# Quantitative and chemical fingerprint analysis for quality evaluation of the dried bark of wild Phellodendron amurense Rupr. based on HPLC-DAD-MS combined with chemometrics methods


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# Quantitative and chemical fingerprint analysis for quality evaluation of the dried bark of wild *Phellodendron amurense* Rupr. based on HPLC-DAD-MS combined with chemometrics methods

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## Abstract

The dried bark of *Phellodendron amurense* Rupr., known as "Guanhuangbo" in China, has been widely used as traditional Chinese medicine for thousands of years. In this paper, an accurate and reliable high performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD-MS) method was developed for quality evaluation of wild Guanhuangbo. Six bioactive compounds, including chlorogenic acid, phellodendrine, magnoflorine, jatrorrhizine, palmatine and berberine, were determined simultaneously in 37 batches of wild Guanhuangbo samples collected from different locations of China. The chromatographic conditions and extraction procedures were optimized by an orthogonal design during the study whereas the identities of compounds were confirmed by LC-MS. Moreover, similarity analysis and hierarchical clustering analysis were successfully applied to demonstrate the variability of these wild Guanhuangbo samples, and data from different analysis showed good consistency. The results indicated that the developed multi-compounds determination method in combination with fingerprint analysis was suitable to quantitative analysis and quality evaluation of the dried bark of wild *Phellodendron amurense* Rupr..


**Keywords** *Phellodendron amurense* Rupr., Fingerprint analysis, HPLC-DAD-MS, Quality evaluation, Chemometrics

## 1. Introduction

Traditional Chinese medicine (TCM) has played an indispensable role in preventing and treating human diseases for a long time, which have already attracted global attention [1]. In the process of "modernization" and "globalization" of TCM, a key issue is the consistency and controllability of quality of TCM [2]. Traditionally, the identification of TCM is performed according to its morphology, one or two markers' TLC identification and/or content determination. However, this method does not provide a complete profile of the drug, so it cannot distinguish drugs with similar appearance and/or similar main chemical constitution [3]. Therefore, quantitative analysis of multi-compounds coupled with qualitative analysis of chromatographic fingerprinting is the development trend to evaluate TCM quality.

The dried bark of *Phellodendron amurense* Rupr., known as "Guanhuangbo" in China, has been widely used as traditional Chinese medicine, which is officially listed in Chinese Pharmacopoeia (2010 version) [4]. Guanhuangbo shows the function of clearing heats and dampness, purging fire and eliminating steaming of bone, relieving toxicity and curing sores from the viewpoint of TCM theory [5-7]. Pharmacologically, the main active ingredients of Guanhuangbo are attributed to alkaloids, such as magnoflorine can protect human high density lipoprotein against lipid peroxidation [8]; phellodendrine, magnoflorine, jatrorrhizine, palmatine and berberine exhibit the effects of anti-Alzheimer, antioxidant, analgesic, anti-inflammatory and antihyperglycemic and so on [9-12]. Meanwhile, studies have shown the beneficial properties to humans such as antioxidant, hypoglycaemic, antiviral and hepatoprotective activities have been attributed to chlorogenic acid in *in vitro*, *in vivo* and epidemiological studies [13]. In Chinese Pharmacopoeia (2010 version), only palmatine and berberine are included to evaluate the quality of Guanhuangbo. Nonetheless, such a means of quality control is not sufficient to evaluate the quality of Guanhuangbo, considering that *P. amurense* Rupr. is widely distributed in many geographical locations in China and the diverse geographical sources which has different ecological environments and other factitious factors could possibly result in great variations of their chemical constituents. Therefore, it is highly desirable to develop an accurate and systematical method for quality evaluation.

Although several analytical methods have been employed to quantify chemical markers based on HPLC [14-16, 18], the former methods have simultaneously determined only two or three compounds in wild Guanhuangbo. A rapid and validated multi-components analytical method is

yet highly desirable for the systematical evaluation of quality, as a result, in the study, a reliable and accurate method by HPLC-DAD-MS was developed for quantitative analysis of six bioactive compounds, including chlorogenic acid, phellodendrine, magnoflorine, jatrorrhizine, palmatine and berberine, which chlorogenic acid is phenylpropanoids, and the other 5 compounds are alkaloids, in Guanhuangbo. Furthermore, similarity analysis (SA) and hierarchical clustering analysis (HCA) were successfully applied to demonstrate the variability of the six bioactive compounds in the 37 batches of wild Guanhuangbo samples which were collected from different locations in China. The current developed method has the advantages of higher extraction efficiency, greater resolution and more compounds determination. Moreover the chemometrics-assisted HPLC-DAD-MS was firstly applied in evaluating the quality of Guanhuangbo.

## 2. Materials and methods

### 2.1. Materials and reagents

Thirty-two batches Guanhuangbo samples were collected at DBH (diameter at breast height) of *P. amurense* Rupr. in July 2013, which distributed in Jilin, Liaoning and Heilongjiang Province of China with the growth years being over ten years whereas the other 5 batches Guanhuangbo samples were obtained from Beijing City, Hebei and Anhui Province, respectively. All of these specimens, identified by Prof. Bengang Zhang, were kept at our laboratory for future reference. The air-dried samples stored at room temperature until analysis. Six reference compounds, chlorogenic acid, phellodendrine, magnoflorine, jatrorrhizine chloride, palmatine chloride, berberine chloride, were purchased from Phytomarker Ltd. (Tianjin, China). The purities of all the reference compounds were more than 98%.

Acetonitrile was purchased from Honeywell Burdick & Jackson (Muskegon, USA). Analytical grade of methanol and hydrochloric acid were purchased from Beijing Chemical Works (Beijing, China). Chromatographic grade of acetic acid was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Ammonium acetate was obtained from Xilong Chemical Co., Ltd. Pure water (18.2MΩ) for the HPLC analysis was obtained from a Milli-Q System (Millipore, Billerica, MA, USA).

### 2.2. Preparation of standard solutions and samples preparation

The standard stock solutions of six reference compounds were prepared by weighing

accurately and dissolving them with methanol, and then the standard stock solutions were diluted to generate an appropriate concentration range to establish calibration curves. All the stock and working standard solutions were stored at 4 °C until use.

All the air-dried Guanhuangbo samples were pulverized to powder, sieved (65-mesh) and oven-dried to constant mass at 45°C. Powdered sample (0.5000 g) was suspended in 50 ml 1% hydrochloric acid within methanol in a capped conical flask, weighed accurately, and extracted with ultrasonic thrice (40 minutes for each time). After cooling, weigh again, and compensate the loss of the weigh with extraction solvent, and mix well. The sample solution was filtered through a 0.22 μm membrane filter prior to HPLC analysis.

**2.3. Instrumentation and chromatographic conditions**

Chromatographic analysis was performed by a Waters 2695 high performance liquid chromatography system (Milford, MA, USA) coupled with a 2996 photodiode array detector, and chromatographic data were processed by Waters Empower 2 data station. Chromatographic separation was performed on a Agilent ZORBAX SB-$C_{18}$ column (250 mm×4.6 mm i.d., 5 μm). A linear gradient elution of solvent A (Water contains 0.3% acetic acid, 4 mM ammonium acetate) and solvent B (Acetonitrile) were applied with the following program: 0-5 min, 5 to 10% B; 5-18 min, 10 to 13% B; 18-20 min, 13 to 18% B; 20-30 min, 18-40% B and 30-40 min, 40 to 90% B. A pre-equilibration period of 10 min was used between individual runs. The flow rate was at 1.0 $mL \cdot min^{-1}$ and the injection volume was 4 μL. The wavelength was set at 280 nm. The column and auto-sampler were maintained at 30°C and 25°C, respectively.

**2.4. HPLC-DAD-MS analysis**

HPLC-DAD-MS analysis was carried out with Applied Biosystem 3200 Q-Trap mass spectrometer (Foster City, CA, USA) connected to an Agilent 1200 HPLC system via electro-spray ionization interface. The chromatographic conditions were as described above. Electro-spray ionization was applied in positive ion modes for MS and MS/MS with an ion spray voltage of 4000 V, curtain gas of 10 psi, nebulizer gas of 70 psi and auxiliary gas 40 psi. The ion source temperature was set at 400°C. Ultrapure nitrogen was used as nebulizer, heater, curtain and collision-activated dissociation (CAD) gas. Moreover chlorogenic acid was identified in negative ion modes. Data were processed by the Analyst 1.4 software (Applied Biosystems / MDSSciex). MS data, retention time and UV-Vis spectra were used to identify the bioactive compounds

contained in Guanhuangbo. The assignments were validated by co-elution with the corresponding reference compounds and by comparison with published data.

### 2.5. Method validation and Chemometrics analysis

The calibration curves were constructed by using five different concentrations. Analytical method was validated for the calibration curves, limit of detection and quantification (LOD and LOQ), repeatability, stability, and accuracy of the six bioactive compounds.

SA was performed by the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), which was recommended by China's State Food and Drug Administration (CFDA). HCA was applied to demonstrate the variability of the content of six bioactive compounds in 37 batches of wild Guanhuangbo samples by using SPSS (Version 19.0).

## 3. Results

### 3.1. Optimization of HPLC conditions

In general, a suitable chromatographic column, mobile phase, elution mode and detection wavelength are critically important for chromatographic separation. Therefore, to obtain accurate and optimal chromatographic conditions, different HPLC parameters were examined and compared, including various columns (Waters XBridge $C_{18}$ 250 mm×4.6 mm, 5 μm, Dikma Diamonsil $C_{18}$ 250 mm×4.6 mm, 5 μm, Kromasil KR100-5 $C_{18}$ 250 mm×4.6 mm, 5 μm and Agilent ZORBAX SB-$C_{18}$ 250 mm×4.6 mm, 5 μm), mobile phases (methanol-water and acetonitrile-water with different modifiers, including phosphoric acid, phosphoric buffer, formic acid, and acetic acid solutions adjusted by ammonium acetate or triethylamine with different pH values), column temperatures (25°C, 30°C and 35°C), and mobile phase flow rates (0.8, 1.0 and 1.2mL·min$^{-1}$). The monitoring wavelength was set at 280 nm, where most of compounds could be detected and had adequate absorption. As a result, the optimized HPLC condition was established by comparing the resolution, baseline, elution time and the number of characteristic peaks in each chromatogram after repeated experiments. Typical chromatograms for chemical analysis were shown in **Fig. 1**.

### 3.2. Optimization of the extraction methods

To obtain satisfactory extraction efficiency, ultrasonic, heat refluxing, and soxhlet extraction were compared. It was found that ultrasonic extraction was simpler and more effective for the six

bioactive compounds extraction than any other ways and thus was used in further experiments. The other factors of extraction procedures were optimized by an orthogonal ($L_9 3^4$) experimental design, including extraction solvents (60% methanol, 100% methanol and 1% hydrochloride within methanol), sample-solvent ratios (1:50, 1:100 and 1:150, w/v), and extraction time (20, 30 and 40 min) and extraction cycles (1, 2 and 3 cycles). Each extract combination was tested in triplicate. Comparing the numbers, areas and resolution of the chromatographic peaks obtained by different extraction procedures, the optimal extraction procedures were established. The samples were extracted in 1% hydrochloride within methanol of sample/solvent ratio (w/v) 1:100 by ultrasonic extraction, the process carried out three cycles (40 min each time).

### 3.3. LC-MS identity confirmation

HPLC-DAD-MS was used to identify the six bioactive compounds from the extract of Guanhuangbo samples. By comparison with retention time, ultraviolet spectra, precursor ions, and diagnostic fragment ions of the corresponding reference compounds, six bioactive compounds in HPLC-chromatogram of Guanhuangbo were unambiguously identified as chlorogenic acid (**1**), phellodendrine (**7**), magnoflorine (**8**), palmatine chloride (**13**), jatrorrhizine chloride (**14**), berberine chloride (**15**), respectively **(Table 1)**. The results further revealed that the six investigated compounds were the main chemical constituents of Guanhuangbo, which was of great importance to establish a relatively accurate and feasible method for its quality evaluation.

### 3.4. Method validation of quantitative analysis

#### 3.4.1. Calibration curves, LOD and LOQ

Calibration curves of six bioactive compounds were determined by using the developed method. Their correlation coefficient values ($R^2 \geq 0.9991$) indicated appropriate correlations between concentrations of each analytes and their peak areas within the investigated ranges for all the analytes. The LODs and LOQs were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively. The reference calibration curves, linear range, $R^2$, LOD and LOQ were listed in **Table 2**.

#### 3.4.2. Precision, repeatability, stability and recovery test

The intra- and inter-day precisions were investigated by analyzing a mixed standard solution in five replicates in one day and by duplicating the experiments once a day for five consecutive days. The relative standard deviation (RSD) values of the six analytes were all less than 1.23%. To

confirm the repeatability of the developed assay, six independently prepared samples (S1) were analyzed to test the repeatability of the above method. The RSD values were all less than 1.16%. Stability of sample solution was tested at the time interval of 0, 8, 16, 24, 32, 48 and 72 h at room temperature. The results (RSD<2.19%) showed that the sample solutions were stable within 3 days. The results were shown in **Table 3**.

Recoveries were tested to investigate the accuracy of the method by adding the mixed standard solutions to known amounts samples (S1). The resultant samples were then extracted and analyzed (n=6) by using the proposed procedure. The ratio of determined and add amount were used to calculate the recovery. The results were shown in **Table 4**, and the recoveries of the six bioactive compounds were ranged from 97.05% to 103.68%, and their RSD values were less than 1.82%.

### 3.5. Sample analysis

#### 3.5.1. Quantitative analysis

The developed assay method was subsequently applied to quantitative analysis of six bioactive compounds in 37 batches of wild Guanhuangbo samples collected from different locations of China. Each sample was analyzed three times to determine the mean content (mg/g) and the data were summarized in **Table 5**. The results indicated that the content of six bioactive compounds varied greatly among the samples collected from different locations, and the total content of six bioactive compounds was higher in S3, S16, S19, S29 and S36, and lower in S10, S15, S26, S31, S32 and S34. In Chinese Pharmacopoeia (2010 version), it stipulates that the content of palmatine should not be less than 3 mg/g and that of berberine should not less than 6 mg/g in Guanhuangbo. The present results suggested that ten wild Guanhuangbo samples did not meet the requirement with the palmatine content being lower than 3 mg/g and/or the berberine content being lower than 6 mg/g. But the means content of analytes were higher than stipulation and superior to the purchase samples. Meanwhile phellodendrine and magnoflorine were deemed to be the main chemical constituents in the term of content as well, which might be beneficial to evaluate the quality of Guanhuangbo comprehensively. Six bioactive compounds were identified and quantified simultaneously. The established method was simple and accurate for quality evaluation of Guanhuangbo.

#### 3.5.2. Similarity analysis

A total of 37 batches of wild Guanhuangbo samples from different geographical locations were investigated. Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A) was performed based on their HPLC fingerprints. Fifteen peaks that existed in all 37 batches of wild Guanhuangbo samples were assigned as "characteristic peaks". The reference fingerprint (marked with R) is generated by the chromatograms of 37 batches of wild Guanhuangbo samples using median method. The similarities were compared to the R (**Fig. 2**). The closer the cosine values approached 1, the more similar the two chromatograms were. The similarity values during tested samples ranged from 0.826 to 0.998 (**Table 5**), indicating chemical constituents of Guanhuangbo from different sources varied significantly in terms of identities and quantities. S8 that collected from Jilin Province and S32 collected from Liaoning Province were markedly different from others for the content of their palmatine was higher than berberine. Besides, most of samples which collected from different geographical locations were similar to the ones that purchased from drug stores. Therefore, chromatographic fingerprint combined with similarity analysis was an efficient method to judge the consistency of samples.

### 3.5.3. Hierarchical cluster analysis

In order to validate the results of similarity analysis and further elucidate the resemblance relationship among samples, HCA was applied by SPSS 19.0. The results of HCA data which were acquired by submitting the fifteen characteristic peak areas to analysis showed that 37 batches of wild Guanhuangbo samples were divided into three clusters obviously (**Fig. 3**).The distance between cluster I and cluster II was shorter than the distance between cluster I and cluster III, which indicated cluster III was less similar to that of cluster I and cluster II. According to **Table 5**, cluster III was formed by the samples which the total content was lower, and/or the similarity value was less than 0.90. The samples in cluster II were S3, S16, S19, S29 and S36, which the contents of six bioactive compounds in the samples were higher than other clusters. Cluster I consisted of the remaining samples including the purchased one which implied that most of samples had satisfied drug store's requirements. The result was very similar to the quantitative analysis and similarity analysis. Hence, HCA was helpful to differentiate and evaluate the consistency of Guanhuangbo.

## 4. Conclusion

A fast and validated HPLC-DAD-MS method combined with chemometrics tools was first developed for the comprehensive quality evaluation of Guanhuangbo. The proposed method which combined fingerprint analysis with quantitative analysis was successfully applied to determined simultaneously six bioactive compounds in 37 batches of wild Guanhuangbo samples collected from different locations in China, and fifteen peaks in the extract solution of Guanhuangbo were assigned as "characteristic peaks". The results indicated that the samples from different locations shared a similar HPLC pattern but the contents of the six bioactive compounds in the samples varied greatly. Based on the fingerprints, 37 batches of wild Guanhuangbo samples were classified or discriminated by chemometric tools (SA and HCA) objectively and successfully. Therefore the developed HPLC-DAD-MS method displayed good precision, stability, sensitivity and recovery, and was suitable to evaluate the quality of Guanhuangbo, especially in combination with chemometric tools.

In addition, the total contents of the six bioactive compounds in samples can change with the growth year of *P. amurense* Rupr.. The contents of compounds are positively correlated with the growth year of *P. amurense* Rupr. [17-18]. The ecological factors can affect the quality of Guanhuangbo as well [19-22]. Hence we need further research the relationship between ecological factors and the quality evaluation of Guanhuangbo perfectly.

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**Table 1** Identification of the six bioactive compounds in Guanhuangbo

| Peak No. | Identification | RT (min) | λ max (nm) | m/z(MS) | m/z ($MS^n$) |
|---|---|---|---|---|---|
| 1 | Chlorogenic acid* | 10.95 | 220,240,326 | 353$[M-H]^+$ | 191 |
| 7 | Phellodendrine | 18.77 | 205,285, | 343$[M+H]^+$ | 280,199 |
| 8 | Magnoflorine | 24.99 | 223,270,303 | 343$[M+H]^+$ | 313, 265 |
| 13 | Jatrorrhizine chloride | 35.18 | 227,266,347 | 339$[M+H]^+$ | 323, 294, |
| 14 | Palmatine chloride | 37.57 | 227,275,347 | 353$[M+H]^+$ | 337,322, 294 |
| 15 | Berberine chloride | 38.38 | 230,266,348 | 337$[M+H]^+$ | 321,306,278 |

* negative ion mode

**Table 2** Linearity, LODs and LOQs for six bioactive compounds

| Compound | Calibration curve | $R^2$ | Linear range (μg/ml) | LOD (μg/ml) | LOQ (μg/ml) |
|---|---|---|---|---|---|
| Chlorogenic acid | y = 1238495.5804 x - 22299.2873 | 0.9998 | 1.15-34.5 | 0.06 | 0.19 |
| Phellodendrine | y = 803590.4898 x - 2134.4801 | 0.9992 | 1.8-54 | 0.08 | 0.26 |
| Magnoflorine | y = 1647959.1336 x - 94021.7334 | 0.9994 | 10.1-303 | 0.04 | 0.13 |
| Jatrorrhizine chloride | y = 5568942.8620 x + 739.5792 | 0.9991 | 0.12-3.6 | 0.01 | 0.04 |
| Palmatine chloride | y = 3756362.6306 x - 131546.7061 | 0.9998 | 6.2-186 | 0.02 | 0.05 |
| Berberine chloride | y = 2968997.0134 x + 18869.1690 | 0.9994 | 3.65-109.5 | 0.02 | 0.06 |

**Table 3** Precisions, stability and repeatability of six bioactive compounds

| Compound | Precisions (n=6) | | Repeatability (n=6) | Stability (n=6) |
|---|---|---|---|---|
| | Intra-day RSD (%) | Inter-day RSD (%) | RSD (%) | RSD (%) |
| Chlorogenic acid | 0.88 | 0.98 | 1.16 | 2.19 |
| Phellodendrine | 0.65 | 0.77 | 0.68 | 0.72 |
| Magnoflorine | 0.95 | 1.23 | 1.01 | 1.93 |
| Jatrorrhizine chloride | 0.61 | 0.69 | 0.99 | 2.10 |
| Palmatine chloride | 0.54 | 0.96 | 0.97 | 1.92 |
| Berberine chloride | 0.32 | 0.93 | 1.15 | 1.78 |

**Table 4** Recovery of the six bioactive compounds. (n=6)

| Compound | Original (mg) | Spiked (mg) | Found* (mg) | Recovery (%) | Average recovery (%) | RSD (%) |
|---|---|---|---|---|---|---|
| Chlorogenic acid | 0.2425 | 0.2300 | 0.4698 | 98.84 | 97.91 | 0.83 |
| | | | 0.4678 | 97.96 | | |
| | | | 0.4662 | 97.28 | | |
| | | | 0.4701 | 98.94 | | |
| | | | 0.4661 | 97.24 | | |
| | | | 0.4660 | 97.19 | | |
| Phellodendrine | 0.3042 | 0.3600 | 0.6591 | 98.57 | 101.03 | 1.82 |
| | | | 0.6752 | 103.05 | | |
| | | | 0.6690 | 101.33 | | |
| | | | 0.6604 | 98.96 | | |
| | | | 0.6722 | 102.23 | | |
| | | | 0.6716 | 102.05 | | |
| Magnoflorine | 1.9203 | 2.0200 | 3.8790 | 96.97 | 97.05 | 1.30 |
| | | | 3.9101 | 98.51 | | |
| | | | 3.8851 | 97.27 | | |
| | | | 3.9056 | 98.28 | | |
| | | | 3.8585 | 95.95 | | |
| | | | 3.8451 | 95.29 | | |
| Jatrorrhizine chloride | 0.0161 | 0.0240 | 0.0411 | 103.99 | 103.68 | 0.77 |
| | | | 0.0408 | 102.76 | | |
| | | | 0.0410 | 103.62 | | |
| | | | 0.0413 | 104.77 | | |
| | | | 0.0408 | 102.79 | | |
| | | | 0.0411 | 104.17 | | |
| Palmatine chloride | 1.0170 | 1.2400 | 2.2286 | 97.71 | 99.59 | 1.18 |
| | | | 2.2451 | 99.04 | | |
| | | | 2.2484 | 99.31 | | |
| | | | 2.2639 | 100.56 | | |
| | | | 2.2565 | 99.96 | | |
| | | | 2.2690 | 100.97 | | |
| Berberine chloride | 0.7104 | 0.7300 | 1.4277 | 98.26 | 98.47 | 0.79 |
| | | | 1.4261 | 98.04 | | |
| | | | 1.4261 | 98.03 | | |
| | | | 1.4243 | 97.79 | | |
| | | | 1.4314 | 98.77 | | |
| | | | 1.4398 | 99.91 | | |

* Found is the sum of the Original and Spiked quantities.

**Table 5** Content (mg/g) of six bioactive compounds in Guanhuangbo collected from different locations (n=3).

| Sample No. | Origin | Similarity value | Content (mg/g) | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 7 | 8 | 13 | 14 | 15 | |
| S1 | Tonghua city, Jilin Province | 0.947 | 1.17 | 2.29 | 9.96 | 0.23 | 5.14 | 10.83 | 29.62 |
| S2 | Tonghua city, Jilin Province | 0.990 | 2.98 | 3.21 | 5.59 | 0.51 | 6.76 | 20.85 | 39.89 |
| S3 | Tonghua city, Jilin Province | 0.980 | 3.30 | 4.14 | 8.61 | 0.76 | 9.52 | 22.26 | 48.59 |
| S4 | Tonghua city, Jilin Province | 0.974 | 0.91 | 1.87 | 8.21 | 0.39 | 4.21 | 11.27 | 26.86 |
| S5 | Tonghua city, Jilin Province | 0.998 | 1.30 | 2.88 | 6.92 | 0.56 | 4.78 | 20.01 | 36.46 |
| S6 | Tonghua city, Jilin Province | 0.976 | 2.43 | 3.11 | 9.00 | 0.35 | 2.88 | 19.63 | 37.41 |
| S7 | Tonghua city, Jilin Province | 0.994 | 1.04 | 2.41 | 7.85 | 0.35 | 3.08 | 17.58 | 32.31 |
| S8 | Tonghua city, Jilin Province | 0.826 | 0.97 | 2.53 | 7.24 | 0.42 | 9.62 | 8.71 | 29.49 |
| S9 | Tonghua city, Jilin Province | 0.988 | 0.40 | 2.07 | 3.19 | 0.27 | 2.96 | 16.11 | 25.01 |
| S10 | Tonghua city, Jilin Province | 0.983 | 0.83 | 1.70 | 8.00 | 0.18 | 3.79 | 9.51 | 24.02 |
| S11 | Tonghua city, Jilin Province | 0.992 | 1.51 | 3.20 | 3.70 | 0.60 | 5.54 | 21.32 | 35.87 |
| S12 | Tonghua city, Jilin Province | 0.995 | 1.22 | 2.04 | 3.25 | 0.32 | 2.98 | 12.87 | 22.67 |
| S13 | Tonghua city, Jilin Province | 0.990 | 1.27 | 1.99 | 5.04 | 0.29 | 2.71 | 11.95 | 23.24 |
| S14 | Tonghua city, Jilin Province | 0.990 | 1.56 | 2.31 | 4.37 | 0.32 | 2.73 | 14.75 | 26.03 |
| S15 | Tonghua city, Jilin Province | 0.984 | 0.77 | 1.35 | 3.87 | 0.24 | 1.81 | 9.28 | 17.32 |
| S16 | Tonghua city, Jilin Province | 0.956 | 2.22 | 5.09 | 9.67 | 0.36 | 11.83 | 21.72 | 50.88 |
| S17 | Tonghua city, Jilin Province | 0.981 | 1.21 | 3.85 | 6.86 | 0.31 | 7.57 | 19.73 | 39.52 |
| S18 | Tonghua city, Jilin Province | 0.994 | 3.38 | 3.87 | 11.32 | 0.37 | 5.88 | 21.16 | 45.99 |
| S19 | Tonghua city, Jilin Province | 0.922 | 4.08 | 4.63 | 15.53 | 0.40 | 4.94 | 28.42 | 58.01 |
| S20 | Tonghua city, Jilin Province | 0.987 | 2.48 | 2.94 | 7.75 | 0.31 | 3.47 | 18.49 | 35.46 |
| S21 | Baoding city, Hebei Province | 0.995 | Tr* | 2.46 | 7.73 | 0.31 | 5.46 | 16.94 | 32.91 |
| S22 | Xiyuan Hospital CACMS, Beijing | 0.990 | Tr* | 3.04 | 8.65 | 0.29 | 7.23 | 17.53 | 36.74 |
| S23 | Bozhou city, Anhui Province | 0.991 | Tr* | 2.71 | 6.88 | 0.31 | 6.59 | 18.08 | 34.57 |
| S24 | Peking University Third Hospital, Beijing | 0.981 | Tr* | 3.20 | 9.05 | 0.29 | 7.88 | 17.14 | 37.55 |
| S25 | Anguo city, Hebei Province | 0.993 | Tr* | 2.04 | 6.97 | 0.28 | 4.95 | 14.68 | 28.91 |
| S26 | Fushun city, Liaoning Province | 0.980 | 0.67 | 0.77 | 4.11 | 0.08 | 2.73 | 5.27 | 13.62 |
| S27 | Fushun city, Liaoning Province | 0.929 | 1.92 | 1.97 | 11.86 | 0.41 | 7.41 | 10.28 | 33.86 |
| S28 | Fushun city, Liaoning Province | 0.992 | 2.31 | 2.16 | 7.92 | 0.32 | 4.17 | 17.98 | 34.85 |
| S29 | Fushun city, Liaoning Province | 0.922 | 6.01 | 4.71 | 16.92 | 0.67 | 15.25 | 20.54 | 64.10 |
| S30 | Fushun city, Liaoning Province | 0.984 | 1.77 | 1.47 | 7.01 | 0.24 | 2.74 | 9.06 | 22.29 |
| S31 | Fushun city, Liaoning Province | 0.957 | 1.16 | 0.56 | 4.43 | 0.07 | 1.54 | 4.15 | 11.91 |
| S32 | Fushun city, Liaoning Province | 0.840 | 1.24 | 1.22 | 7.39 | 0.13 | 5.11 | 4.26 | 19.36 |
| S33 | Fushun city, Liaoning Province | 0.993 | 3.04 | 3.09 | 12.99 | 0.57 | 6.26 | 18.94 | 44.91 |
| S34 | Hailun city, Heilongjiang Province | 0.953 | 1.27 | 2.12 | 6.34 | 0.29 | 3.95 | 7.97 | 21.90 |
| S35 | Hailun city, Heilongjiang Province | 0.915 | 1.08 | 2.08 | 7.12 | 0.35 | 6.95 | 8.72 | 26.30 |
| S36 | Hailun city, Heilongjiang Province | 0.923 | 2.48 | 3.67 | 16.15 | 0.30 | 8.18 | 15.59 | 46.38 |
| S37 | Hailun city, Heilongjiang Province | 0.962 | 1.74 | 2.86 | 6.74 | 0.57 | 4.86 | 12.05 | 28.81 |

Tr*: below LOQ.