The high-performance liquid chromatographic fingerprints study of Awei capsules

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ABSTRACT

Background: Awei Capusules are Hospital preparation for Hyperlipidemia. It was composed by Awei, Magnoliae Officinalis and Polygonum Bistoral etc. Manufacture and quality standard of Awei Capusules had been studied. Results of animal pharmacodynamic and clinical study all displayed that Awei Capusules can reduce serum levels of TC, TG, LDL-C, increases HDL-C/TC. It was safe. It could improve hemorrheology and vessel function of blood stasis animal. On the basis of these, we studied on fingerprint of Awei capsule. **Materials and Methods:** The gradient elution method was used for analyzing samples on HPLC. Fingerprint similarity calculation software was used for data analysis. **Results:** We got a good separation of Awei Capusules peaks. There were 15 peaks in fingerprint of Awei capusles. Gallic acid, magnolol and honokiol were identified. **Conclusion:** HPLC fingerprinting of Awei Capusules can provide to reference. It can control preparations quality of Awei Capusules.

Key words: Awei capsules, fingerprints, high-performance liquid chromatographic

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INTRODUCTION

Awei Capsules are Hospital preparation for Hyperlipidemia. It was based on Traditional Chinese Medicine (TCM) theory of "phlegm", "dampness", "obesity", with spleen and stomach disorders.[1] It was composed by Xinjiang Awei (*Ferula sinkiangensis K. M. Shen*), Cortex Magnoliae Officinalis (*Magnolia officinalis Rehd. et Wils.)*, Bistort Rhizome (*Polygonum bistorta L.*), Radix Lithospermi Root of Sinkiang Arnebia (*Arnebia euchroma (Royle) Johnst.)*, Pseudobulbus Cremastrae seu Pleiones (*Cremastra appendiculata (D. Don) Makino*) and Imperata cylindrical (*Imperata cylindrical Beauv.var.major (Nees) C.E.Hubb*). It was composed by the Awei, Magnoliae Officinalis and Polygonum Bistoral, etc. After clinical application for many years, it has good effect. Manufacture[2] and quality standard[3] of Awei Capsules had been studied. In animal pharmacodynamic test, it could reduce levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C), increases high-density lipoprotein cholesterol (HDL-C)/TC, especially decreased of TG obviously. At the same time, it improved blood stasis hemorrheology's index and vessel's function of the animal models.[4] Through acute toxicity and chronic toxicity tests, it was proved to be safety in clinical dosimetry.[5,6] Through clinical studying of 340 cases of primary hyperlipidemia, it could reduce levels of TC, TG, LDL-C, increases HDL-C/TC. By molecular biology techniques, the regulating lipid mechanism of Awei Capsules was lifting of Hepatic lipase (HL) and cardiac lipoprotein lipase (LPL) activity and inhibiting of three hydroxy three methyl amyl two acyl coenzyme A (HMG-CoA) activity *in vivo*.[7] It could improve Apo-A4 gene transcription level obviously.[8] There were essential oil composition[9] gallate acid[10,11] in Awei Capsules. There were magnolol and honokiol in Magnolia of Awei Capsules.[12-16] Magnolia has increased gastric motility, central inhibition, antibacterial, and antiinflammatory.[17]

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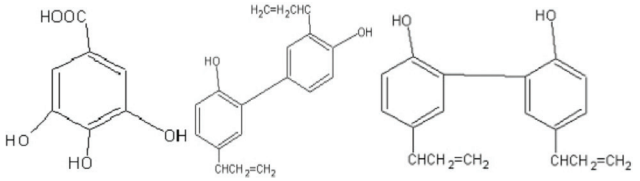
The constructor of some ingredients in Awei Capsules are:



Gallate acid, magnolol, and honokiol

Awei Capsules have been the medical institution preparation (registration number: ZJL20100013). On the basis of this, we studied on finger prints of Awei Capsules. Fingerprint is an effective means for the quality control of Awei Capsules.[18-20] A high-performance liquid chromatographic-diode array

detector (HPLC-DAD) method was established for the simultaneous evaluation of Awei Capsules.[21]

MATERIALS AND METHODS

Materials and reagents

Awei Capsules were afforded by Affiliated Hospital of traditional Chinese medicine of Xinjiang Medical University, Urumqi, Xinjiang, China. (lot:20090117). Standard substance of ferulic acid (lot:0773-9910), gallic acid (lot:110831-200302), magnolol (lot:110729-200310), honokiol (lot:110730-200609), and shikonin (lot:110769-200405) were bought from the national institute for the control of pharmaceutical and biological products, Beijing, China. Acetonitrile and methanol (chromatographic purity, American Fisher company); ultra pure water (18 MΩ, Millipore company); phosphoric acid (analysis pure, Tianjin Chemical Reagent Factory); high pure nitrogen (> 99.999%, Xinjiang Kondit Industrial Development Company Limited), Urumqi, Xinjiang, China.

Preparation of sample solutions

The content powder of Awei Capsules samples (0.1 g) were extracted by methanol in dark brown calibrated flasks (25 ml) in an ultrasonic bath for 30 min. After extracting solution cooled down, added methanol to the volume (25 ml). The solution was filtered through membrane filter (0.45 μm).

Preparation of standard solutions

Standard stock solutions of gallate acid (0.04 μg/ml), magnolo l(12.4 μg/ml), and honokiol (4.05 μg/ml), were prepared in methanol.

Chromatographis analysis

Analyses were performed using HPLC system SHIMADZU (LC-20 AB pump, SPD-M20 A diode array detector, CBM-20 A system controller, CTO-20 A column temperature box, SIL-20 A auto sample injector, DGU-20 A3 on-line degassing device). Detection wavelengths were set at 273 nm for gallate acid, 294 nm for both magnolol and honokiol. An Inertsil ODS-SP chromatographic column (250 × 4.6 mm, 5 μm) was used with a flow rate of 1.0 ml/min. The injection volume was 20 μl and the column temperature was maintained at 25°C. Mobile phase was composed of (A) aqueous phosphoric acid (0.4%, v/v) and (B) acetonitrile using a gradient elution of 0–25 min, 5–20%A; 25–40 min, 20–30%A; 40–60 min, 30–75%A; 60–100 min, 75–100%A.

Results and discussion optimization of HPLC chromatography conditions selection of the detection wavelength

In references, gallic acid, the index components of Awei Capsules, had maximum absorption at 273 nm. Honokiol and magnololat at 294 nm had maximum absorption. The study used DAD detector to scan UV wavelength. The chromatographic peaks in the sample concentrated at 190–400 nm. Impurity existed great absorption peaks at about 200 nm, solvent had peaks of absorption too. Background interference was big. Hence, established fingerprints at the maximum absorption of all the index components. The wavelength at which more peaks existed as the test wavelength was selected.

Selection of the mobile phase

Optimization of gradient elution separation condition is the key to establish fingerprints. Suitable mobile phase can adjust the retention time and modulate peak shape. Awei Capsules contain gallic acid, honokiol and magnolol, and other ingredients of great difference of polarities. Gallic acid have larger polar, hence, it can be dissociated with polar solvent easily. Adding appropriate proportion of phosphoric acid to the mobile phase can obtain stable chromatographic peak. Separation effect of mobile phases of methanol (A)–water (B); acetonitrile (A)–water (B); methanol:acetonitrile (1:1A)-0.1% phosphoric acid solution (B) were compared. The chromatogram of different wavelengths between 200 and 400 nm were compared. According to the number and size of chromatographic peak, 294 nm was selected as the detection wavelength and chose three gradient elution programs. Specific conditions are shown in Tables 1–3.

In methanol–water system elution program, there were fewer peaks, which expressed incomplete information and bad shape. In the acetonitrile–water system elution

Table 1: Gradient elution procedures

Time (min)	Methanol	Water
0	5	95
60	100	0
70	100	0

Table 2: Gradient elution procedures

Time (min)	Acetonitrile	Water
0	5	95
60	100	0
70	100	0

Table 3: Gradient elution procedures

Time (min)	Methanol:acetonitrile (1:1)	0.1%phosphoric acid aqueous solution
0	5	95
60	100	0
70	100	0

program, chromatographic peaks appeared in 50 min but stacked together. Hence, methanol acetonitrile-0.1% phosphoric acid solution system is considered. The baseline was separated.

Figure 1 shows that there were many chromatographic peaks in methanol–acetonitrile-0.1% phosphoric acid system. But chromatographic peaks accumulated between 15–35, 45–65 min. Hence, it needed to optimize elution program.

Optimization of elution program

Optimization condition 1, see Table 4. Main peaks concentrated before 30 min or after 70 min. Fewer peaks appeared in the middle. Optimization conditions 2, see Table 5.

Figure 2 shows that chromatographic peak expressed information completely. Baseline of main peaks could be separated, hence, chose the chromatographic conditions to Awei Capsules HPLC finger prints.

Selection of the temperature of the column

During the experiment, it was found that the temperature have great effect on the separation. 25°C, 30°C, 35°C, and 40°C were investigated in the same chromatographic condition on chromatographic peak separation effect. Finally, at 30°C, the effect is best.

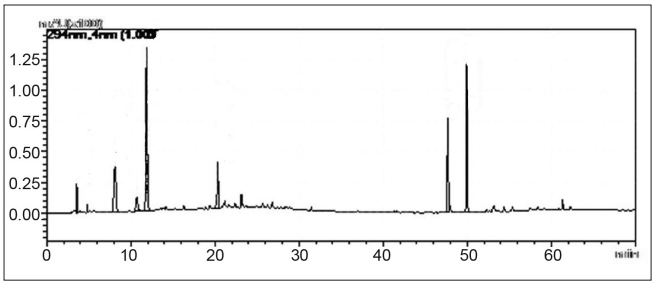


Figure 1: HPLC diagram of gradient elution program 3

Table 4: Gradient elution procedures

Time (min)	Methanol:acetonitrile (1:1)	0.1%phosphoric acid aqueous solution
0	5	95
20	20	80
50	25	75
60	55	45
80	90	10

Table 5: Gradient elution procedures

Time (min)	Methanol:acetonitrile(1:1)	0.1%phosphoric acid aqueous solution
0	5	95
25	20	80
40	30	70
60	75	25
100	100	0

Choice of the flow rate

Separation effect at 0.8, 1, and 1.2 mL/min in the same conditions were investigated. When the flow rate was quick, the peak separation is bad; 1.0 mL/min was the best velocities found.

Methodological study

The precision test

The same test solution was taken, sampled five times continuously, and recorded fingerprints. The peak at retention time 70.4 min was taken as the reference peak. The RSD of relative retention time and relative peak area of mutual peaks between 0.55–1.13% and 1.16–1.99% was calculated. It was accorded with the fingerprint requirement.

The reproducibility test

Five portions of Awei Capsules were precisely weighed and five portions of sample solution according to the preparing method of sample solution were prepared. Samples were tested respectively and their fingerprints were recorded. The peak of retention time at 70.4 min as the reference peak was taken. The RSD of the relative retention time and relative peak area of common peak between 0.96–1.21% and 1.16–2.13% was calculated. It was accorded with the fingerprint requirement.

The stability test

The sample solutions at 0, 2, 6, 12, 24, and 36 h, respectively, were injected, and chromatograms were recorded. The RSD of the relative retention time and relative peak area of common peak between 0.19–0.73% and 0.96–1.53% were calculated. The sample solution has stability in 36 h.

Identification of chromatographic peak of index component

In order to control further quality of Awei Capsules, fingerprints of Awei Capsules by identifying chromatographic peak of index component was established.

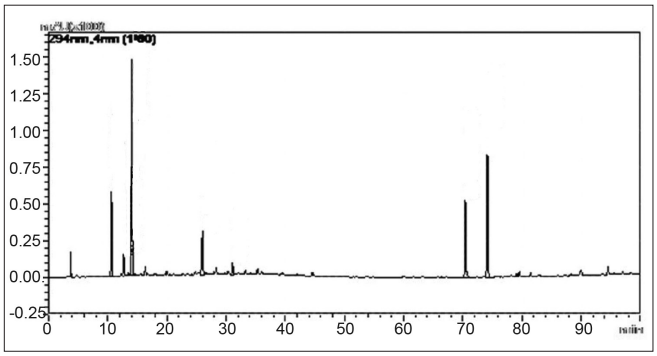


Figure 2: HPLC chromatogram of gradient elution program 5

The determination of gallic acid

Ultraviolet scanning results showed that gallic acid had maximum absorption wavelength at 273 nm, hence, the chromatographic peaks at 273 nm were contrasted. There was no interference in blank solvent of HPLC chromatogram. The results are shown in Figure 3.

Determination of magnolol and honokiol

Ultraviolet scanning results, honokiol and magnololhad had maximum absorption wavelength at about 294 nm, hence, chromatographic peak at 294 nm was compared. Blank solvent of HPLC chromatogram had no interference. The results are showed in Figure 4.

Fingerprints of 10 Awei Capsules were studied, and calculated the similarity by using the Chromatographic fingerprints Similarity Evaluation System of Traditional Chinese Medicine, which was set by National Pharmacopoeia Committee in 2004. See Figure 5. The tenth (S_{10}) was taken as the reference peak. In the figure, fingerprints chromatographic of Awei Capsules had 21 component peaks. Chromatographic peaks through multipoint correction were matched. The results are shown in Table 6.

Table 6 shows that there were only 15 common peaks in the chromatographic of the 10 groups of Awei Capsules finger prints. Although there were different chromatographic peaks and peak areas, there were three index ingredients: gallic acid (12 min), magnolol (70 min), and honokiol (73 min). It indicates that, with these three kind of components as the index components, it was feasible to control Awei Capsules product quality by establishing Awei Capsules HPLC finger prints. It is feasible to control the quality of Awei Capsules by setup of HPLC fingerprints.

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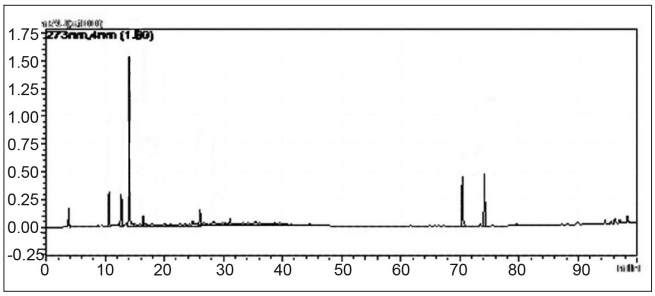

Figure 3: HPLC chromatogram of Awei Capsules at the 273 nm

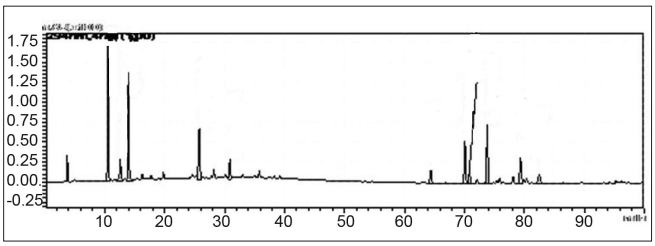

Figure 4: HPLC chromatogram of Awei Capsules at the 294 nm

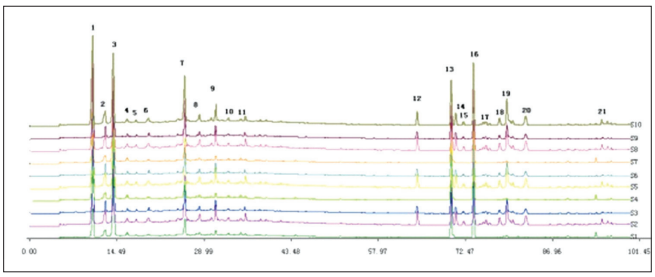

Figure 5: The stacking chart of 10 times detection fingerprints of Awei Capsules

Table 6: The matching results of chromatographic peaks of 10 groups of awei capsule

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Control fingerprints
S1	1	0.853	0.852	1	0.853	0.852	1	0.853	0.852	0.853	0.921
S2	0.853	1	0.994	0.853	1	0.994	0.853	1	0.994	1	0.989
S3	0.852	0.994	1	0.852	0.994	1	0.852	0.994	1	0.994	0.987
S4	1	0.853	0.852	1	0.853	0.852	1	0.853	0.852	0.853	0.921
S5	0.853	1	0.994	0.853	1	0.994	0.853	1	0.994	1	0.989
S6	0.852	0.994	1	0.852	0.994	1	0.852	0.994	1	0.994	0.987
S7	1	0.853	0.852	1	0.853	0.852	1	0.853	0.852	0.853	0.921
S8	0.853	1	0.994	0.853	1	0.994	0.853	1	0.994	1	0.989
S9	0.852	0.994	1	0.852	0.994	1	0.852	0.994	1	0.994	0.987
S10	0.853	1	0.994	0.853	1	0.994	0.853	1	0.994	1	0.989
Control fingerprints	0.921	0.989	0.987	0.921	0.989	0.987	0.921	0.989	0.987	0.989	1

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Conflict of Interest: No.