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HPLC Study on HPLC Characteristic Spectrum of Huoxiang (Herba Agastachis) Eliminating Summer-heat Soft Capsule

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1. Introduction

Huoxiang (Herba Agastachis) Eliminating Summer-heat Soft Capsule is a commonly-used drug relieving summer-heat in summer, which can dispel summer heat and resolve dampness, relieve exterior syndrome and regulate the middle warmer and thus is mainly used for intrinsic wet hysteresis, fever with aversion to cold caused by heatstroke and cold, headache and adiapneustia, aching and tired limbs, nausea and vomiting, abdominal pain and diarrhea and other symptoms [1]. The prescription of Huoxiang Eliminating Summer-heat Soft Capsule includes 12 medicinal materials including Pogostemonis Herba; Moslae Herba; Angelicae Dahuricae Radix; Perillae Folium; Atractylodis Rhizoma; Caryophylli Flos; Citri Reticulatae Pericarpium; Arecae Pericarpium; Poria; Glycyrrhizae Radix Et Rhizoma; Pinelliae Rhizoma Praeparatum and Zingiberis Rhizoma Recens [1]. At present, current standards only test content of hesperidin in the preparation [2,3], while quality standard evaluation of traditional Chinese medicine is developing from single component analysis to multi-index and multi-component analysis because single component of one medicinal material cannot effectively controls quality of preparations [4-7]. High performance liquid chromatography (HPLC) and high performance liquid chromatography/diode array detector (HPLC/DAD) are the most effective methods used in Characteristic Spectrum analysis and quality control of Chinese traditional medicine [8-10]. Hence, this project study characteristic chromatogram of Huoxiang Eliminating Summer-heat Soft Capsule by HPLC/DAD and provide methods and
theoretical basis for comprehensive quality control of Huoxiang Eliminating Summer-heat Soft Capsule.

2. Experimental

2.1 Materials and Reagents

All experiments were performed using ‘A class’ volumetric glassware. Acetonitrile (HPLC grade /chromatographically pure, MERCK), and other analytical grade reagents (China National Pharmaceutical (Group) Shanghai Chemical Reagent Company) were used in the preparation of the mobile phase for gradient elution. HPLC grade Milli Q water was used in the preparation of the mobile phase for gradient elution. The mobile phase was filtered through a 0.45 µm PVDF filter (Welch Materials, Inc.) and degassed under vacuum, prior to use. For the preparation of the reference solution and reference crude herb solution, pharmaceutical grade reference standards were used.

Liquiritin (C21H22O9, lot number: 111610-201106); Ammonium Glycyrrhizinate (C42H65NO16∙5H2O, lot number:110731-201116); Hesperidin (C28H34O15, lot number: 110721-201316); Eugenol (C10H12O2, lot number: 110725-201112); Imperatorin (C16H14O4, lot number: 0826-9502) were provided by National institutes for food and drug control. Pogostemonis Herba (lot number: 121135-201005); Moslae Herba (lot number: 121456-200401); Angelicae Dahuricae Radix; Perillae Folium; Atractylodis Rhizoma; Caryophylli Flos; Citri Reticulatae Pericarpium; Arecae Pericarpium; Poria; Glycyrrhizae Radix Et Rhizoma; Pinelliae Rhizaoma Praeparatum and Zingiberis Rhizoma Recens were provided by National institutes for food and drug control. The other two medicinal materials, Pinelliae Rhizoma Praeparatum and Zingiberis Rhizoma Recens were provided by the manufacturer.

2.2 Analytical Solutions

Test Sample Solution

Took 2 g of drug, accurately weighed, placed in 100 ml stoppered conical flask, added 70% methanol 50 ml, sonicated for 30 min, shook, filtered through a 0.45 µm PVDF filter.

Reference Crude Herb Solution

The control medicinal materials extract was prepared by the preparation process of Huoxiang Eliminating Summer-heat Soft Capsule, which included Pogostemonis Herba; Moslae Herba; Angelicae Dahuricae Radix; Perillae Folium; Atractylodis Rhizoma; Caryophylli Flos; Citri Reticulatae Pericarpium; Arecae Pericarpium; Poria; Glycyrrhizae Radix Et Rhizoma; Pinelliae Rhizaoma Praeparatum and Zingiberis Rhizoma Recens and each of them was prepared into control medicinal material solution according to the preparation method of sample solution.

Stock Solutions

Stock solution of each single reference substance (Liquiritin; Ammonium Glycyrrhizinate; Hesperidin; Eugenol; Imperatorin) was suitably diluted by 70% methanol to get concentrations of 0.8 mg ml⁻¹ respectively.

Reference Substance Solution

A solution containing 40 μg ml⁻¹ of mixed reference substances was used as a reference solution.

3. Results

3.1 The Choice of Measuring Wavelength

Huoxiang Eliminating Summer-heat Soft Capsule contains 12 medicinal materials, each of which has a great difference in ultraviolet absorption wavelength. For example, “Chinese Pharmacopoeia” 2015 Edition predetermined detection the wavelength of Liquiritin, Chenpi (Citri reticulatae pericarpium), Baizhi (Angelicae dahuricae radix), Cangzhu (Atractylodis rhizoma) are 237 nm, 283 nm, 300 nm, 340 nm respectively. In order to detect two or more different ingredients and reduce interference, five wavelengths were chosen for investigation (237 nm, 250 nm, 280 nm, 300 nm, 270 nm), through experimental comparison, the chromatographic peak information obtained at 270 nm is relatively rich, and its peak height is relatively homogenous, thus it selected as the most suitable wavelength for the test. (Figure 1, wavelength Selection investigation).

3.2 The Choice of Mobile Phase for Gradient Elution

Acetonitrile-0.05% phosphoric acid was chosen as flow phase system [2,11], and three gradient of elution methods were investigated. The gradient elution programs are showed in Table 1 as follow. According to experimental results (Figure 2), chromatographic peaks of Elution Method 3 had good degree of separation, and its elution baseline was flat. Thus, the Elution Method 3 was chosen as formal elution method.
Figure 1. Wavelength Selection investigation

Figure 2. Chromatogram of three gradient elution methods (1 Gradient elution program ①, 2 Gradient elution program ②, 3 Gradient elution program ③)

Figure 3. Chromatogram of three sample extraction method (1 sample extraction method ①, 2 sample extraction method ②, 3 sample extraction method ③)
Table 1. Gradient elution program

| Gradient elution program ① | Time/min | Acetonitrile (%) | 0.05% phosphoric acid (%) |
|---------------------------|----------|------------------|--------------------------|
| 0 ~ 33                    | 5 → 40   | 95 → 60          |
| 33 ~ 50                   | 40 → 75  | 60 → 25          |
| 50 ~ 62                   | 75       | 25               |
| 62 ~ 64                   | 75 → 20  | 25 → 80          |

| Gradient elution program ② | Time/min | Acetonitrile (%) | 0.05% phosphoric acid (%) |
|---------------------------|----------|------------------|--------------------------|
| 0 ~ 5                     | 5 → 10   | 95 → 90          |
| 5 ~ 30                    | 10 → 20  | 90 → 80          |
| 30 ~ 35                   | 20 → 30  | 80 → 70          |
| 35 ~ 37                   | 30 → 45  | 70 → 55          |
| 37 ~ 55                   | 45 → 75  | 55 → 25          |
| 55 ~ 60                   | 75 → 5   | 25 → 95          |

| Gradient elution program ③ | Time/min | Acetonitrile (%) | 0.05% phosphoric acid (%) |
|---------------------------|----------|------------------|--------------------------|
| 0 ~ 30                    | 5 → 30   | 95 → 70          |
| 30 ~ 35                   | 30 → 40  | 70 → 60          |
| 35 ~ 55                   | 40 → 75  | 60 → 25          |
| 55 ~ 60                   | 75 → 5   | 25 → 95          |

3.3 Suitable Sample Extraction Method

The aim of this study was to find a suitable sample extraction method. Three parallel samples (Pharmaceutical manufacturer A, batch number: 127160205) with 2 g each was taken for the follow three extraction methods: ① Add 25 ml petroleum ether, shake for 30 min, filter, discard the petroleum ether solution, evaporate the filter residue, add 50 ml methanol, and ultrasonic treatment for 30 min; ② 70% methanol ultrasonic treatment for 30 minutes; ③ 50 ml pure methanol ultrasonic treatment for 30 minutes [2,11,12]. According to experimental results shown in Figure 3, method ① has fewer chromatographic peaks and insufficient peak information. Although method ② and method ③ have little difference in liquid chromatograms, the baseline of chromatogram of method ② is more smoother than method ③. Thus, this method (method ②) was selected as extraction method of test sample solution.

3.4 Investigation the Concentration Condition of Phosphoric Acid Solution in Mobile Phase

Since the concentration of phosphoric acid varies slightly in each actual operation, it is necessary to investigate whether the chromatographic peak information is different under the condition of different concentration of phosphoric acid when phosphoric acid is used as the mobile phase the concentrations of 0.04%, 0.05% and 0.06 phosphoric acid were investigated in the experiment. According to experimental results (Figure 4), it shows little difference in liquid chromatogram obtained from three phosphoric acid solution concentrations, which does not affect overall information of chromatographic peaks.
Considering the simplicity of calculation, the 0.05% phosphoric acid was used in the subsequent experiments.

3.5 Identification and Attribution of Characteristic Peaks

Preparation of solution: according to analytical solutions preparation, test sample solution, reference crude herb solution and reference substance solution were prepared.

After successively prepared test sample solution, reference crude herb solution and reference substance solution, the solutions were tested under chromatographic conditions of characteristic chromatogram. By comparing the chromatogram of three solutions, that include test sample, reference crude herb and reference substance, and their DAD (Diode Array Detector) scanning (200 nm-400 nm) results, the characteristic peaks and each peak were affirmed. A total of 11 characteristic peaks were found in the chromatogram of the test sample (Figure 5) after comparing with chromatographic peaks of reference crude herb, which respectively represented Caryophylli Flos (Peak 1), Glycyrrhizae Radix Et (Peak 2, Liquiritin), Caryophylli Flos (Peak 3), Moslae Herba (Peak 4), Citri Reticulatae Pericarpium (Peak 5, Hesperidin), Caryophylli Flos (Peak 6, Eugenol), Glycyrrhizae Radix Et Rhizoma (Peak 7, Ammonium Glycyrrhizinate), Angelicae Dahuricae Radix (Peak 8, Imperatorin), Angelicae Dahuricae Radix (Peak 9), Atractylodis Rhizoma (Peak 10 and 11).

The chromatographic peak of Hesperidin (Peak 5) was glaringly obvious in the chromatogram, so the Hesperidin peak (Peak 5) was chosen as the S peak. A solution containing 40 μg ml⁻¹ of Hesperidin was used as a reference solution in identification experiment mentioned above.
Table 2. Relative retention time and identification of chromatographic peaks

| Peak Number | relative retention time | The RSD of relative retention time | ± 5% range of relative retention time | Identification of peak | Identification of crude herb |
|-------------|-------------------------|-----------------------------------|--------------------------------------|------------------------|-------------------------------|
| 1           | 0.2351                  | 1.75%(n=80)                       | 0.2235-0.2471                       | Peak 1                 | Caryophylli Flos              |
| 2           | 0.8407                  | 0.19%(n=75)                       | 0.7987-0.8827                       | Peak 2 (Liciritin)     | Glycyrrhizae Radix Et Rhizoma |
| 3           | 0.8668                  | 0.26%(n=80)                       | 0.8236-0.9102                       | Peak 3                 | Caryophylli Flos              |
| 4           | 0.9005                  | 0.27%(n=80)                       | 0.8546-0.9456                       | Peak 4                 | Moslae Herba                  |
| 5           | 1.0000                  | 0.00%(n=80)                       | 0.950-1.050                         | Peak 5 (Hesperidin)    | Citri Reticulatae Pericarpium |
| 6           | 1.5399                  | 0.40%(n=80)                       | 1.4632-1.6172                       | Peak 6 (Eugenol)       | Caryophylli Flos              |
| 7           | 1.6215                  | 0.29%(n=80)                       | 1.5408-1.7030                       | Peak 7 (Ammonium Glycyrrhizinate) | Glycyrrhizae Radix Et Rhizoma |
| 8           | 1.8456                  | 0.31%(n=75)                       | 1.7533-1.9379                       | Peak 8 (Imperatorin)   | Angelicae Dahuricae Radix     |
| 9           | 1.8959                  | 0.23%(n=75)                       | 1.8011-1.9907                       | Peak 9                 | Angelicae Dahuricae Radix     |
| 10          | 2.0300                  | 0.21%(n=75)                       | 1.9285-2.1315                       | Peak 10                | Atractylodis Rhizoma          |
| 11          | 2.1836                  | 0.30%(n=66)                       | 2.0744-2.2928                       | Peak 11                | Atractylodis Rhizoma          |

Figure 7. DAD scan pattern of chromatographic peaks
The HPLC characteristic spectrum of the drug should be similar to the control characteristic map, and should show 11 chromatographic peaks. The retention time of peak 5 and the peak of hesperidin should be the same as well. Using the reference hesperidin peak as the S peak calculate the relative retention time of the 11 characteristic peaks. The relative retention time of each characteristic peak should be within ± 5% of the specified value. The specified value is: 0.2351 (Peak 1), 0.8407 (Peak 2), 0.8668 (Peak 3), 0.9005 (Peak 4), 1.0000 (Peak 5, hesperidin, S), 1.5399 (Peak 6), 1.6215 (Peak 7), 1.8456 (Peak 8), 1.8959 (Peak 9), 2.0300 (Peak 10), 2.1836 (Peak 11). And the DAD scan pattern of each characteristic peak is consistent with the following pattern.

The typical chromatogram of sample characteristic spectrum is shown in Figure 6, which contains 11 characteristic peaks marked from 1 to 11. Compared chromatographic peaks between the sample and reference, the attribution of 11 characteristic peaks were determined (Table 2), and the DAD scan pattern illustrates in Figure 7.

### 3.6 Stability of Analytical Solutions

Stability of analytical solutions: the aim of this study is to prove the stability of the sample solution and reference solution at room temperature\[13\]. For the study, duplicate sets of spiked sample preparations and sample preparations as per the test method were prepared and kept on a bench top (25 ± 2 °C) and analyzed initially (0day,0h), after 2h, 4h, 8h, 12h and 24h by a single injection of each sample preparations into chromatography column, and chromatograms were recorded. Using 5# Hesperidin Peak as referenced retention time to calculate relative retention time of different common peaks. According to experimental results (Table 3), relative standard deviation on relative retention time of different common peaks was less than 2%, indicating the test sample solution basically remains stable within 24h.

#### 3.7 Repeatability Study

Method precision (Repeatability): a group of samples were taken again to conduct repetitive investigation. Six test sample solutions were prepared and tested according to set chromatographic conditions, and calculated relative retention time of different common peaks. According to experimental results (Table 4), relative standard deviation (RSD) on relative retention time of different common peaks was less than 2%, providing the experiment has good repeatability and could be repeated.

### Table 3. Relative retention time of different common peaks in Stability study

| Number | 0h     | 2h     | 4h     | 8h     | 12h    | 24h    | Average value | RSD (%) |
|--------|--------|--------|--------|--------|--------|--------|---------------|---------|
| Peak 1 | 0.2312 | 0.2314 | 0.2311 | 0.2313 | 0.2318 | 0.2321 | 0.2315        | 0.16%   |
| Peak 2 | 0.8388 | 0.8388 | 0.8386 | 0.8386 | 0.8387 | 0.8298 | 0.8389        | 0.05%   |
| Peak 3 | 0.8654 | 0.8654 | 0.8650 | 0.8647 | 0.8650 | 0.8659 | 0.8652        | 0.05%   |
| Peak 4 | 0.8997 | 0.8998 | 0.8996 | 0.8994 | 0.8996 | 0.9001 | 0.8997        | 0.03%   |
| Peak 5 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000        | 0.00%   |
| Peak 6 | 1.5340 | 1.5339 | 1.5338 | 1.5358 | 1.5348 | 1.5351 | 1.5346        | 0.05%   |
| Peak 7 | 1.6181 | 1.6181 | 1.6183 | 1.6208 | 1.6190 | 1.6177 | 1.6187        | 0.07%   |
| Peak 8 | 1.8427 | 1.8422 | 1.8423 | 1.8448 | 1.8436 | 1.8429 | 1.8431        | 0.05%   |
| Peak 9 | 1.8916 | 1.8910 | 1.8911 | 1.8938 | 1.8922 | 1.8914 | 1.8919        | 0.06%   |
| Peak 10| 2.0271 | 2.0264 | 2.0270 | 2.0299 | 2.0267 | 2.0260 | 2.0272        | 0.07%   |
| Peak 11| 2.1840 | 2.1829 | 2.1831 | 2.1860 | 2.1829 | 2.1819 | 2.1835        | 0.06%   |

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3.8 Instrument Precision Study

Instrument precision (suitability of the system): the suitability of the system was checked by a single injection of the resolution solution and five replication injections of the reference solution. The %RSD, theoretical plates, tailing factor and resolution were optimized as the system suitability parameters. Mixed reference substance solution was implemented as sample for six times during test according to set chromatographic conditions, and calculated relative retention time of different common peaks. According to experimental results (Table 5), relative standard deviation on relative retention time of different common peaks was also below 2%, indicating the experimental instrument had good accuracy and would not influence experimental results.

| Number | 1     | 2     | 3     | 4     | 5     | 6     | Average value | RSD (%) |
|--------|-------|-------|-------|-------|-------|-------|---------------|---------|
| Peak 1 | 0.2312| 0.2311| 0.2311| 0.2309| 0.2311| 0.2311| 0.2311        | 0.03%   |
| Peak 2 | 0.8389| 0.8388| 0.8387| 0.8384| 0.8386| 0.8287| 0.8387        | 0.02%   |
| Peak 3 | 0.8656| 0.8654| 0.8654| 0.8651| 0.8652| 0.8653| 0.8653        | 0.02%   |
| Peak 4 | 0.8999| 0.8998| 0.8998| 0.8996| 0.8995| 0.8996| 0.8997        | 0.02%   |
| Peak 5 | 1.0000| 1.0000| 1.0000| 1.0000| 1.0000| 1.0000| 1.0000        | 0.00%   |
| Peak 6 | 1.5338| 1.5336| 1.5334| 1.5336| 1.5337| 1.5341| 1.5337        | 0.02%   |
| Peak 7 | 1.6178| 1.6176| 1.6172| 1.6173| 1.6178| 1.6181| 1.6176        | 0.02%   |
| Peak 8 | 1.8416| 1.8417| 1.8419| 1.8422| 1.8426| 1.8428| 1.8421        | 0.03%   |
| Peak 9 | 1.8904| 1.8906| 1.8907| 1.8909| 1.8913| 1.8917| 1.8909        | 0.03%   |
| Peak 10| 2.0262| 2.0264| 2.0267| 2.0270| 2.0275| 2.0268| 2.0268        | 0.02%   |
| Peak 11| 2.1839| 2.1839| 2.1838| 2.1838| 2.1843| 2.1840| 2.1840        | 0.01%   |

Table 4. Relative retention time of different common peaks in Repeatability study

| Number | 1     | 2     | 3     | 4     | 5     | 6     | Average value | RSD (%) |
|--------|-------|-------|-------|-------|-------|-------|---------------|---------|
| Peak 1 | -     | -     | -     | -     | -     | -     | -             | -       |
| Peak 2 | 0.8401| 0.8395| 0.8393| 0.8392| 0.8392| 0.8390| 0.8394        | 0.025%  |
| Peak 3 | -     | -     | -     | -     | -     | -     | -             | -       |
| Peak 4 | -     | -     | -     | -     | -     | -     | -             | -       |
| Peak 5 | 1.0000| 1.0000| 1.0000| 1.0000| 1.0000| 1.0000| 1.0000        | 0.00%   |
| Peak 6 | 1.5404| 1.5419| 1.5415| 1.5393| 1.5392| 1.5380| 1.5400        | 0.10%   |
| Peak 7 | 1.6238| 1.6255| 1.6246| 1.6221| 1.6216| 1.6198| 1.6229        | 0.13%   |
| Peak 8 | 1.8495| 1.8523| 1.8518| 1.8486| 1.8482| 1.8473| 1.8496        | 0.11%   |
| Peak 9 | -     | -     | -     | -     | -     | -     | -             | -       |
| Peak 10| -     | -     | -     | -     | -     | -     | -             | -       |
| Peak 11| -     | -     | -     | -     | -     | -     | -             | -       |
3.9 The Universally of HPLC Instruments and Chromatograph Column

To test the universally of HPLC instruments and chromatograph column, two different instruments and three chromatograph columns were chosen in the test. Took 1 batch of this product (Pharmaceutical manufacturer A, batch number: 127160205), prepared the test solution according to the method in the text, and investigated the HPLC instruments and chromatographic columns according to the proposed mobile phase elution method: ① Agilent 1260 high performance liquid chromatograph, ② Diane Ultimate 3000 high performance liquid chromatograph; ① Tech Mate C18-ST, 5 um, 4.6 × 250 nm , ② Innovaal ODS-2, 5 um, 4.6 × 250 nm, ③ Alltima C18, 5 um, 4.6 × 250 nm. Shows in Figure 8 Chromatographic Column Investigation, Table 6 Chromatographic Column Investigation Results and Table 7 Different Instrument Investigation Results. The results indicated that the resolution of those three columns were the same. Each of the columns could be used in characteristic spectrum test of Huoxiang Eliminating Summer-heat Soft Capsule because of their well separation. Considered the peak shape and separation situation, the chromatographic column ① and HPLC instrument ① were finally selected for sample detection.

![Figure 8. Chromatograms of Chromatographic Column Investigation](image)

| Number | Column 1 | Column 2 | Column 3 |
|--------|---------|---------|---------|
| Peak 1 | 0.2312  | 0.2518  | 0.2717  |
| Peak 2 | 0.8389  | 0.8650  | 0.8801  |
| Peak 3 | 0.8656  | 0.8986  | 0.9007  |
| Peak 4 | 0.8999  | 0.9248  | 0.9216  |
| Peak 5 | 1.0000  | 1.0000  | 1.0000  |
| Peak 6 | 1.5338  | 1.5593  | 1.5038  |
| Peak 7 | 1.6178  | 1.5843  | 1.5790  |
| Peak 8 | 1.8416  | 1.8760  | 1.8522  |
| Peak 9 | 1.8904  | 1.9304  | 1.8992  |
| Peak 10| 2.0262  | 2.0597  | 2.0111  |
| Peak 11| 2.1839  | 2.1824  | 2.1582  |

Table 6. Relative retention time of different common peaks in three columns
3.10 Test Sample

All collected samples (80 different samples from 5 manufacturers) were tested by applying HPLC method. Tech Mate C18-ST Chromatographic Column and Agilent 1260 HPLC instrument were chosen to detect the samples. After detection of all samples, a quality conclusion about all those Huoxiang Eliminating Summer-heat Soft Capsules can be drawn from the five manufacturers. 11 characteristic peaks were shown out in products which came from 3 manufacturers, and the result indicated those three manufacturers’ Huoxiang Eliminating Summer-heat Soft Capsules have well quality. The remaining two manufacturers’ products were shown out 10 and 6 characteristic peaks respectively. The reason may be that the manufacturer did not use all 12 herbs specified on the prescription, or some herbs were of poor quality.

4. Discussions

A challenging, versatile HPLC method was developed for the simultaneous determination of multi-component in Huoxiang Eliminating Summer-heat Soft Capsule. The method was very simple and effective, and based on the validation data that can be concluded within an analysis time of 60 min, 11 characteristic peaks were determined accurately and precisely. Convenient operation, economy and high efficiency and providing method have been achieved to comprehensive quality control of Huoxiang Eliminating Summer-heat Soft Capsule.

This HPLC Characteristic Spectrum method compares the locations of the reference substance and different prescription medicines to determine 11 characteristic peaks with identification significance. By DAD scanning (200-400 nm) on the 11 peaks in the sample, the DAD scanning pattern of each characteristic peak is shown out. Via chromatogram contrast of three solutions included test sample, reference crude herb and reference substance, and their DAD scanning results, to affirm characteristic peaks and each peak.

The retention time of the mobile phase prepares at different times (0 h, 2 h, 4 h, 8 h, 12 h and 24 h), since these 11 characteristic peaks have little changes. Among these 11 characteristic peaks, hesperidin (peak 5) is relatively stable, and has a large peak area which is easy to identify. Therefore, hesperidin (peak 5) is selected to calculate the relative retention time. The relative retention time of each characteristic peak is basically stable. Meanwhile, different chromatographic columns are used to investigate the durability of the method. The HPLC Characteristic Spectrum can evaluate the quality of the products in more economical, efficient and scientific way.

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| Number | Agilent 1260 | Diane Ultimate 3000 |
|--------|--------------|----------------------|
| Peak 1 | 0.2312       | 0.2305               |
| Peak 2 | 0.8389       | 0.8483               |
| Peak 3 | 0.8656       | 0.8865               |
| Peak 4 | 0.8999       | 0.9132               |
| Peak 5 | 1.0000       | 1.0000               |
| Peak 6 | 1.5338       | 1.5131               |
| Peak 7 | 1.6178       | 1.5762               |
| Peak 8 | 1.8416       | 1.8176               |
| Peak 9 | 1.8904       | 1.8663               |
| Peak 10| 2.0262       | 2.0001               |
| Peak 11| 2.1839       | 2.1603               |
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