Quantitative $^1$H NMR for the direct quantification of saikosaponins in *Bupleurum chinense* DC.

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Abstract

Saikosaponin a and saikosaponin d are used as chemical standards for the quality evaluation of *Bupleurum chinense* DC. by the high-performance liquid chromatography method in current Chinese Pharmacopoeia. However, other saikosaponins, such as saikosaponin c and saikosaponin b₂, also possess pharmaceutical activity but are not used as chemical standards. In this study, a quantitative proton nuclear magnetic resonance (¹H NMR) method was developed to determine the total mass percentage (mg/g) of SSa, SSb₁, SSb₂ and SSd in *B. chinense* DC., using the H-24 (δ₁H 0.71) signal. Furthermore, the molality (mol/kg) of type I saikosaponins (epoxy-ether structure) was also determined by quantitative ¹H NMR in the area of H-11 (δ₁H 5.95) for a more accurate quality evaluation. Validation of the method confirmed that it had acceptable selectivity, precision, stability, and repeatability. The results indicated that this method has the potential to be a reliable method for the quantification of saikosaponins in *Bupleurum scorzonerifolium* Willd., vinegar baked *B. Chinense* DC. and *B. scorzonerifolium* Willd., Chaihu Koufuye (oral liquid of Chaihu).

**Keywords:** *Bupleurum chinense* DC.; quantitative ¹H NMR; molality; saikosaponin; quality evaluation
Introduction

*Radix Bupleuri* (RB, Chinese name *Chaihu*) is derived from the dried roots of *Bupleurum chinense* DC. or *Bupleurum scorzonerifolium* Willd. (*Apiaceae*) according to the Chinese Pharmacopoeia.\(^1\) The former is known as “beichaihu” (northern Chinese thorowax root) and the latter is known as “nanchaihu” (southern Chinese thorowax root), due to the differences in their origin and description.\(^2\) As one of the most common traditional Chinese herbal medicines, it has been used widely for the treatment of cold, fever,\(^3\) inflammation,\(^4\) malaria\(^5\) and liver disease\(^6\) in East Asia for about 2,000 years. To improve its clinical effectiveness, *R. Bupleuri* is also combined with other herbs in many classical formulas, such as Xiao Yao San, Xiao Chai Hu Tang and Chai Hu Shu Gan Yin.\(^1\)

With the development of modern pharmacology, many valuable and important properties of *R. Bupleuri* have been discovered, such as antitumor,\(^7\) antidepressant,\(^8\) antimicrobial,\(^9\) immunoregulation\(^10\) and neuromodulation activities.\(^11\) These biological and pharmacological activities are attributed to triterpenoid saponins, flavonoids, and essential oils.\(^2\) The saikosaponins are acknowledged to be the principle bioactive components.\(^12,13\)

The aglycones of saikosaponins are closely related oxygenated pentacyclic triterpenoidal structures (oleanane type) that can be divided into six types\(^3\), as shown in Figure 1 (in some articles,\(^14,15\) there are seven types of saikosaponins, type II was divided into two types). Among these saikosaponins, saikosaponin a (SSa), saikosaponin d (SSd), saikosaponin c (SSc), saikosaponin b\(_1\) (SSb\(_1\)) and saikosaponin b\(_2\) (SSb\(_2\)) are the major saponins in *R. Bupleuri* and are believed to be responsible for the most pharmacological actitivities.\(^6,15-18\) Saikosaponin a, SSd and SSc are epoxy-ether
saikosaponins (type I), while SSb₁ and SSb₂, with a different aglycone, are heterocyclic diene saikosaponins (type II).\textsuperscript{15} Saikosaponin a and SSD can be converted into SSb₁ and SSb₂, respectively, by a mild acid treatment or heating.\textsuperscript{19,20} There is about 0.05% SSb₂ in \textit{B. Chinense} DC., while the content is higher in vinegar baked \textit{B. Chinense} DC.\textsuperscript{21} Only the SSa and SSD contents are used for the quality evaluation of \textit{Bupleuri} by a high performance liquid chromatography (HPLC) method in the current Chinese Pharmacopoeia. Ideally, a method that could determine the content of all saikosaponins would be preferable for the quality estimation of \textit{R. Bupleuri}. Although many articles have reported HPLC methods for the simultaneous determination of many saikosaponins,\textsuperscript{3,21,22} all of these methods are time consuming and use expensive standard compounds.

In general, HPLC, capillary electrophoresis (CE) and thin layer chromatography (TLC) are considered to be the traditional quantitative techniques. Nuclear magnetic resonance (NMR) is the essential qualitative technique for the determination of chemical structure\textsuperscript{23}. Because the intensity of the $^1$H NMR signal is directly proportional to the number of protons that give rise to the signal, the molar proportion of different compounds with an unambiguous structure can be obtained. Quantification is achieved with the help of an appropriate internal standard.\textsuperscript{24} Quantitative $^1$H NMR (qNMR) has various other advantages. One of the main advantages is that no calibration curve is needed because the signal intensity is absolutely proportional to the molar concentration of the chemicals in a $^1$H NMR spectrum; therefore, specific pure reference standards are not necessary.\textsuperscript{25} In addition, the qNMR method is nondestructive.\textsuperscript{26} Moreover, sample preparation and handling are simple and easy because either organic or inorganic impurities will not affect the purity measurement of the analyte if they do not possess any resonance.\textsuperscript{27,28} Therefore, in the past decade,
qNMR had been widely used for the quantitative analysis of the active ingredients in complex plant extracts, such as the quantitative determination of praeruptorin A and B in *Peucedani Radix*, catechins in green tea, essential oils in *Ocimum* and artemisinin in *Artemisia annua*. To the best of our knowledge, no literature is available regarding the use of $^1$H NMR to quantify the saikosaponins in *B. chinense* DC. Therefore, the aims of this study were as follows: (1) to develop a facile and effective quantitative $^1$H NMR method for the quality control of *B. chinense* DC. and *B. scorzonerifolium* Willd.; and (2) to determine a rapid and accurate analytical method for the quantitative determination of saikosaponins.

**Experimental**

*Materials and Chemicals*

The dried roots of *B. chinense* DC. were harvested from Gansu and Shanxi provinces. Two samples were authenticated by Zhiming Gao (a professor at Henan Agricultural University, Zhengzhou, Henan, China), and voucher specimens were deposited in High & New Technology Research Center of Henan Academy of Sciences.

Saikosaponin a (Lot: C10104122, 98%) was purchased from Shanghai Macklin BioChemical Co. Ltd. and the National Institutes for Food and Drug Control (ID: CL91-H6ZN, 91.1%). Saikosaponin c (Lot: H1419038, 98.0%), Saikosaponin d (Lot: G1211012, 98.0 %) and 2,3,5-triiodobenzoic acid (Lot: D1725025, 98.0 %) were obtained from Aladdin Industrial Corporation (Shanghai, China). Saikosaponin b$_1$, b$_2$, b$_4$ and g were purchased from Chengdu Must Biotechnology Co. Ltd., Chengdu, China, with a purity of 98.0%.

Methanol-$d_4$ (CD$_3$OD, 99.8%, Cambridge Isotope Laboratories, Andover, MA, USA)
and 5mm NMR tubes (Norell, Landisville, NJ, USA) were purchased from Qingdao Tenglong Weibo Technology Co. Ltd. (Qingdao, China). For HPLC analysis, HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q System (Darmstadt, Germany). Analytical grade ammonium hydroxide and methanol were purchased from Tianjin Kemiou Chemical Reagent Co. Ltd. (Tianjin, China).

**Preparation of internal standard solutions and samples for NMR spectroscopic analysis**

A quantity of 2,3,5-triiodobenzoic acid (20.3 and 21.4 mg) was dissolved in methanol-$d_4$ (2.0 mL) to produce internal standard solutions with concentrations of 10.15 and 10.70 mg/mL, respectively. The standard solutions were prepared before use and stored in a refrigerator.

*B. chinense* DC. Samples were pulverized using an electric grinder, and then passed through a 60 mesh sieve. The pulverized crude materials (about 50 mg) were extracted with methanol-$d_4$ (0.4 mL × 3) using a vortex mixer for 2 min. The extracting solution was then centrifuged at 10,000 × g for 5 min, and the supernatant was transferred into a 5 mm NMR tube. Before the NMR analysis, 80 µL of internal standard solution was added to each NMR tube.

**Proton nuclear magnetic resonance analysis and data processing**

All $^1$H NMR spectra were recorded on a 400 MR 400MHz NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA) operating at a proton NMR frequency of 399.79 MHz. A presaturation sequence was applied to suppress the residual water signal (irradiation position: $\delta_H$ 4.87, width: 10 Hz). The spectra were measured without sample spinning at a temperature of 298K. Methanol-$d_4$ was used as the internal lock. The following parameters were used in all $^1$H NMR experiments: 32 scans of 64K data points were acquired, with a spectral width of 3592 Hz (9 ppm), an acquisition time of
2.28s, and a relaxation delay of 30 s to ensure the relaxation of all signals. The exponential window function was selected and the line broadening (LB) was set to 0.3 Hz. Phase and baseline corrections were performed manually prior to signal integration using MestReNova software (version 10.0.1, Mestrelabs Research SL, Santiago de Compostela, Spain). For a quantitative analysis, the standard function (sum) of MestRenova to calculate the peak area. The peak area was determined and the integration limits of each peak were selected manually. For internal standard, the integration width is 0.034 ppm (δ_H 8.13). For the extraction of *Bupleurum chinense* DC., the integration width is 0.053 ppm for H-24 (δ_H 0.71) and 0.076 ppm for H-11 (δ_H 5.95). The chemical shift was referenced to the solvent signal of methanol-d_4. In addition, spin-lattice relaxation time (T_1) values of the quantified protons of the analytes and the internal standard were measured using a standard inversion-recovery pulse sequence.

**Quantification of saikosaponins by the \(^1\text{H} \text{NMR method}\)**

Because the area of a signal appearing on a \(^1\text{H} \text{NMR}\) spectrum is directly proportional to the number of resonant nuclei evoking the signal, the quantification of saikosaponins could be performed using the following equation:27,33

$$\text{Mass percent (mg/mg)} = \frac{Ax}{A_{IS}} \times \frac{N_{IS}}{N_x} \times \frac{M_x}{M_{IS}} \times \frac{W_{IS}}{W_x} \times P_{IS}$$  \hspace{1cm} (1)

where Ax and A_{IS} represent the integral areas of the analyte and internal standard (IS), respectively; N_{IS} and N_x correspond to the number of spinning protons of the internal standard and the analyte, respectively; M_x and M_{IS} are the molecular masses of the analyte and IS, respectively; W_{IS} is the weighed mass of IS; W_x is the weighed mass of the analyte; and P_{IS} is the purity of the internal standard.

**Preparation of samples for HPLC-diode-array detector (DAD) analysis**

The sample used for HPLC-DAD detection was prepared according to the method
described in the Chinese Pharmacopoeia.  

Quantification of saikosaponins by the HPLC-DAD method

The HPLC analysis was conducted according to the method described in the Chinese Pharmacopoeia.  
Chromatographic separation of SSa, SSc and SSd was performed on a Shimadzu LC-20A HPLC system (Shimadzu, Kyoto, Japan), which consisted of a model LC 20AT pump and a model SPD-20 AU UV detector. The samples were separated on an Inertsil ODS-3 column (250 × 4.6 mm, 5μm) at a column temperature of 30°C and flow rate of 1.0 mL min⁻¹. Chromatographic separation of SSb₁ and SSb₂ was performed on an ALLIANCE e2695 HPLC system (Waters, Singapore), which included a model 2489 UV/Vis detector. The samples were separated on an XTERRA-RP18 column (250 × 4.6 mm, 5 μm) at a column temperature of 30°C and flow rate of 1.0 mL min⁻¹. The mobile phase was acetonitrile (A) and water (B), with a gradient elution program of 25–90% A at 0–50 min, and 90% A at 50–55 min. The detection wavelength was set at 210 nm for SSa, SSc and SSd, and 254 nm for SSb₁ and SSb₂. The saikosaponin concentrations in each sample were determined using the calibration curves established from the standard compounds. Each sample was analysed in triplicate and the results were averaged. 

Results and Discussion

Selection of signals for the quantification of saikosaponins

A representative ¹H NMR spectrum of the extraction of B. Chinense DC., together with the internal standard is shown in Figure 2. Using the ¹H NMR spectrum of pure SSa, SSb₁, SSb₂, SSb₄, SSc, SSd and SSg, the protons at δ₁ 0.71 (H-24 signals of SSa, SSb₁, SSb₂ and SSd, Figure S4) and δ 5.95 (H-11 signals of SSa, SSc and SSd) were
selected for the quantification of saikosaponins. The H-24 signals at $\delta_H$ 0.71 were not suitable for quantification because they were overlapped. Fortunately, SSA, SSB1, SSB2 and SSD have the same molecular weight, and therefore the total contents of SSA, SSB1, SSB2 and SSD could be calculated by the H-24 signals. The H-11 proton of SSA, SSC and SSD were highly overlapped at $\delta_H$ 5.95 and appeared as a doublet peak. This result indicated that the signals of the H-11 proton of all type I structure saikosaponins will overlap. The $^1$H and $^{13}$C NMR data of SSA, SSB1, SSB2, SSB4, SSc, SSD and SSg in methanol-$d_4$ are listed in Tables S17 and S18. In addition, 2,3,5-triiodobenzoic acid was chosen as an internal standard for the $^1$H NMR quantitative analysis because it is not present in B. chinense DC., and is a conveniently weighable solid, with good solubility and stability in methanol-$d_4$. It also has the same approximate molecular weight as saikosaponin. Most importantly, compared with saikosaponins, the signals of 2,3,5-triiodobenzoic acid were in a lower field ($\delta_H$ 8.13), which was well separated from any other signals, and thus eliminated any interference.

_Determination of the relaxation time_

The value of the relaxation delay is very important in quantitative $^1$H NMR. It is necessary to wait at least five times the longest $T_1$ in the sample between scans in order to recover 99% of the equilibrium magnetization. The relaxation time $T_1$ was determined experimentally by an inversion recovery experiment for all the monitored protons of the targeted compounds and internal standard (Table 1). The longest relaxation time was 5.089 s for the proton at $\delta_H$ 8.13 (H-6) of 2,3,5-triiodobenzoic acid (internal standard). Based on the $T_1$ data, the relaxation delay was set as 30s to ensure an accurate quantification.

_Confirmation of the extraction method_

To extract all the saikosaponins in the B. chinense DC., the extraction conditions were
confirmed as follows. The crude materials were treated four times by the method described in section 2.4, and each time the supernatant was transferred into a 3mm NMR tube for $^1$H NMR test. There were some saikosaponins ($\delta_1$ 5.95, H-11 as the marker) present in the second supernatant (monitored by $^1$H NMR), while for the third treatment there was only a slight bulge above the baseline at 5.95 ppm. The fourth treatment had nothing above the baseline at 5.95 ppm (Figure S5). The pulverized crude materials were extracted three times and the supernatant was combined for a quantitative $^1$H NMR test. Furthermore, DMSO-$d_6$ and Pyridine-$d_5$, were also used as the extraction solvents, it showed clearly that they were not suitable for quantification (Figure S6).

Method validation

Selectivity: In a method with good selectivity, even when other components are present the analytes of interest can be measured unambiguously. For qNMR, this means that the signals used for integration can be clearly assigned. In this study, selectivity was assessed by a visual comparison between the $^1$H NMR spectra of the extraction of B. chinense DC. with and without an internal standard. Additionally, the $^1$H-$^1$H correlated spectroscopy (COSY) spectra (Figure S1) were used to confirm the specificity of the qNMR method. No proton had a cross-peak with H-24 in the $^1$H-$^1$H COSY spectrum, which means that no fatty acids and amino acids were obscured under H-24. The 2,3,5-triiodobenzoic acid (H-6) and saikosaponins (H-11 and H-24) signals were not overlapped with any other signals in the $^1$H-$^1$H COSY, which indicated a good selectivity for the qNMR method.

Linearity: To determine the linearity of the qNMR method, five SSa solutions in the range of 0.16–3.19 mg/mL were prepared and analysed in triplicate (Table S1, S2). The calibration curve was constructed by plotting the ratio (Y) between the peak areas of
SSa and the internal standard against the SSa concentration in mg/mL (X). The linear regression equations (correlation coefficient) were Y=0.4547X+0.0016 (R²=0.9999) for H-11 and Y=1.3755X+0.0024 (R²=0.9999) for H-24, indicating a good linearity for the qNMR method within the test ranges. The limit of quantitation (LOQ) was set to 0.16 mg/mL (0.1 mg SSa in 0.6 mL methanol-d₄) for 32 scans, where the S/N ratios were 128 (for H-24) and 13 (for H-11),³¹,³⁴ and the relative standard deviation (RSD) values were 0.574% (for H-24) and 2.84% (for H-11). The limit of detection (LOD) was set to 0.037 mg/mL (the S/N ratio was 3 for H-11).

Repeatability: The method repeatability was evaluated by analysing six independently prepared sample solutions. The RSD value of the total SSa, SSb₁, SSb₂ and SSd concentrations was 2.78% (Table S3).

Accuracy: Accuracy expresses the closeness of agreement between a measured value and its true value. In general, recovery tests are performed to determine the accuracy of quantitative methods. Three different SSa concentrations (low-, medium- and high-level solutions) were spiked into a known amount of sample that had previously been analysed (Table S4, S5). The spiked samples were then extracted, processed and quantified in accordance with the established methods mentioned above (H-24). The results are shown in Table 2. The average SSa recoveries were 99.8% and 92.5%, with RSD values less than 3% for two test compounds.

Precision and stability: The precision of the qNMR method was evaluated by six replicate assays of the same sample solution at 8 h intervals. The RSD value of the total SSa, SSb₁, SSb₂ and SSd concentration was 0.43%. These results proved that the samples remained stable within 48 h (Table S6).

Analysis results for the qNMR and HPLC-DAD methods

To verify the veracity of the qNMR method, a HPLC method was applied to
determine the SSa, SSb1, SSb2 and SSd concentrations in the *B. chinense* DC. (Table S11–S16). The results of the HPLC analysis are summarized in Table 3. The total SSa, SSb1, SSb2 and SSd concentrations were consistent with those obtained by qNMR (Table S7, S8), thereby cross-validating the two analytical approaches. The total SSa, SSb1, SSb2 and SSd concentrations obtained by qNMR were slightly higher than those obtained by HPLC, possibly because of the different sample preparation methods used in the two methods.

**Total amount of type I saikosaponins**

From the $^1$H NMR spectra of saikosaponins and the extraction of *B. chinense* DC. (Figure 2), it was found that the H-11 signals of SSa, SSc and SSd were overlapped, although they had different $R_1$, $R_2$ and $R_3$ substituted groups. There were 14 type I saikosaponins identified (Figure S7), and it could be inferred from their structure that the H-11 signals had the same chemical shift in $^1$H NMR. The molality (mol/kg) of type I saikosaponins could therefore be easily calculated (Table 4) by Equation 2 (simply by erasing the Mx item of Equation 1) from the area of the H-11 signal (Table S9, S10).

$$\text{Molality (mol/kg)} = \frac{A_x}{A_{IS}} \times \frac{N_{IS}}{N_x} \times \frac{1}{M_{IS}} \times \frac{W_{IS}}{W_x} \times P_{IS}$$

(2)

The molalities of total type I saikosaponins were $37.09 \pm 0.27$ mmol/kg for Gansu Province and $20.17 \pm 0.38$ mmol/kg for Shanxi Province, respectively. This result was useful for the quality control of *B. chinense* DC. because it expressed the concentrations of all type I saikosaponins in *B. chinense* DC.

**Conclusions**

A quantitative $^1$H NMR method with good linearity, accuracy, repeatability and
precision was successfully developed for the quality control of *B. chinense* DC. A conventional HPLC-DAD analysis was also conducted for comparative purposes based on the analytical method described in the Chinese Pharmacopoeia. The results showed that, compared with the HPLC method, the established qNMR method had several advantages. For example, the method was rapid, requiring about 20 min per sample, which was only half the analysis time of HPLC (about 50 min). The qNMR method also provided accurate mass percentages (mg/g) of SSa, SSb₁, SSb₂ and SSD, especially the molality of total type I saikosaponins (mmol/kg) in one spectrum. No expensive standard compounds were required to prepare the calibration curve for the quantitative determination of the saikosaponins in this study due to the reasons mentioned above. This is a major advantage, especially in situations where it is difficult to obtain pure standard compounds. These results indicate that this method has the potential to be a reliable method for the quality evaluation of *B. chinense* DC., and it is also useful for the quality evaluation of saikosaponins in *B. scorzonerifolium* Willd., vinegar baked *B. Chinense* DC. and *B. scorzonerifolium* Willd., Chaihu Koufuye (oral liquid of Chaihu). We will try to quantitate saikosaponins in traditional Chinese medicine formulas with *B. Chinense* DC. (such as Chaihu Shugan pill, Chaihuang tablet, Xiaochaihu capsule, and so on) for quality evaluation in the future.

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Supporting Information includes Figure S1-S6 (NMR spectra of the extraction of B. chinense DC.), Figure S7 (Chemical structures of type I saikosaponins), Figure S8-S10 (HPLC for the determination of SSa, SSc, SSD, SSb1 and SSb2 in B. chinense DC. from Gansu Province), Table S1-S6 (Data of the 1H NMR validation method: linearity, repeatability, accuracy, stability of analyte and internal standard in methanol-d4), Table S7-S10 (Data of the quantification of saikosaponins in B. chinense DC. samples by 1H NMR), Table S11-S12 (Data of the standard curve (linearity) of HPLC method), Table S13 - S16 (Data of the quantification of saikosaponins in B. chinense DC. samples by HPLC method), Table S17-S18 (1H and 13C NMR data of saikosaponins). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
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Table 1. The $T_1$ values of the monitored protons

| Substance                  | Proton     | $T_1$/s     |
|----------------------------|------------|-------------|
| 2,3,5-triiodobenzoic acid  | H-4, H-6   | 4.688, 5.089|
| SSa                        | H-11, H-24 | 0.479, 0.484|
| SSc                        | H-11       | 0.516       |
| SSD                        | H-11, H-24 | 0.494, 0.488|

Table 2. Recovery of two test samples

| Origin | Original /mg | Spiked /mg | Calculated /mg | Recovery $^a$, % | Average recovery mean±SD, % | RSD, % |
|--------|---------------|------------|----------------|------------------|-----------------------------|--------|
| Gansu  | 1.355         | 1.196      | 2.511          | 96.7             |                             |        |
|        | 1.406         | 0.478      | 1.888          | 100.8            | 99.8±2.7                    | 2.71   |
|        | 1.355         | 0.167      | 1.525          | 101.8            |                             |        |
|        | 0.684         | 0.478      | 1.118          | 90.8             |                             |        |
| Shanxi | 0.698         | 0.239      | 0.917          | 91.6             | 92.5±2.3                    | 2.49   |
|        | 0.692         | 0.143      | 0.828          | 95.1             |                             |        |

$^a$ Recovery (%) = [(calculated – original)/spiked] × 100.

Table 3. Total SSa, SSb₁, SSb₂ and SSD concentrations (mg/g) in *B. chinense* DC. samples from Gansu and Shanxi provinces as determined by qNMR and HPLC

| Origin | Method | SSa         | SSb₁        | SSb₂         | SSD         | sum         |
|--------|--------|-------------|-------------|-------------|-------------|-------------|
| Gansu  | qNMR   | 27.166±0.090|             |             |             |             |
|        | HPLC   | 12.074±0.248| 0.0352±0.0032| 0.0447±0.0006| 14.289±0.311| 26.443      |
| Shanxi | qNMR   |             |             |             |             | 13.868±0.189|
|        | HPLC   | 5.231±0.244 | 0.0598±0.0007| 0.3414±0.0071| 7.512±0.311 | 13.144      |
Table 4. Total concentrations (mmol/kg) of type I saikosaponins and SSa, SSc, SSd in *B. chinense* DC. samples from Gansu and Shanxi provinces as determined by qNMR and HPLC

| Origin | Method | SSa    | SSc    | SSd    | sum     | Type I SS |
|--------|--------|--------|--------|--------|---------|-----------|
| Gansu  | qNMR   |        |        |        | 37.09±0.27 |
|        | HPLC   | 15.46±0.32 | 1.44±0.02 | 18.30±0.40 | 35.20   |
| Shanxi | qNMR   |        |        |        | 20.17±0.38 |
|        | HPLC   | 6.70±0.31 | 1.77±0.10 | 9.67±0.40  | 18.14   |
Figure Captions

Fig. 1 The chemical structures of six saikosaponins.

Fig. 2 The $^1$H NMR spectrum of saikosaponins and extraction of *B. chinense* DC. with an internal standard.
Fig. 1 The chemical structures of six saikosaponins.

Fig. 2 The $^1$H NMR spectrum of saikosaponins and extraction of *B. chinense* DC. with an internal standard.
Graphical Index

Bupleurum Chinense DC.

Quality control of Bupleurum Chinense DC.