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# Simultaneous determination of nucleobases, nucleosides and saponins in *Panax notoginseng* using multiple columns high performance liquid chromatography

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ABSTRACT

A new multiple columns HPLC method for simultaneous determination of 16 characteristic components, 5 nucleobases and nucleosides (uracil, cytidine, uridine, guanosine and adenosine), and 11 saponins (notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, notoginsenoside R4, notoginsenoside Fa, ginsenoside Rb1, notoginsenoside R2, ginsenoside Rg2, ginsenoside Rh1, ginsenoside Rd and notoginsenoside K), in the root of *Panax notoginseng*, a valued traditional Chinese medicinal herb, were developed. Notoginsenoside R4, Fa and K were first quantitatively determined in *P. notoginseng*. The 5 nucleobases and nucleosides compounds were separated on a Zorbax SB-Aq column (150 × 4.6 mm, 5.0 μm) and 11 saponins were analyzed using a Zorbax Bonus-RP column (150 × 4.6 mm, 5.0 μm) with column switching. The column temperature was set at 30 °C. Mobile phase was composed of 5 mM ammonium acetate aqueous (A), water (B) and acetonitrile (C) using a gradient elution. The flow rate was 1.5 mL/min and detection wavelengths were set at 260 nm for nucleobases and nucleosides, and 203 nm for saponins. The developed method had good repeatability and sensitivity for quantification of 16 analytes with overall precision (including intra- and inter-day) less than 3% (RSD), and LOD and LOQ were less than 1.33 μg/mL and 5.12 μg/mL, respectively. The method was successfully applied to the simultaneous determination of 16 analytes in 15 samples of *P. notoginseng* collected from different places of China, which indicated that multiple columns HPLC can be used for comprehensive quality control of *P. notoginseng*.



## 1. Introduction

*Panax notoginseng*, called Sanqi or Tianqi in Chinese, is one of the valued traditional Chinese medicines (TCMs) [1]. It is well known for its efficacy in promoting blood circulation, removing blood stasis, inducing blood clotting, relieving swelling, and alleviating pain [2–4]. Modern pharmacological studies have demonstrated that *P. notoginseng* possesses wide pharmacological activities such as preventive and therapeutic effects on cardiovascular and cerebrovascular diseases [4,5]. Generally, saponins are considered as the major bioactive compounds in *P. notoginseng* [6–8]. Several methods, including high-performance liquid chromatography [9–12] and micellar electrokinetic chromatography [13], have been reported for quality control of *P. notoginseng* based on the analysis of saponins. However, the therapeutic effects of TCMs are usually attributed to multiple bioactive components. Recently, nucleobases and nucleosides have been proven as important bioactive compounds related to multiple activities such as anti-platelet aggregation, anti-arrhythmic and anti-seizure effects [14–17], and have also been selected as the quality control marker of several TCMs, such as *Ganoderma lucidumn* and *Cordyceps sinensis* [18,19]. Our previous study showed that the nucleosides were important anti-platelet aggregation agents in *P. notoginseng* [17]. Therefore, simultaneous determination of nucleobases, nucleosides and saponins is beneficial for comprehensive quality evaluation of *P. notoginseng*, which has no report based on our knowledge.

Actually, TCM usually contains a myriad of bioactive compounds with different polarity, which makes a challenge for HPLC separation. It is difficult to separate these compounds using a single column in HPLC analysis. Fortunately, column switching technique provides a resolve method, which allows separation of complex mixtures on different columns, and has been successfully applied in the analysis of complex mixtures such as environmental water, plasma and plant extracts recently [20–23]. However, these studies mainly focused on sample on-line enrichment or clean-up [24,25], and few was used for multiple columns HPLC analysis [26].

In the present study, a multiple columns HPLC method using column switching technique was developed for simultaneous quantification of 16 compounds, including 5 nucleobases and nucleosides (uracil, cytidine, uridine, guanosine and adenosine)

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and 11 saponins (notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, notoginsenoside R4, notoginsenoside Fa, ginsenoside Rb1, notoginsenoside R2, ginsenoside Rg2, ginsenoside Rh1, ginsenoside Rd and notoginsenoside K) in 15 samples of *P. notoginseng* collected from different places in China.

## 2. Experimental

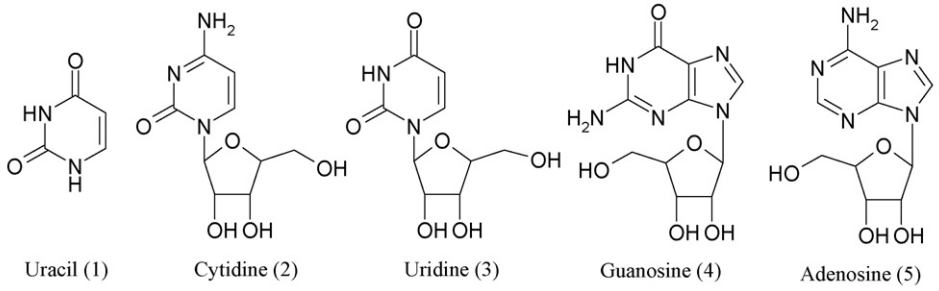
### 2.1. Chemicals, reagents and materials

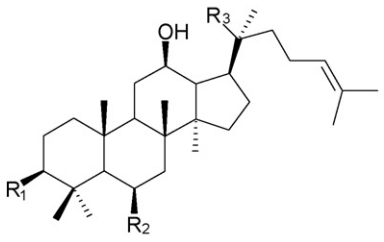
Fifteen samples (S1–S15) of *P. notoginseng*, i.e. S1–S10, S11–S13, S14 and S15, were collected from Wenshan, Maguan, Mengzi and Xichou of Yunnan Province, China, respectively. The botanical origin of materials was identified by Dr. Cui Xiuming, Wenshan Prefecture Sanqi Research Institute (Yunnan Province, China). The voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

Uracil (1), cytidine (2), uridine (3), guanosine (4) and adenosine (5) were purchased from Sigma (St. Louis, MO, USA). Notoginsenoside R1 (6), ginsenoside Rg1 (7), Re (8), Rb1 (11) and Rg2 (13) were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ginsenosides Rf (17), Rh1 (14) and Rd (15) were purchased from Chromadex (Santa Anna, CA, USA). Ginsenosides Rc (18) were obtained from International Laboratory (San Bruno, CA, USA). Notoginsenoside R4 (9), Fa (10), R2 (12) and K (16) were isolated in our lab, and the purity of each compound was more than 98% determined by HPLC. The chemical structures of these reference compounds were shown in Fig. 1. Acetonitrile was HPLC-grade from Merck (Darmstadt, Germany). Ammonium acetate was purchased from Fluka (Buchs, France) and deionized water was purified by a Milli-Q purification system (Millipore, Bedford, MA, USA).

### 2.2. Sample preparation

Sample preparation was performed using an ultrasonic cleaning bath (Branson Ultrasonics Corporation, Danbury, USA). In brief, the dried samples were ground using Knifetec™ 1095 Sample Mill




| NO. | Saponins | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| 6 | Notoginsenoside R1 | OH | Oglc(2-1)xyl | Oglc |
| 7 | Ginsenoside Rg1 | OH | Oglc | Oglc |
| 8 | Ginsenoside Re | OH | Oglc(2-1)rha | Oglc |
| 9 | Notoginsenoside R4 | Oglc(2-1)glc | H | Oglc(6-1)glc(6-1)xyl |
| 10 | Notoginsenoside Fa | Oglc(2-1)glc(2-1)xyl | H | Oglc(6-1)glc |
| 11 | Ginsenoside Rb1 | Oglc(2-1)glc | H | Oglc(6-1)glc |
| 12 | Notoinsenoside R2 | OH | Oglc(2-1)xyl | OH |
| 13 | Ginsenoside Rg2 | OH | Oglc(2-1)rha | OH |
| 14 | Ginsenoside Rh1 | OH | Oglc | OH |
| 15 | Ginsenoside Rd | Oglc(2-1)glc | H | Oglc |
| 16 | Notoginsenoside K | Oglc(6-1)glc | H | Oglc |
| 17 | Ginsenoside Rf | OH | Oglc(2-1)glc | OH |
| 18 | Ginsenoside Rc | Oglc(2-1)glc | H | Oglc(6-1)araf |

**Fig. 1.** Chemical structures of the investigated compounds in *Panax notoginseng*. glc, β-D-glucose; rha, α-L-rhamnose; araf, α-L-arabinose (furanose); xyl, β-D-xylose.

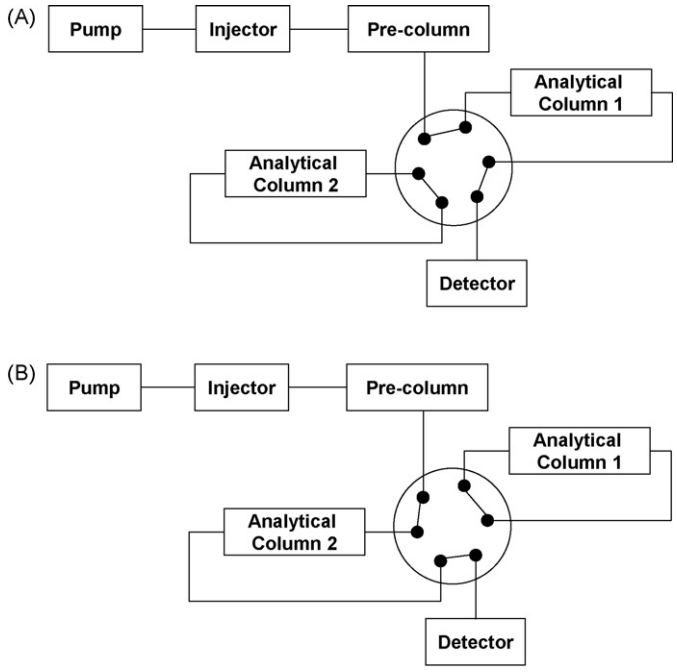



**Fig. 2.** The schematic diagrams of the multiple columns HPLC system for simultaneous determination of nucleobases, nucleosides and saponins in *Panax notoginseng*. (A) The system consists of pre-column (Zorbax SB-Aq C18, 12.5 mm × 4.6 mm i.d., 5 μm) and column 1 (Zorbax SB-Aq C18, 150 mm × 4.6 mm i.d., 5 μm) for analysis of nucleobases and nucleosides. (B) The system included the pre-column and column 2 (Zorbax Bonus-RP, 150 mm × 4.6 mm i.d., 5 μm) was used for the analysis of saponins.

(Foss Tector, Sweden). One gram of ground powder (0.30–0.45 mm) was transferred into a 50 mL conical flask with stopper, and 20 mL water was added. After accurately weighing, ultrasonication (44 KHz, 250 W) was performed at room temperature for 25 min, and then same solvent was added to compensate for the lost weight during the extraction. After centrifugation (2300 × *g*, 10 min), the supernatant stored at 4 °C and filtered through a 0.45 μm filter (Agilent Technologies, USA) before injected into the HPLC system for analysis.

### 2.3. Apparatus

Agilent 1100 series HPLC apparatus (Palo Alto, CA, USA), equipped with vacuum degasser, quaternary gradient pump and autosampler, was used. Fig. 2 shows the schematic diagram of the multiple columns HPLC system. After pre-column (Zorbax SB-Aq C18 column, 12.5 mm × 4.6 mm i.d., 5 μm), a six-port, two-position valve was used for column switching between the two analysis columns. First, 0–13 min, the valve was set at position A (Fig. 2A), mobile phase eluted through pre-column and analytical column 1 (Zorbax SB-Aq C18 column, 150 mm × 4.6 mm i.d., 5 μm) for the analysis of nucleobases and nucleosides. Then, 13–60 min, the valve was turned to position B (Fig. 2B), which allowed mobile phase flow through pre-column and analytical column 2 (Zorbax Bonus-RP column, 150 mm × 4.6 mm i.d., 5 μm) for saponins analysis. The columns temperature was maintained at 30 °C. The detection wavelengths were set at 260 nm during 0–13 min for analysis of nucleobases and nucleosides, and 203 nm between 13–60 min for the analysis of saponins. The mobile phases were 5 mM/L ammonium acetate aqueous (A), water (B) and acetonitrile (C), and the separation was achieved using gradient program (Table 1). The flow-rate was at 1.5 mL/min and sample injection volume was 15 μL.

HPLC–MS was used to confirm the peaks in the profiles obtained by UV detection. HPLC–MS analysis was performed on an Agilent 1100 Series LC/MSD Trap system (Palo Alto, CA, USA) equipped with an ESI interface. The same chromatographic conditions described above were used, but solvent B was 5 mM/L ammonium acetate aqueous instead of water. MS analysis of the nucleobases and nucleosides were monitored in positive mode and saponins were in negative mode. The mass range was set at 100–1500 U. The conditions of ESI source were as follows: drying gas ($N_2$) flow rate, 8.5 L/min; drying gas temperature, 350 °C; nebulizer, 35 psi. ESI–MS/MS conditions: isolation width, 4; fragment amplification, 1.5.

### 2.4. Calibration curves

Aqueous stock solutions containing 16 analytes were prepared and diluted to appropriate concentration for construction of calibration curves. At least 6 concentration of the 16 analytes' solution were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the concentration of each analyte.

### 2.5. Limits of detection and quantification

The lowest concentration of working solution was diluted with water to a series of appropriate concentrations, and aliquots of diluted solutions were injected into HPLC for analysis. The limits of detection (LOD) and quantification (LOQ) for each analyte under

**Table 1**
The gradient program of multiple column HPLC for simultaneous determination of nucleobases, nucleosides and saponins in *Panax notoginseng*.

| Time (min) | Column | Mobile phases (%) | | | Detection (nm) |
|---|---|---|---|---|---|
| | | 5 mM $NH_4Ac$ (A) | Water (B) | Acetonitrile (C) | |
| 0 | Pre-column + colum 1 | 100 | 0 | 0 | 260 |
| 4 | Pre-column + colum 1 | 100 | 0 | 0 | 260 |
| 5 | Pre-column + colum 1 | 80 | 18 | 2 | 260 |
| 7 | Pre-column + colum 1 | 40 | 53 | 7 | 260 |
| 10 | Pre-column + colum 1 | 0 | 87 | 13 | 260 |
| 13 | Pre-column + colum 1 | 0 | 87 | 13 | 260 |
| 14 | Pre-column + colum 2 | 0 | 83 | 17 | 203 |
| 30 | Pre-column + colum 2 | 0 | 82 | 18 | 203 |
| 35 | Pre-column + colum 2 | 0 | 80 | 20 | 203 |
| 45 | Pre-column + colum 2 | 0 | 65 | 35 | 203 |
| 50 | Pre-column + colum 2 | 0 | 60 | 40 | 203 |
| 60 | Pre-column + colum 2 | 0 | 0 | 100 | 203 |

Pre-column, Zorbax SB-Aq C18 (12.5 mm × 4.6 mm i.d., 5 μm); Column 1: Zorbax SB-Aq C18 (150 mm × 4.6 mm i.d., 5 μm); Column 2: Zorbax Bonus-RP (150 mm × 4.6 mm i.d., 5 μm).

**Table 2**
Regression date, LODs and LOQs of the 16 analytes.

| Analytes | Regressive equation | $r^2$ | Test range (μg/mL) | LOQ (μg/mL) | LOD (μg/mL) |
|---|---|---|---|---|---|
| Uracil | $y = 25.81x - 0.42$ | 1.0000 | 0.40–16.00 | 0.16 | 0.04 |
| Cytidine | $y = 10.40x - 0.46$ | 0.9996 | 0.30–12.00 | 0.32 | 0.08 |
| Uridine | $y = 15.42x - 0.53$ | 0.9999 | 0.40–16.00 | 0.24 | 0.06 |
| Guanosine | $y = 13.86x - 0.29$ | 1.0000 | 1.00–20.00 | 0.32 | 0.08 |
| Adenosine | $y = 18.23 + 0.66$ | 1.0000 | 1.00–20.00 | 0.24 | 0.06 |
| Notoginsenoside R1 | $y = 1.80x - 6.73$ | 0.9999 | 30.00–600.00 | 2.32 | 0.58 |
| Ginsenoside Rg1 | $y = 1.48x - 2.51$ | 0.9997 | 110.00–2200.00 | 2.14 | 0.54 |
| Ginsenoside Re | $y = 1.48x + 9.45$ | 0.9995 | 20.00–400.00 | 1.86 | 0.47 |
| Notoginsenoside R4 | $y = 1.29x + 1.22$ | 0.9991 | 18.00–360.00 | 2.59 | 0.65 |
| Notoginsenoside Fa | $y = 1.43x - 2.03$ | 1.0000 | 20.00–400.00 | 2.46 | 0.65 |
| Ginsenoside Rb1 | $y = 1.55x - 1.73$ | 0.9999 | 90.00–1800.00 | 2.69 | 0.67 |
| Notoginsenoside R2 | $y = 2.53x + 7.13$ | 0.9986 | 10.00–200.00 | 1.42 | 0.36 |
| Ginsenoside Rg2 | $y = 2.34x - 4.25$ | 0.9999 | 10.00–200.00 | 2.36 | 0.59 |
| Ginsenoside Rh1 | $y = 2.01x + 0.25$ | 0.9998 | 10.00–200.00 | 2.16 | 0.54 |
| Ginsenoside Rd | $y = 2.08x + 0.02$ | 0.9998 | 16.00–320.00 | 2.15 | 0.54 |
| Notoginsenoside K | $y = 0.64x - 1.05$ | 0.9997 | 8.00–160.00 | 5.12 | 1.33 |

the chromatographic conditions were determined at the signal-to-noise ratio (S/N) of 3 and 10, respectively.

### 2.6. Precision, repeatability, accuracy and stability

The measurement of intra- and inter-day variability was utilized to determine the precision of the developed method. The intra-day precision was examined on the mixed standards for six times within one day, and inter-day precision was determined in duplicates for consecutive three days. The relative standard deviation (RSD) was taken as a measure of precision.

The repeatability of the developed method was evaluated at three levels (0.8 g, 1.0 g and 1.2 g) of the sample S1 which were extracted and analyzed as mentioned above triplicates. The repeatability present as RSD ($n = 3$).

A recovery test was used to evaluate the accuracy of the developed method. Known amount of the 16 analytes were added to approximate 0.5 g of *P. notoginseng* powder (sample S1), and then extracted and analyzed as described above. Each sample was analyzed in triplicate. The average percentage recoveries were calculated as follow formula: Recovery (%) = 100 × (amount found − original amount)/amount spiked.

Stability of sample solution was tested, The sample solution was analyzed in every 4 h within 24 h. Variation was expressed as RSD.

## 3. Results and discussions

### 3.1. Optimization of multiple columns HPLC conditions

In order to obtain the good separation and short analysis time of the experiment, the multiple columns HPLC conditions were optimized. The nucleobases and nucleosides are the compounds with high polarity, which are easily separated on Zorbax SB-Aq column with high ratio of aqueous mobile phase. Especially, aqueous ammonium acetate can improve the separation of nucleobases and nucleosides [27]. Therefore, 5 mM ammonium acetate aqueous solution was selected as one of the mobile phases for the analysis of nucleobases and nucleosides. However, the gradient elution with aqueous ammonium acetate could induce the baseline drift at 203 nm at which the saponins in *P. notoginseng* were detected because of their poor UV absorbability. Thus water instead of aqueous ammonium acetate was used as the mobile phase in order to decrease the baseline drift during saponin analysis. In addition, several columns, including Zorbax Bonus-RP column (150 mm × 4.6 mm i.d., 5 μm) which was a amide group embedded in a long alkyl chain, Zorbax SB-C18 (150 mm × 4.6 mm i.d., 5 μm), silanes with di-isobutyl side chain group, and Eclipsed XDB-C18 (150 mm × 4.6 mm i.d., 5 μm) of silanes with dimethyl side chain group, were tested for optimization of the saponins analysis. The results showed that the best separation of saponins was achieved on

**Table 3**
Precision, recovery, stability and repeatability of 16 analytes.

| Analytes | Precision (RSD, %, $n = 6$) | | Recovery (%, $n = 3$) | | Stability (RSD, %, $n = 6$) | Repeatability (RSD, %, $n = 3$) | | |
|---|---|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | Mean | RSD (%) | | LL | ML | HL |
| Uracil | 0.83 | 0.68 | 104.44 | 2.50 | 3.09 | 4.16 | 4.51 | 3.53 |
| Cytidine | 0.74 | 1.39 | 93.57 | 3.55 | 3.05 | 4.42 | 4.02 | 4.67 |
| Uridine | 0.83 | 2.41 | 105.97 | 2.63 | 2.97 | 2.42 | 1.84 | 3.58 |
| Guanosine | 0.55 | 0.88 | 99.71 | 2.92 | 3.05 | 3.09 | 1.39 | 1.81 |
| Adenosine | 0.56 | 1.01 | 91.48 | 2.65 | 2.54 | 2.96 | 0.85 | 2.09 |
| Notoginsenosid R1 | 1.13 | 2.81 | 98.99 | 2.28 | 2.06 | 2.65 | 1.59 | 0.70 |
| Ginsenoside Rg1 | 1.34 | 1.65 | 90.77 | 1.58 | 1.01 | 3.69 | 2.27 | 0.66 |
| Ginsenoside Re | 0.90 | 1.32 | 93.58 | 3.11 | 0.62 | 2.20 | 2.45 | 0.97 |
| Notoginsenoside R4 | 0.75 | 2.45 | 97.09 | 2.98 | 2.83 | 4.29 | 2.67 | 3.34 |
| Notoginsenoside Fa | 0.88 | 1.23 | 93.70 | 2.95 | 2.85 | 2.98 | 3.26 | 2.79 |
| Ginsenoside Rb1 | 0.52 | 1.60 | 97.79 | 1.13 | 0.51 | 2.56 | 2.34 | 1.02 |
| Notoginsenoside R2 | 0.53 | 0.94 | 95.24 | 1.68 | 2.66 | 3.57 | 0.80 | 1.93 |
| Ginsenoside Rg2 | 1.02 | 1.31 | 104.06 | 2.69 | 2.85 | 2.42 | 1.25 | 1.16 |
| Ginsenoside Rh1 | 1.41 | 2.35 | 95.63 | 3.18 | 2.77 | 2.55 | 2.78 | 3.25 |
| Ginsenoside Rd | 1.05 | 1.46 | 102.46 | 0.96 | 0.78 | 3.16 | 2.24 | 0.95 |
| Notoginsenoside K | 2.06 | 2.84 | 100.77 | 2.36 | 1.67 | 3.46 | 4.11 | 2.02 |

LL (low lever), ML (middle lever) and HL (high lever), 0.8 g, 1.0 g and 1.2 g of sample S1 extracted with 20 mL water for analysis, respectively.

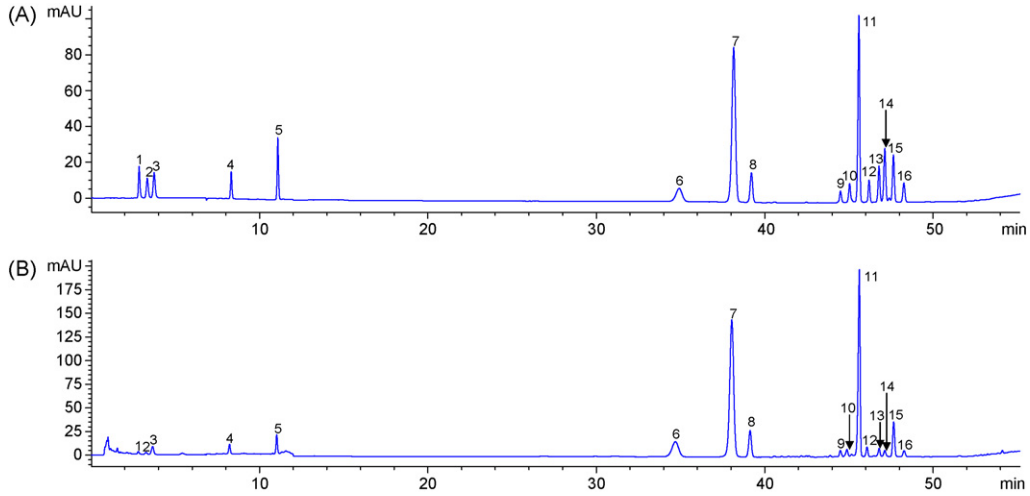



**Fig. 3.** Typical multiple columns HPLC chromatograms of (A) mixed standards and (B) *Panax notoginseng*. Uracil (1), Cytidine (2), Uridine (3), Guanosine (4), Adenosine (5), Ginsenoside Rg1 (7), Re (8), Rb1 (11), Rg2 (13), Rh1 (14) and Rd (15); Notoginsenoside R1 (6), R4 (9), Fa (10), R2 (12) and K (16).

Zorbax Bonus-RP column. Finally, Zorbax SB-Aq and Zorbax Bonus-RP column were chosen for the analysis of nucleosides/nucleobases and saponins, and detected at 260 nm and 203 nm, respectively. The optimized multiple columns HPLC conditions were list in Table 1.

### 3.2. Validation of the method

All calibration curves showed good linear regression ($r^2 > 0.998$) within test ranges, and the overall LODs and LOQs were less than 1.33 μg/mL and 5.12 μg/mL, respectively (Table 2). The intra- and inter-day variations, repeatability and recoveries of all analytes were shown in Table 3. The analytes in sample solution were stable within 24 h (RSD < 4%).

### 3.3. Identification of the analytes in P. notoginseng

Chromatograms of the standards and water extract of *P. notoginseng* were shown in Fig. 3A and B, respectively. The identification of investigated compounds was carried out by comparison of their retention time and their UV spectra (except saponins) with those obtained injecting standards in the same conditions or by spiking *P. notoginseng* samples with stock standard solutions. Actually, TCM is a complex matrix, which contained tens to hundreds of components and some of them had the similar retention time. Therefore, the means mentioned above could not get unambiguous identification of the chromatographic peaks. MS data usually offer a good supplement for confirmation of peak identification.

Herein, HPLC-DAD-MS analysis was carried out. In HPLC-DAD profile, the retention time of peak 10 was similar to those of ginsenoside Rf and notoginsenoside Fa, while peak 12 had same retention time with ginsenoside Rc and notoginsenoside R2. Therefore, unambiguous identification of peaks 10 and 12 was difficult based on their retention time, though peaks 10 and 12 were considered as ginsenoside Rf and Rc, respectively, in previous reports [13,28]. Actually, ultra-performance liquid chromatography (UPLC) analysis indicated that ginsenoside Rf and Rc were not found in *P. notoginseng* [29]. Actually, MS data of peaks 10 and 12 highly matched with those of notoginsenoside Fa and notoginsenoside R2, respectively. Therefore, peak 10 and peak 12 were considered as notoginsenoside Fa and R2. The identification of other peaks was also confirmed by comparing their MS data with those of reference compounds (Table 4).

**Table 4**
MS and UV data of the 16 analytes in *Panax notoginseng*.

| Peaks no. | Identification | Retention time (min) | MW | MS data (*m*/*z*) | UV $\lambda_{max}$ (nm) |
|---|---|---|---|---|---|
| 1 | Uracil | 2.9 | 112 | –[a] | 260 |
| 2 | Cytidine | 3.4 | 243 | 244 $[M+H]^+$, 266 $[M+Na]^+$, 112 $[M+H\text{-}Rib]^+$ | 270 |
| 3 | Uridine | 3.8 | 244 | 245 $[M+H]^+$, 267 $[M+Na]^+$, 113 $[M+H\text{-}Rib]^+$ | 260 |
| 4 | Guanosine | 8.4 | 283 | 284 $[M+H]^+$, 306 $[M+Na]^+$, 152 $[M+H\text{-}Rib]^+$ | 255 |
| 5 | Adenosine | 11.1 | 267 | 268 $[M+H]^+$, 290 $[M+Na]^+$, 136 $[M+H\text{-}Rib]^+$ | 260 |
| 6 | Notoginsenosid R1 | 35.1 | 932 | 931 $[M\text{-}H]^-$, 799 $[M\text{-}H\text{-}Xyl]^-$, 769 $[M\text{-}H\text{-}Glc]^-$, 637 $[M\text{-}H\text{-}Glc]^-$, 475 Agl | – |
| 7 | Ginsenoside Rg1 | 38.2 | 800 | 799 $[M\text{-}H]^-$, 637 $[M\text{-}H\text{-}Glc]^-$, 475 Agl | – |
| 8 | Ginsenoside Re | 39.3 | 946 | 945 $[M\text{-}H]^-$, 799 $[M\text{-}H\text{-}Rha]^-$, 783 $[M\text{-}H\text{-}Glc]^-$, 765 $[M\text{-}H\text{-}Glc\text{-}H_2O]^-$, 637 $[M\text{-}H\text{-}Glc\text{-}Rha]^-$, 619 $[M\text{-}H\text{-}Glc\text{-}Rha\text{-}H2O]^-$, 475 Agl | – |
| 9 | Notoginsenoside R4 | 44.5 | 1240 | 1239 $[M\text{-}H]^-$, 1107 $[M\text{-}H\text{-}Xyl]^-$, 1077 $[M\text{-}H\text{-}Glc]^-$, 945 $[M\text{-}H\text{-}Xyl\text{-}Glc]^-$, 783 $[M\text{-}H\text{-}Xyl\text{-}2Glc]^-$ | – |
| 10 | Notoginsenoside Fa | 44.9 | 1240 | 1239 $[M\text{-}H]^-$, 1107 $[M\text{-}H\text{-}Xyl]^-$, 945 $[M\text{-}H\text{-}Xyl\text{-}Glc]^-$, 783 $[M\text{-}H\text{-}Xyl\text{-}2Glc]^-$ | – |
| 11 | Ginsenoside Rb1 | 45.7 | 1108 | 1107 $[M\text{-}H]^-$, 945 $[M\text{-}H\text{-}Glc]^-$, 783 $[M\text{-}H\text{-}2Glc]^-$, 621 $[M\text{-}H\text{-}3Glc]^-$ | – |
| 12 | Notoginsenoside R2 | 46.1 | 770 | 769 $[M\text{-}H]^-$, 637 $[M\text{-}H\text{-}Xyl]^-$, 475 Agl | – |
| 13 | Ginsenoside Rg2 | 46.8 | 784 | 783 $[M\text{-}H]^-$, 637 $[M\text{-}H\text{-}Rha]^-$, 621 $[M\text{-}H\text{-}Glc]^-$ | – |
| 14 | Ginsenoside Rh1 | 47.1 | 638 | 637 $[M\text{-}H]^-$, 475 Agl | – |
| 15 | Ginsenoside Rd | 47.7 | 946 | 945 $[M\text{-}H]^-$, 783 $[M\text{-}H\text{-}Glc]^-$, 621 $[M\text{-}H\text{-}2Glc]^-$, 459 Agl | – |
| 16 | Notoginsenoside K | 48.3 | 946 | 945 $[M\text{-}H]^-$, 783 $[M\text{-}H\text{-}Glc]^-$, 621 $[M\text{-}H\text{-}2Glc]^-$, 459 Agl | – |

Rib, ribose; Xyl, xylose; Glc, glucose; Rha, rhamnose; Agl, aglycone.
[a] Not found.

**Table 5**
The contents (mg/g) of 16 investigated compounds in *P. notoginseng*.

| Analytes | Samples | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S1[a] | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 |
| Uracil | 0.04[b] (3.01[c]) | 0.04 (2.17) | 0.04 (2.43) | 0.03 (1.93) | 0.03 (1.79) | 0.04 (2.81) | 0.03 (2.95) | 0.02 (3.46) | 0.03 (2.54) | 0.02 (4.36) | 0.02 (2.75) | 0.04 (2.89) | 0.02 (3.29) | 0.03 (2.94) | 0.02 (1.76) |
| Cytidine | 0.08 (4.12) | 0.05 (1.47) | 0.05 (2.38) | 0.06 (2.54) | 0.05 (1.96) | 0.06 (1.79) | 0.06 (3.87) | 0.06 (3.26) | 0.06 (1.62) | 0.07 (2.31) | 0.07 (1.72) | 0.05 (2.29) | 0.05 (2.75) | 0.06 (1.93) | 0.07 (3.45) |
| Uridine | 0.17 (1.72) | 0.08 (2.35) | 0.09 (3.08) | 0.10 (1.77) | 0.09 (2.43) | 0.08 (4.02) | 0.09 (1.32) | 0.15 (2.61) | 0.10 (2.09) | 0.13 (1.74) | 0.17 (1.98) | 0.08 (2.01) | 0.13 (2.11) | 0.09 (2.14) | 0.16 (3.08) |
| Guanosine | 0.15 (1.90) | 0.10 (3.01) | 0.10 (2.82) | 0.10 (2.06) | 0.09 (2.91) | 0.09 (2.17) | 0.11 (1.78) | 0.15 (2.66) | 0.13 (3.15) | 0.12 (2.81) | 0.15 (2.17) | 0.09 (2.93) | 0.12 (2.22) | 0.11 (1.82) | 0.17 (1.94) |
| Adenosine | 0.21 (3.33) | 0.20 (1.00) | 0.19 (2.63) | 0.23 (1.74) | 0.22 (0.91) | 0.21 (1.43) | 0.22 (2.73) | 0.25 (1.20) | 0.22 (0.45) | 0.25 (0.40) | 0.26 (1.15) | 0.22 (1.82) | 0.23 (0.43) | 0.23 (2.61) | 0.28 (1.07) |
| Total nucleosides[d] | 0.65 | 0.47 | 0.47 | 0.52 | 0.48 | 0.48 | 0.51 | 0.63 | 0.54 | 0.59 | 0.67 | 0.48 | 0.55 | 0.52 | 0.70 |
| Notoginsenosid R1 | 7.73 (1.94) | 6.50 (1.69) | 7.42 (0.94) | 7.04 (1.42) | 7.77 (2.32) | 6.98 (0.86) | 8.41 (0.71) | 7.29 (2.47) | 7.50 (1.73) | 7.68 (0.78) | 6.90 (2.75) | 7.23 (0.41) | 7.32 (1.50) | 8.30 (2.53) | 6.72 (0.45) |
| Ginsenoside Rg1 | 43.09 (0.42) | 39.41 (1.50) | 41.39 (1.33) | 43.27 (1.02) | 42.25 (0.57) | 42.90 (2.12) | 45.98 (0.28) | 34.92 (1.43) | 41.83 (1.58) | 49.29 (1.10) | 41.11 (1.02) | 42.62 (0.16) | 40.96 (1.90) | 41.40 (1.23) | 40.98 (1.59) |
| Ginsenoside Re | 6.19 (1.13) | 4.71 (1.49) | 3.98 (1.26) | 4.82 (1.87) | 4.13 (0.97) | 4.68 (0.64) | 5.61 (1.78) | 3.73 (1.61) | 4.69 (1.28) | 5.52 (0.91) | 4.71 (1.27) | 5.25 (1.33) | 4.28 (2.10) | 4.96 (0.40) | 4.36 (1.15) |
| Notoginsenoside R4 | 1.37 (1.46) | 1.04 (0.96) | 1.01 (2.97) | 1.10 (1.82) | 1.27 (2.36) | 1.07 (1.87) | 1.35 (2.22) | 0.98 (3.06) | 1.03 (3.88) | 1.02 (1.96) | 0.96 (3.13) | 1.33 (3.01) | 0.91 (2.20) | 1.00 (3.00) | 0.84 (1.19) |
| Notoginsenoside Fa | 1.68 (1.79) | 1.30 (1.54) | 1.49 (2.68) | 1.46 (1.37) | 1.49 (1.34) | 1.64 (1.22) | 1.56 (1.92) | 1.27 (2.36) | 1.35 (2.22) | 1.51 (0.66) | 1.11 (2.70) | 1.46 (2.05) | 1.30 (2.31) | 1.15 (2.61) | 1.40 (2.14) |
| Ginsenoside Rb1 | 31.66 (0.44) | 23.29 (0.39) | 26.84 (0.22) | 25.94 (0.54) | 32.96 (0.49) | 26.51 (0.30) | 31.26 (1.63) | 23.01 (1.35) | 26.97 (0.85) | 27.17 (0.52) | 21.12 (2.41) | 28.50 (1.89) | 24.68 (2.03) | 27.86 (1.79) | 25.72 (1.21) |
| Notoginsenoside R2 | 1.02 (1.96) | 0.67 (2.99) | 0.87 (3.45) | 0.86 (3.49) | 0.95 (2.11) | 0.90 (2.22) | 0.98 (4.08) | 0.88 (1.14) | 0.99 (4.04) | 0.97 (2.06) | 0.77 (2.60) | 0.90 (2.22) | 0.76 (2.63) | 0.96 (2.08) | 0.88 (2.27) |
| Ginsenoside Rg2 | 1.04 (1.92) | 0.55 (5.45) | 0.62 (1.61) | 0.74 (2.70) | 0.65 (3.08) | 0.78 (1.28) | 0.81 (2.47) | 0.60 (3.33) | 0.77 (2.60) | 0.87 (2.30) | 0.62 (3.23) | 0.74 (4.05) | 0.56 (5.36) | 0.71 (2.82) | 0.71 (1.41) |
| Ginsenoside Rh1 | 0.92 (2.17) | 0.67 (1.49) | 0.86 (1.16) | 0.90 (1.11) | 0.92 (4.35) | 0.98 (1.02) | 1.04 (1.92) | 0.78 (1.28) | 1.02 (1.96) | 1.07 (1.87) | 0.78 (1.28) | 0.84 (2.38) | 0.77 (2.60) | 0.88 (1.14) | 0.91 (2.20) |
| Ginsenoside Rd | 4.54 (0.44) | 4.60 (0.87) | 5.65 (0.53) | 5.22 (0.57) | 6.75 (0.15) | 5.64 (0.35) | 7.50 (0.40) | 4.53 (0.88) | 5.22 (0.77) | 5.90 (0.51) | 4.63 (0.65) | 6.18 (0.49) | 5.25 (1.14) | 6.27 (0.80) | 4.85 (0.82) |
| Notoginsenoside K | 2.61 (1.15) | 2.08 (1.92) | 1.85 (1.08) | 2.03 (1.97) | 1.92 (1.04) | 2.71 (1.11) | 3.54 (1.13) | 1.32 (1.52) | 2.19 (1.37) | 1.24 (1.61) | 0.62 (3.23) | 2.47 (1.21) | 0.92 (2.17) | 2.88 (1.04) | 1.22 (0.82) |
| Total saponins | 101.85 | 84.82 | 91.98 | 93.38 | 101.06 | 94.79 | 108.04 | 79.31 | 93.56 | 102.24 | 83.33 | 97.52 | 87.71 | 96.37 | 88.59 |

[a] S1–S10, S11–S13, S14 and S15, were collected from Wenshan, Maguan, Mengzi and Xichou of Yunnan Province, China, respectively.
[b] Average of two determinations.
[c] RD, relative deviation (%) = (|measured value−mean|/mean) × 100.
[d] Uracil, the nucleobase, was also included.

### 3.4. Quantification of the analytes in P. notoginseng

The developed multiple columns HPLC method was applied to simultaneous quantification of the investigated compounds (5 nucleobases and nucleosides, and 11 saponins) in 15 samples of *P. notoginseng* collected from different places in China. The typical multiple columns HPLC profile of *P. notoginseng* was shown in Fig. 3B and the results were summarized in Table 5. In brief, notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd were five abundant components in *P. notoginseng*, which accounted for more than 80% of the content of total saponins. Notoginsenoside R4 (9), Fa (10) and K (16) were first quantitatively determined, and they were detected in all *P. notoginseng* samples. In addition, five nucleosides and nucleobases were detected and their contents were similar to the previous report [27]. The RSDs of total saponins and total nucleosides (include 1 nucleobase) were 8.47% ($n = 15$) and 14.22% ($n = 15$), respectively, which suggested that the consistency of quality of *P. notoginseng* were rather good. The results were also in agreement with our previous reports [28,29]. Actually, the cultivation of *P. notoginseng* have been guided under Good Agriculture Practice (GAP) for 9 years, and more than 85% yield of *P. notoginseng* in China is from Wenshan, Yunnan Province, which may be the reason of stable quality of *P. notoginseng*.

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## 4. Conclusion

In this study, a multiple columns HPLC method using column switching technique was developed for simultaneous determination of 16 compounds, including 5 nucleobases and nucleosides and 11 saponins, in *P. notoginseng*. Among the 16 analytes, notoginsenoside R4 (9), Fa (10) and K (16) were first quantitatively determined. The developed method is helpful to comprehensively control the quality of *P. notoginseng*.

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## References

[1] Pharmacopoeia of the People's Republic of China, Chemical Industry Press, Peking, 2005, p. 10.
[2] Q. Li, W.H. Ye, Y.Q. Xing, Gao Deng Xue Xiao Hua Xue Xue Bao 17 (1996) 1886–1892.
[3] Z.G. Yang, A.Q. Chen, S.D. Yu, Shanghai Zhong Yi Yao Za Zhi 39 (2005) 59–62.
[4] T.B. Ng, J. Pharm. Pharmacol. 58 (2006) 1007–1019.
[5] P. Chan, G.N. Thomas, B. Tomlinson, Acta Pharmacol. Sin. 23 (2002) 1157–1162.
[6] G. Yu, H.B. Xu, X.B. Sun, Shi Zhen Guo Yi Guo Yao 18 (2007) 1251–1252.
[7] K. Sun, C.S. Wang, J. Guo, Y. Horie, S.P. Fang, F. Wang, Y.Y. Liu, L.Y. Liu, J.Y. Yang, J.Y. Fan, J.Y. Han, Life Sci. 81 (2007) 509–518.
[8] H.X. Sun, Z.G. Yang, Y.P. Ye, Int. J. Immunopharmacol. 6 (2006) 14–25.
[9] L. Li, J.L. Zhang, Y.X. Sheng, D.A. Guo, Q. Wang, H.Z. Guo, J. Pharm. Biomed. Anal. 38 (2005) 45–51.
[10] A.J. Lau, B.H. Seo, S.O. Woo, H.L. Koh, J. Chromatogr. A 1057 (2004) 141–149.
[11] J.B. Wan, F.Q. Yang, S.P. Li, Y.T. Wang, X.M. Cui, J. Pharm. Biomed. Anal. 41 (2006) 1596–1601.
[12] A.J. Lau, B.H. Seo, S.O. Woo, H.L. Koh, J. Chromatogr. A 1011 (2003) 77–78.
[13] S.F. Wang, S. Ye, Y.Y. Chen, J. Chromatogr. A 1109 (2006) 279–284.
[14] A.P. Schmidt, D.R. Lara, J.F. Maraschin, A.S. Perla, D.O. Souza, Brain Res. 864 (2000) 40–43.
[15] G. Anfossi, I. Russo, P. Massucco, L. Mattiello, F. Cavalot, A. Balbo, M. Trovati, Thromb. Res. 105 (2002) 71–78.
[16] J.B. Conti, L. Belardinelli, D.B. Utterback, A.B. Curtis, Circulation 91 (1995) 1761–1767.
[17] J. Wang, Z.G. Huang, H. Cao, Y.T. Wang, P. Hui, C. Hoo, S.P. Li, J. Sep. Sci. 31 (2008) 1173–1180.
[18] S.P. Li, F.Q. Yang, K.W.K. Tsim, J. Pharm. Biomed. Anal. 41 (2006) 1571–1584.
[19] W.H. Carmen, D. Xin, J. Chromatogr. B 812 (2004) 241–257.
[20] S. Petr, S. Dalibor, S. Petr, Trends Analyt. Chem. 26 (2007) 375–384.
[21] S.J. Ding, E. Dudley, L.J. Chen, S. Plummer, J.D. Tang, R.P. Newton, A.G. Brenton, Rapid Commun. Mass Spectrom. 20 (2006) 3619–3624.
[22] S. Wang, W. Huang, G.Z. Fang, J.X. He, Y. Zhang, Anal. Chim. Acta 606 (2008) 194–201.
[23] K. Hamase, A. Morikawa, T. Ohgusu, W. Lindner, K. Zaitsu, J. Chromatogr. A 1143 (2007) 105–111.
[24] S. Souverain, S. Rudaz, J.L. Venthey, J Chromatogr. B 801 (2004) 141–156.
[25] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, J. Pharm. Biomed. Anal. 44 (2007) 342–355.
[26] T.T. Zhou, W.Q. Zhao, G.R. Fan, Y.F. Chai, Y.T. Wu, J. Chromatogr. B 858 (2007) 296–301.
[27] J. Wang, Y.T. Wang, S.P. Li, J. Chin. Pharm. Sci. 16 (2007) 79–83.
[28] J.B. Wan, P. Li, S.P. Li, Y.T. Wang, T.T.X. Dong, K.W.K. Tsim, J. Sep. Sci. 29 (2006) 2190–2196.
[29] J. Guan, C.M. Lai, S.P. Li, J. Pharm. Biomed. Anal. 44 (2007) 996–1000.