;

F: relative correction factor (1 for andrographolide, 1.150 for neoandrographolide, 0.779 for deoxyandrographolide and 0.721 for dehydroandrographolide).

10 batches of Xiaoyanlidan tablets samples were detected for 10 times respectively and the results are shown in Table 2, errors are in the range of 10% or less.

Table 2. Comparison of contents measurement results of 10 batches of Xiaoyanlidan tablets

No.	Andrographolide	Neoandrographolide			Deoxyandrographolide			Dehydroandrographolide		
	Content mg/g	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %
1	1.08	5.81	6.22	7.0	3.43	3.70	7.9	10.47	10.88	4.0
2	3.74	5.50	5.81	5.6	2.97	3.16	6.4	8.50	8.72	2.6
3	2.50	5.88	6.23	5.9	4.09	4.37	6.8	11.37	11.70	2.9
4	1.85	4.24	4.50	6.1	2.95	3.15	7.0	8.90	9.18	3.1
5	1.06	5.44	5.82	7.0	3.19	3.44	7.9	9.87	10.26	4.0
6	2.84	5.02	5.31	5.8	2.83	3.02	6.5	8.55	8.79	2.7
7	2.96	4.94	5.22	5.7	3.74	3.99	6.7	9.70	9.96	2.8
8	1.11	4.42	4.73	6.9	4.00	4.31	7.9	9.76	10.14	3.9
9	1.71	4.36	4.63	6.2	2.91	3.11	7.1	8.04	8.30	3.2
10	3.27	5.23	5.52	5.7	3.03	3.23	6.5	8.86	9.09	2.7

Example 3

The contents of *Andrographis paniculata* capsules under Content Uniformity was taken and ground into fine powder, about 0.5g of which was taken and accurately weighed, and placed in 25mL volumetric flask. 40% methanol was added precisely to volume, and the mixture was sealed with stopper and weighed, followed by ultrasonic treatment for 30 minutes, then allowed to be cool, weighed again and complemented weight loss with 40% methanol, shook up and filtered, and the filtrate was filtered through 0.45μm filter membrane. After that, 10μL of the filtrate was taken for sample injection.

Appropriate amount of reference substance of andrographolide was taken, accurately weighed, and placed in a volumetric flask. Adding methanol into the volumetric flask to prepare a reference solution with 0.1 mg of the reference substance contained in per 1 mL of the solution. The reference solution was filtered through 0.45μm filter membrane, and 10μL of the subsequent filtrate was taken for sample injection.

The chromatographic conditions of HPLC are as follows: employing octadecyl bonded silica gel as stationary phase and using continuous gradient

elution, with acetonitrile being mobile phase A and 0.2%v/v phosphoric acid solution being mobile phase B; continuous gradient elution procedure is as follows:

At 0 min, mobile phase A: 20%, mobile phase B: 80%;

5 At 55 mins, mobile phase A: 40%, mobile phase B: 60%; and

At 70 mins, mobile phase A: 85%, mobile phase B: 15%;

Flow rate of mobile phase: 1mL/min; detection wavelength: 205nm; column temperature: room temperature. The peak areas of andrographolide, neoandrographolide, deoxyandrographolide, and dehydroandrographolide were
10 obtained respectively.

The contents of andrographolide, neoandrographolide, deoxyandrographolide and dehydroandrographolide in *Andrographis paniculata* are calculated according to the following equation:

$$\text{Equation: Content (mg/g)} = (C_S/W) \times (A_U/A_S) \times 25F$$

15 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;

20 F : relative correction factor (1 for andrographolide, 1.150 for neoandrographolide, 0.779 for deoxyandrographolide and 0.721 for dehydroandrographolide).

10 batches of *Andrographis paniculata* capsules samples were detected for 10 times respectively and the results are shown in Table 3, errors are in the range
25 of 10% or less.

Table 3. Comparison of contents measurement results of 10 batches of *Andrographis paniculata* capsules

No.	Andrographolide	Neoandrographolide			Deoxyandrographolide			Dehydroandrographolide		
	Content mg/g	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %
1	2.46	1.28	1.36	6.3	2.97	3.07	3.4	12.42	13.02	4.8
2	1.02	3.26	3.17	-2.8	5.34	5.59	4.7	9.76	9.54	-2.3
3	3.05	4.55	4.56	0.2	3.17	3.07	-3.2	10.73	10.67	-0.6
4	1.77	3.19	3.25	1.9	2.48	2.35	-5.2	11.59	12.14	4.7

5	2.86	2.62	2.78	6.1	3.05	3.14	3.0	14.32	14.57	1.7
6	2.32	2.49	2.46	-1.2	2.13	2.19	2.8	12.86	13.08	1.7
7	1.98	4.38	4.21	-3.9	2.34	2.44	4.3	12.74	12.62	-0.9
8	1.81	3.05	2.87	-5.9	2.61	2.57	-1.5	12.08	11.67	-3.4
9	4.73	2.81	2.93	4.3	2.88	2.79	-3.1	12.96	12.35	-4.7
10	3.29	2.57	2.52	-1.9	1.62	1.70	4.9	13.04	12.94	-0.8

Example 4

Appropriate amount of *Andrographis paniculata* pills was taken and ground into fine powder. About 0.5g of the powder was taken and accurately weighed, and placed in a stoppered conical flask. 25 mL of 60% ethanol was added into the conical flask precisely, and the mixture was sealed with stopper and weighed, followed by ultrasonic treatment for 30 minutes, then allowed to be cool, weighed again and complemented weight loss with 60% ethanol, shook up and filtered, and the filtrate was filtered through 0.45µm filter membrane. After that, 10µL of the filtrate was taken for sample injection.

Appropriate amount of reference substance of andrographolide was taken, accurately weighed, and placed in a volumetric flask. Adding methanol into the volumetric flask to prepare a reference solution with 0.1 mg of the reference substance contained in per 1 mL of the solution. The reference solution was filtered through 0.45µm filter membrane, and 10µL of the subsequent filtrate was taken for sample injection.

The chromatographic conditions of HPLC are as follows: employing octadecyl bonded silica gel as stationary phase and using continuous gradient elution, with acetonitrile being mobile phase A and water being mobile phase B; continuous gradient elution procedure is as follows:

At 0 min, mobile phase A: 20%, mobile phase B: 80%;

At 55 mins, mobile phase A: 40%, mobile phase B: 60%; and

At 70 mins, mobile phase A: 65%, mobile phase B: 35%;

Flow rate of mobile phase: 1mL/min; detection wavelength: 205nm; column temperature: 10°C. The peak areas of andrographolide, neoandrographolide, deoxyandrographolide, and dehydroandrographolide were obtained respectively.

The contents of andrographolide, neoandrographolide, deoxyandrographolide and dehydroandrographolide in *Andrographis paniculata*

are calculated according to the following equation:

$$\text{Equation: Content (mg/g)} = (C_S/W) \times (A_U/A_S) \times 25F$$

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

5 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;

F : relative correction factor (1 for andrographolide, 1.150 for neoandrographolide, 0.779 for deoxyandrographolide and 0.721 for
10 dehydroandrographolide).

10 batches of *Andrographis paniculata* pills samples were detected for 10 times respectively and the results are shown in Table 4, errors are in the range of 10% or less.

15 Table 4. Comparison of contents measurement results of 10 batches of *Andrographis paniculata* pills

No.	Andrographolide	Neoandrographolide			Deoxyandrographolide			Dehydroandrographolide		
	Content mg/g	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %
1	3.23	0.54	0.55	1.9	2.29	2.26	-1.1	10.23	10.14	-0.8
2	5.08	1.37	1.34	-2.2	2.70	2.81	3.9	9.91	10.30	3.9
3	1.87	1.68	1.71	1.8	2.40	2.47	3.1	8.12	8.37	3.2
4	2.69	2.61	2.59	-0.8	2.98	3.09	3.5	8.86	9.09	2.7
5	4.65	1.95	1.89	-3.1	2.15	2.23	3.7	8.79	8.69	-1.1
6	3.53	3.57	3.64	2.0	3.78	3.95	4.3	7.60	8.09	6.4
7	6.79	4.67	4.57	-2.1	3.85	4.07	5.5	8.71	8.99	3.2
8	2.16	3.47	3.58	3.2	4.52	4.75	5.1	10.93	11.32	3.6
9	3.82	2.94	2.88	-2.0	3.62	3.78	4.3	11.85	11.69	-1.4
10	1.47	2.93	2.94	0.3	1.20	1.21	1.5	7.93	8.14	2.5

Example 5

2.00mL of *Andrographis paniculata* injection were weighed precisely, loaded onto a neutral alumina column (200 ~ 300 mesh, 5g, inner diameter of
20 1.5cm), and eluted with 80mL methanol. The eluent was collected in a 100mL volumetric flask, diluted with methanol to volume, shook up and filtered. 5mL of the subsequent filtrate was precisely weighed into a 50mL volumetric flask, into which methanol was added to volume (i.e. dilution ratio is 10). The

mixture was shook up and filtered, the filtrate was filtered through 0.45µm filter membrane, and 10µL of the filtrate was taken for sample injection.

Appropriate amount of reference substance of andrographolide was taken, accurately weighed, and placed in a volumetric flask. Adding methanol into the volumetric flask to prepare a reference solution with 0.1 mg of the reference substance contained in per 1 mL of the solution. The reference solution was filtered through 0.45µm filter membrane, and 10µL of the subsequent filtrate was taken for sample injection.

The chromatographic conditions of HPLC are as follows: employing octadecyl bonded silica gel as stationary phase and using continuous gradient elution, with acetonitrile being mobile phase A and 0.2%v/v phosphoric acid solution being mobile phase B; continuous gradient elution procedure is as follows:

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: 205nm; column temperature: 10°C. The peak areas of andrographolide, neoandrographolide, deoxyandrographolide, and dehydroandrographolide were obtained respectively.

The contents of andrographolide, neoandrographolide, deoxyandrographolide and dehydroandrographolide in *Andrographis paniculata* are calculated according to the following equation:

$$\text{Equation: Content (mg/g)} = (C_S/W) \times (A_U/A_S) \times 100 \times 10 \times F$$

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;

F : relative correction factor (1 for andrographolide, 1.150 for neoandrographolide, 0.779 for deoxyandrographolide and 0.721 for dehydroandrographolide).

10 batches of *Andrographis paniculata* injection samples were detected for

10 times respectively and the results are shown in Table 5, errors are in the range of 10% or less.

Table 5. Comparison of contents measurement results of 10 batches of *Andrographis paniculata* injections

No.	Androgra pholide	Neoandrographolide			Deoxyandrographolide			Dehydroandrographolide		
	Content mg/g	Content g/g	QAMS	Error %	Content mg/g	QAMS	Error %	Content mg/g	QAMS	Error %
1	4.34	5.21	5.51	5.6	2.83	3.02	6.5	9.66	9.39	-2.8
2	4.56	5.25	5.55	5.6	3.00	3.20	6.5	10.42	9.92	-4.8
3	3.50	4.15	4.44	7.0	4.16	4.03	-3.2	11.27	11.85	5.2
4	4.20	4.21	4.50	7.0	4.04	4.36	7.9	11.02	11.19	1.5
5	3.35	6.81	6.56	-3.7	2.91	3.11	7.1	11.94	12.28	2.9
6	2.39	6.79	6.56	-3.3	2.99	3.18	6.5	12.11	12.46	2.9
7	2.43	4.49	4.38	-2.5	3.32	3.19	-3.9	12.89	12.44	-3.5
8	3.37	4.01	4.29	7.0	4.34	4.69	8.1	11.91	12.27	3.0
9	3.98	6.72	7.12	6.0	3.50	3.81	8.8	12.07	12.76	5.7
10	2.38	6.86	7.27	5.9	3.97	4.30	8.5	11.39	12.00	5.4

- 5 The above are only preferred embodiments of the present invention, and it should be noted that the above-described preferred embodiments should not be construed as limiting the present invention, the scope of which should be defined by the claims.

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.

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:

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

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. 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.

9. The method according to claim 7 or claim 8, 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. 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 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. The method according to any one of claims 1 to 10, 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.