# Application Note

## Determination of the ginsenoside composition of *Panax ginseng* by UHPLC

| | |
|---|---|
| **Category** | Pharmaceutical Analysis |
| **Matrix** | Ginseng extract |
| **Method** | UHPLC |
| **Keywords** | Ginseng, Ginsenosides, |
| **Analytes** | Ginsenoside: $Rg_1$, Re, Rf, $Rb_1$, $Rg_2$, Rc, $Rh_1$, $Rb_2$, $F_1$, Rd, $Rg_6$, $F_4$, $Rk_3$, $Rh_4$, (20S) $Rg_3$, (20R) $Rg_3$, $Rk_1$, $Rg_5$, Compound K, $Rh_2$ |
| **ID** | VPH0055N, May 2013 |

PLATIN blue by Knauer


### Summary

This application note describes a fast UHPLC method for the determination of 20 ginsenosides. The developed method allows for the determination of 20 ginsenosides in less than 35 minutes. A binary high pressure gradient is used at a flow rate of 0.6 ml/min. By applying a Bluespher 100-2 C18 column in the dimension 150 x 2 mm ID, the total run time could be reduced to less than 41 minutes when a washing step and re-equilibration of the column is included. This means that 70 % of the eluent and 50 % of analysis time could be saved compared to the classical HPLC method.


### Introduction

Ginseng (*Panax ginseng*) is known as a medicinal herb that is sweet in taste and has the opportunity to warm up the body moderately and to help keeping the lungs and spleen healthy.[1] Ginseng contains more than 30 different ginseng saponins displaying various physiological activities: Polyacetylenes are known to display antitumor activities on various cancers, phenolic compounds featuring antioxidant activities, proteins showing radioprotective effects on victims of an atomic air raid and acidic polysaccharides featuring immune controlling activities.[1] But the main pharmacological components of ginseng are the ginsenosides, which show various biochemical and pharmacological efficacies.[2] Their chemical structure has been confirmed clearly by the studies of Shibata et al. in 1966.[4] Several research groups reported that ginsenoside exhibits medicinal actions including anti-cancer and anti-diabetic activities, constraint effect on central nervous system, prevention of arterial hardening and hypertension, promotion of liver function and protein synthesis, anti-fatigue, anti-stress, anti-oxidative and anti-inflammatory activities as well as enhancement of immunity. This leads to a great public and also academic interest in ginsenoside components.[3]

Ginsenosides can be divided into two groups: Protopanaxadiols and Protopanaxatriols. The main component of the Protopanaxadiols is ginsenoside $Rb_1$ which is known to supress the superactivity of the nervous system. The main component of the Protopanaxatriols is ginsenoside $Rg_1$ which stimulates the central nervous system and is deeply involved in the adaptogen activity of ginseng.[1]

Many studies on ginseng have been done regarding the age of the root and the cultivation area for the composition of ginsenosides.[3] To figure out the ginsenoside composition of roots, flower buds, berries and seeds, a reliable HPLC method is needed. Caused by the complex composition of the ginseng extracts, typical HPLC runs take up to 120 min.[1, 2, 3]

In this application note we present a method for the determination of 20 main ginsenosides and also trace ingredients in red ginseng extracts in less than 35 min.

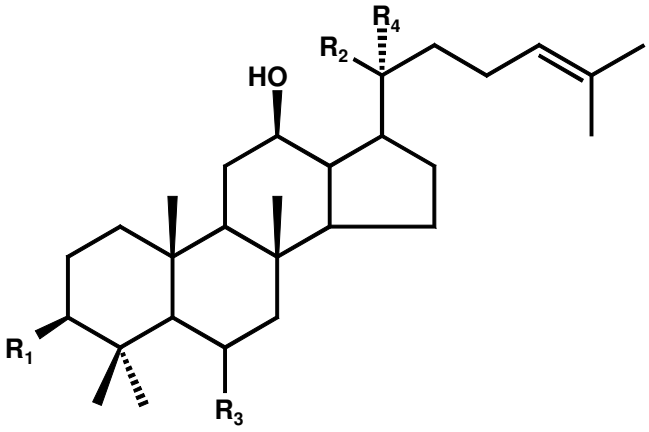


**Fig. 1**

Chemical structures of the analyzed Ginsenosides

| Ginsenoside | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Rb1 | - O glc (2-1) glc | - O glc (6-1) glc | - H | - $CH_3$ |
| Rc | - O glc (2-1) glc | - O glc (6-1) araf | - H | - $CH_3$ |
| Rb2 | - O glc (2-1) glc | - O glc (6-1) arap | - H | - $CH_3$ |
| Rd | - O glc (2-1) glc | - O glc | - H | - $CH_3$ |
| (20S) Rg3 | - O glc (2-1) glc | - OH | - H | - $CH_3$ |
| (20R) Rg3 | - O glc (2-1) glc | - $CH_3$ | - H | - OH |
| Rh2 | - O glc | - H | - H | - $CH_3$ |
| Rg5 | - O glc (2-1) glc | - $CH_3$ | - OH | - H |
| Rk1 | - O glc (2-1) glc | - O | - OH | - H |
| Rg1 | - OH | - glc | - glc | - $CH_3$ |
| Rg2 | - OH | - H | - glc (2-1) rha | - $CH_3$ |
| Rf | - OH | - H | - glc (2-1) glc | - $CH_3$ |
| Re | - OH | - glc | - glc (2-1) rha | - $CH_3$ |
| Rh1 | - OH | - H | - glc | - $CH_3$ |
| Compound K | - OH | - O glc | - H | - $CH_3$ |
| Rh4 | - OH | - $CH_3$ | - O glc | - H |
| Rk3 | - OH | - O | - O glc | - H |
| F4 | - OH | - $CH_3$ | - O glc (2-1) rha | - H |
| Rg6 | - OH | - O | - O glc (2-1) rha | - H |
| F1 | - OH | - O glc | - OH | - $CH_3$ |

Glc: β-d-glucose, Rha: α-1-Rhamnose, Arap: α-l-Arabinose (Pyranose), Araf: α-l-Arabinose (Furanose)

## Experimental preparation of standard solution

The stock standard mixture was received in the concentration of 27.77 ppm per ginsenoside dissolved in water from a customer. This standard was ready for analysis by the UHPLC system.
For calibration, the stock standard was diluted to the required concentrations using a water/acetonitrile mixture as the solvent. Calibration was done for concentrations in the range of 2.78 – 27.77 ppm.

## Experimental Sample Preparation

Ginsenosides can be extracted by steaming and liquid extraction procedures from ginseng by various methods. These techniques have already been described elsewhere.[1]

## Method parameters

| | | | |
|---|---|---|---|
| **Column** | Bluespher 100-2 C18, 150 x 2 mm ID | | |
| **Eluent A** | Water | | |
| **Eluent B** | Acetonitrile | | |
| **Gradient** | **Time [min]** | **% A** | **% B** |
| | 0.00 | 83 | 17 |
| | 15.30 | 80 | 20 |
| | 18.90 | 75 | 25 |
| | 26.00 | 65 | 35 |
| | 32.00 | 40 | 60 |
| | 33.00 | 30 | 70 |
| | 34.00 | 0 | 100 |
| | 37.00 | 0 | 100 |
| **Flow rate** | 0.7 ml/min | | |
| **Injection volume** | 2 µl | | |
| **Column temperature** | 40 °C | | |
| **Run time** | 37.0 min | | |
| **Detection** | PDA-1, 204 nm, 10mm cell, 50 Hz, 0,02 s | | |

## Results

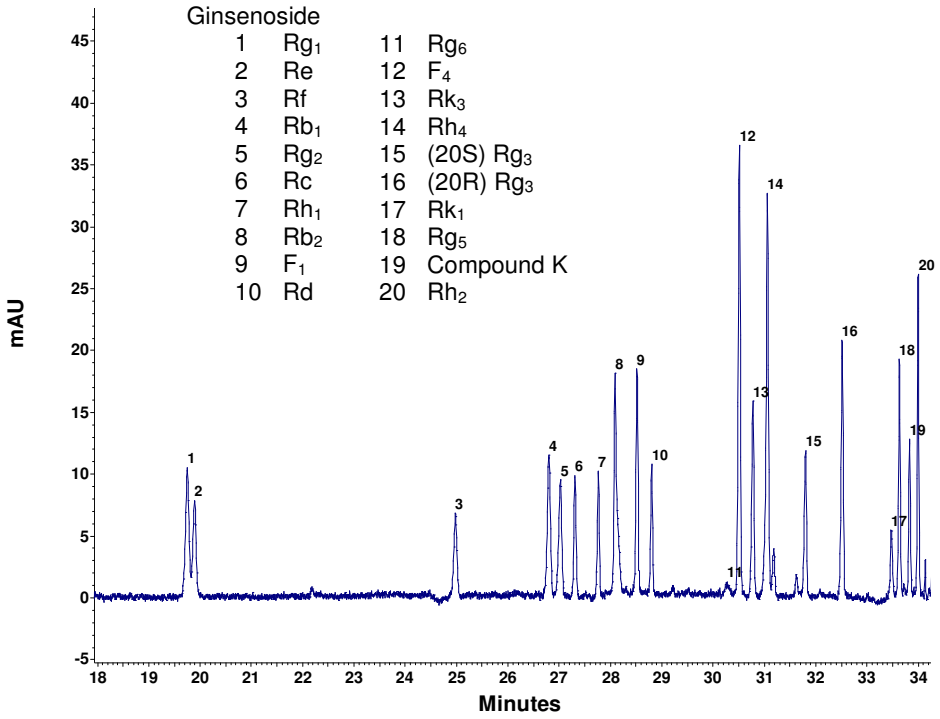


**Fig. 2**

Chromatogram of the ginsenoside standard mix

The standard mixture of the ginsenosides could successfully be separated using the PLATINblue UHPLC system in combination with a UHPLC column. 50 % of the really expensive standard solution can be saved compared to the original HPLC method, because only 5 µl are injected. Caused by the advantages of the PLATINblue UHPLC system, peaks are sharper and therewith significantly higher compared to a classical HPLC system.

The Bluespher C18 column with 2 µm particle size in the dimension 150 x 2 mm ID reaches a baseline separation of all target compounds. Figure 2 shows the chromatogram of this run. 34 minutes are needed for the separation of all ginsenosides. Including a washing step and reequilibration of the column, 41 minutes are needed for one run. This means that 70 % of time and 73 % of the eluent are saved compared to the original method.

After the successful development of a UHPLC method with the standard solution, calibration was done and a real sample was measured. In the following, the calibration curves are shown exemplarily for 4 selected substances. It can be seen, that a good linearity could be reached with the applied method.

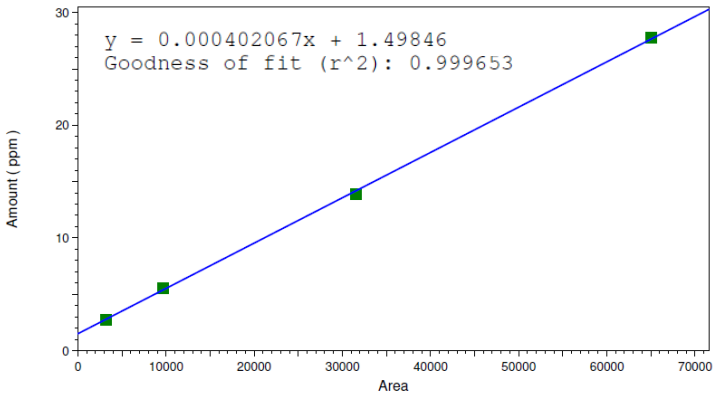


**Fig. 3**

Calibration Curve for Ginsenoside Rg1

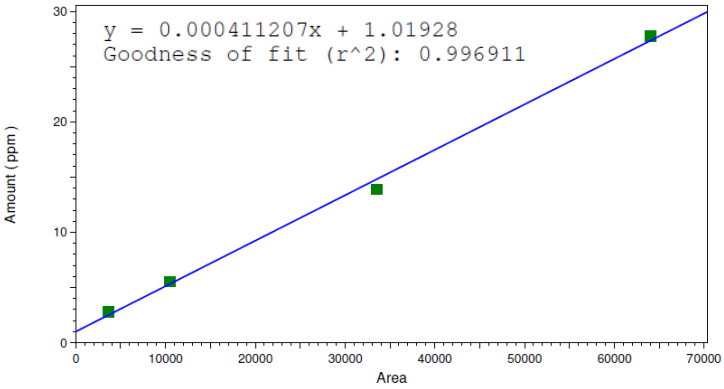


**Fig. 4**

Calibration Curve for Ginsenoside Rb1

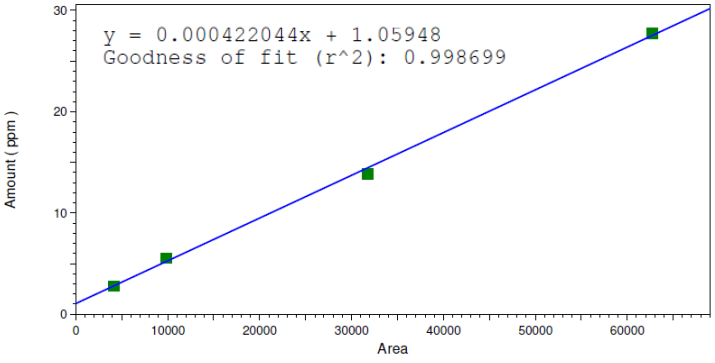


**Fig. 5**

Calibration Curve for Ginsenoside Rk3

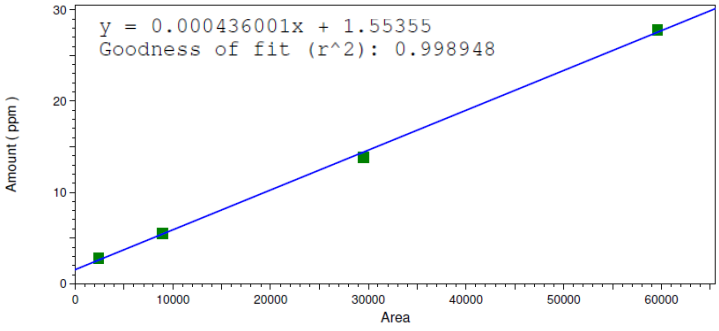


**Fig. 6**

Calibration Curve for Ginsenoside Rg5

The chromatogram shown in figure 7 shows the chromatogram of the real sample of Panax ginseng extract diluted in water and the integration of the ginsenoside peaks. Caused by the higher concentration of ginsenosides Rg1 and Re, the critical peak pair consisting of peak 1 and 2 is not separated as well as in the chromatogram of the standard.

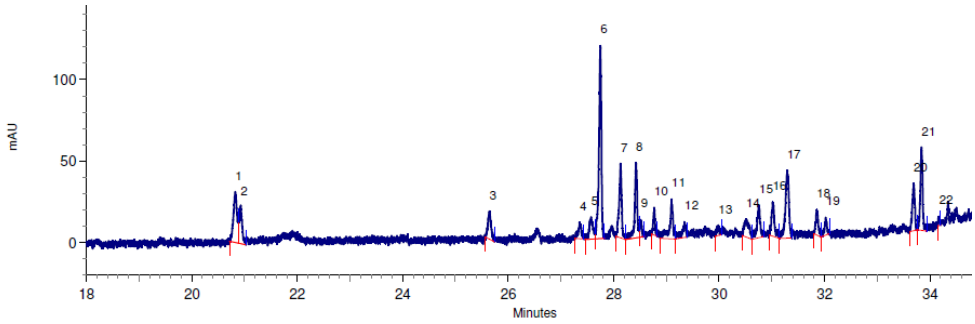


**Fig. 7**

Chromatogram of the real sample Panax ginseng extract

With the calibration of the system, the user is now able to quantify the sample. Table 1 shows the report of the sample analysis.

**Table 1**

Analysis of the Panax ginseng extract real sample

| Peak Nr. | Substance | Calculated Total Amount in the sample [µg] | Concentration in sample Panax ginseng extract [µg/mg] |
|---|---|---|---|
| 1 | Rg1 | 157.35 | 13.56 |
| 2 | Re | 142.925 | 12.32 |
| 3 | Rf | 115.15 | 9.93 |
| 4 | Rb1 | 43.925 | 3.79 |
| 5 | Rg2 | 87.65 | 7.56 |
| 6 | Rc | 627.525 | 54.10 |
| 7 | Rh1 | 294.9 | 25.42 |
| 8 | Rb2 | 145.1 | 12.51 |
| 10 | F1 | 42.625 | 3.67 |
| 11 | Rd | 212.975 | 18.36 |
| 13 | Rg6 | 198.95 | 17.15 |
| 14 | F4 | 36.375 | 3.14 |
| 15 | Rk3 | 95.05 | 8.19 |
| 16 | Rh4 | 44.825 | 3.86 |
| 17 | (20S)Rg3 | 277.4 | 23.91 |
| 18 | (20R)Rg3 | 42.525 | 3.67 |
| | Rk1 | NF | NF |
| 20 | Rg5 | 109.675 | 9.45 |
| 21 | Compound K | 274.9 | 23.70 |
| 22 | Rh2 | 11.6 | 1.00 |

## Conclusion

The separation and determination of the ginsenoside mixture works very well on the KNAUER PLATINblue UHPLC system. The original HPLC method could successfully be converted to a UHPLC method reaching the typical advantages like time and eluent saving and also sharper and higher peaks.
The Bluespher C18 column in the dimension 150 x 2 mm reaches a baseline separation of the ginsenosides and is recommended if the highest resolution of the target compounds is needed. Especially the peaks at the end of the chromatogram are really sharp what leads to a good limit of detection for these compounds.
After calibration of the system with the standard solution, the real sample of a Panax ginseng extract can be analyzed and the included ginsenosides can be quantified without any problem.
Finally, we can summarize that the UHPLC method works very well for the analysis of ginsenosides. The time- and eluent-saving method can surely still be improved concerning resolution and speed depending on the user´s wishes.

## References


1 Sun A Lee et al., J. Ginseng Res. Vol. 36, No. 1, 102-106 (2012)

2 Sung Kwon Ko et al., Food Sci. Biotechnol. Vol. 17, No. 6, 1379 – 1382 (2008)

3 Sung Kwon Ko et al., J- Korean Soc. Appl. Biol. Chem., Vol 54 (1), 154-157 (2011)

4 Shibata S. et al., Chem. Pharm. Bull, Vol. 14, 595 – 600 (1966)


## Authors


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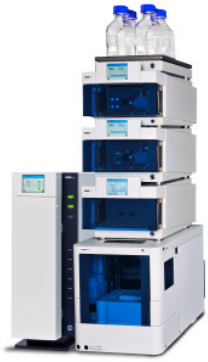
## Physical properties of recommended column

Bluespher columns are packed with an ultra pure silica stationary phase to provide excellent separation performance and are well-suited for either routine analysis or ambitious chromatography in high speed mode where resolution, sensitivity and sample throughput are critical. These columns are your first choice for high-throughput-screening, quality control, and method development.
Bluespher C18 is especially designed for the analysis of acidic, basic and neutral analytes in reversed phase mode.

| | |
|---|---|
| **Stationary phase** | Bluespher 100-2 C18 |
| **USP code** | L1 |
| **Pore size** | 100 Å |
| **Pore volume** | 0.8 ml/g |
| **Particle size** | 2 µm |
| **Form** | spherical |
| **Surface area** | 320 +/- 20 $m^2/g$ |
| **% C** | 16 |
| **Endcapping** | yes |
| **Dimensions** | 150 x 2 mm |
| **Order number** | 15BE181BSF |

## Recommended Instrumentation

This application requires the PLATINblue binary high pressure gradient UHPLC system equipped with degasser, autosampler, column thermostat, and PDA detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.



| **Description** | **Order No.** |
|---|---|
| **PLATINblue UHPLC-System** | A69420 |
| PLATINblue UHPLC Pump P-1 with Smartmix 100 | |
| PLATINblue UHPLC Pump P-1 with Degasser | |
| PLATINblue Autosampler AS-1 | |
| PLATINblue Column Thermostat T-1 | |
| PLATINblue Detector PDA-1 | |
| PDA-1 flow cell (10 mm, 2 µl) | |
| PLATINblue modular eluent tray | |
| PLATINblue CG Data system | |
| PLATINblue CG PDA license | |
| PLATINblue stainless steel capillary kit | |

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