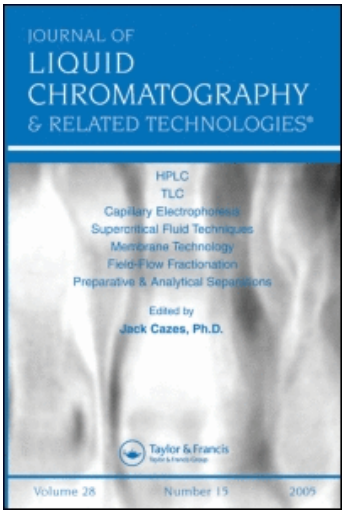

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Separation of Ginsenosides at Elevated Temperature by Ultra High Pressure Liquid Chromatography

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Abstract: Separation of seven ginsenosides at elevated temperature by ultra high pressure liquid chromatography (UHPLC) on reversed phase C_{18}- bonded silica stationary phase was studied. All separations were carried out with a binary mobile phase consisting of CH_3CN/H_2O in isocratic elution mode. It was found that increasing the column temperature resulted in efficient separation and complete resolution of seven ginsenosides. Generally, the separation efficiency increased as a function of temperature. The effect of the mobile phase velocity on the separation efficiency was also investigated.

Keywords: Ultra High Pressure Liquid Chromatography, Ginsenosides, Elevated temperature, van't Hoff plot, Isocratic elution

INTRODUCTION

Ginseng is perhaps the most widely used plant in traditional medicine and now plays a major role in the herbal health care market. There are some indications in

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the literature that for more than 5000 years various forms of ginseng have been used in medicine as a tonic and a panacea that can promote longevity.[1] The main bioactive principles of ginseng are ginsenosides—triterpene saponins by chemical structure. It is derived from the roots and rhizomes of different *Panax* species (Araliaceae). The name *Panax* derives from the Greek word for "all healing" and it is the common name for several plants valued for their medicinal properties. However, originally it was used in Chinese herbals from around AD 600; although herbals of that date do not survive in full.

The most commonly used *Panax* species are *P. ginseng* (Korean or Asian ginseng), *P. quinquefolius* (American ginseng), *P. notoginseng* (Tienchi or Sanchi), *P. vietnamensis* (Vietnamese ginseng) and *P. japonicus* (Japanese ginseng).[2,3] Ginsenosides Re, Rg1, Rf, Rb1, Rc, Rb2, and Rd are the most active and abundant among the other constituents, although more than 80 ginsenosides and its derivatives have been isolated from *Panax* species.[4] In recent years, ginseng has been increasingly used as a health tonic in different forms of health products including ginseng capsules, soups, drinks, and cosmetics, which are distributed in Asia, as well as many other countries around the world.[5] Due to the fact that Ginseng is a very popular phytomedicine used all around the world, a huge quantity of investigations has been carried out during the last 35 years in order to develop analytical methods involving the identification, quantification, and quality control of ginsenosides in natural products, medicinal formulations, and biological samples.[4–7] Although, separation and determination of ginsenosides has been accomplished using different analytical techniques like thin-layer chromatography (TLC)[8,9] and gas chromatography (GC),[10] HPLC remains a widely used analytical method because of its speed, sensitivity, and adaptability to nonvolatile polar compounds, which is ideal for the analysis of saponins and sapogenins.[11] Another advantage is versatility, due to the possibility of using different detection techniques such as ultraviolet (UV),[12,13] evaporative light scattering (ELSD),[14,15] fluorescence,[16] mass spectrometry (MS),[17] and UV-VIS photodiode array detection.

The HPLC technique tends to develop column features in order to increase the separation efficiency while shortening analysis time. Reducing the particle diameter of the packing material and column length in HPLC is the time proven method to achieve both increased separation power and faster analysis times.[18] However, reducing the particle size leads to increasing column backpressure. To get the required flow in columns packed with low micron sized particles requires developing pumps, valves, and columns that can operate at much higher pressures than conventional HPLC systems. To overcome this problem, a new method named ultra high pressure liquid chromatography (UHPLC) was introduced into the analytical sciences, which allows performing separations at very high pressures.[19–21] UHPLC can operate at pressures 15000 psi or more, while conventional HPLC technology is limited to operate below 6000 psi. Packing material size has been reduced from commonly used 5 μm to 1 μm, and it was applied successfully into practice.[22–25] It was proven experimentally that with the decreasing

particle size the column backpressure and the efficiency increases, the retention time decreases linearly while maintaining or even increasing resolution.[19] Reducing the particle diameter from 5 μm to 1 μm yields a five fold increase in theoretical plates and a five fold decrease in analysis time; however, the pressure requirements increase 125 fold.[26]

Temperature is another main factor influencing the column efficiency and backpressure. The use of elevated or high temperatures in C_{18} bonded silica columns can improve separation speed and column efficiency. Low viscosity and high diffusivity of a mobile phase at high temperatures produce much lower mass transfer resistance and backpressure,[27–32] providing more rapid mass transfer and improving efficiency by sharpening peaks.[33]

Many reported HPLC methods used C_{18} columns[7,12,34] with mixtures of acetonitrile and water or buffers as mobile phase, either in gradient or isocratic elution mode, to separate ginsenosides. However, these methods offered partial separation of ginsenosides or, despite good separation, suffered from time consuming chromatographic runs, even with the gradient elution mode. Many reported investigations focused on finding optimum conditions of separating ginsenosides in the shortest possible analysis time while increasing the separation efficiency. More recently, a method for simultaneous determination of six ginsenosides in pharmaceutical preparations, using an improved step gradient reversed phase chromatographic system after solid-phase extraction (SPE), has been reported.[34] HPLC separations using isocratic elution modes on Diol columns were reported by our research group[6] and by the group of M. Bonfill et al.[5]

In this study, we have focused on ginsenosides separation at elevated temperature UHPLC, using two short columns packed with 2.0 and 1.8 μm particle C_{18} bonded silica stationary phases. All separations were carried out with a binary mobile phase consisting of acetonitrile/water 35:65 v/v using the isocratic elution mode.

The aim of the present work was to monitor the column efficiency and the basic factors affecting the performance of ginsenosides separation, such as temperature and mobile phase composition, in order to find the optimum conditions for the separation of these analytes.

EXPERIMENTAL

Materials and Reagents

All solutes were reagent grade and were used without further purification. Reference standards of ginsenosides Re, Rg1, Rf, Rb1, Rc, Rb2, and Rd were purchased from Extrasynthese (Lyon, France), via Funakoshi Co. Ltd. (Tokyo, Japan). Acetonitrile used for the chromatographic mobile phase and methanol for the sample preparations were of HPLC grade and purchased from Kishida Chemical (Osaka, Japan) or Wako Pure Chemical Industries

(Osaka, Japan). Water was purified by a Milli-QWater Purification System (Millipore, Tokyo, Japan). The ginseng samples (powdered crude drug, extract) are representative of several different sorts and brands obtained from a local pharmaceutical company. Powdered samples of crude drugs were purchased from Nakajima Pharmaceutical (Kyoto, Japan). The standard and sample solutions of ginsenosides were prepared as previously reported by D.-W. Lou et al.[6]

UHPLC Instrumentation and Columns

The chromatographic system utilized in these experiments was a JASCO X-LC system consisting of a Model 3085PU Semi-micro pump, Model 3080DG mobile phase degasser, Model 3067CO column oven, Model 3070UV UV/Vis detector, and Model 3080MX Mixing Unit. Columns used in this study were Jasco X-PressPak C18S 2.0 μm, 2.1 mm i.d. × 50 mm and Jasco ODS C18, 1.8 μm, 2.1 mm i.d. × 50 mm.

Chromatographic Conditions

Chromatographic conditions were selected as follows: Mobile phase: acetonitrile/water – 35:65 v/v; Detection: 205 nm; Column temperature was 60°C (as an optimum temperature); all experiments were carried out using two connected columns via 0.1 mm i.d. × 4.0 cm L stainless steel tubes in order to increase the separation efficiency of ginsenosides using the isocratic elution mode.

RESULTS AND DISCUSSIONS

Temperature Effect on Efficiency

Temperature is one of the main factors influencing the separation efficiency of analytes in reversed phase HPLC. The use of temperature became an important variable for improving the peak shape, the solubility of mobile phase, reducing analysis time, and improving reproducibility of the chromatography.[6,30,35] Using elevated temperature reduces the system backpressure because the backpressure is directly proportional to the viscosity of the mobile phase. Lower pressure allows various operational benefits, causing less stress on the system (valves, seals, etc.) and also enables the use of higher flow rates. This becomes important when using the columns packed with smaller sized stationary phase particles that have higher backpressures than the more commonly used 5 μm material. In our experiments, we have also focused on the elevated temperature UHPLC to study the effect of

temperature on the conventional C_{18} bonded stationary phases to obtain good separation of ginsenosides within a reasonable time of analysis. We found that the backpressure decreases approximately 19 MPa when the temperature increased from 25°C to 60°C in a curvilinear fashion.

Previously, our group[6] reported that separation efficiency and resolution of ginsenosides increases using subambient temperatures with a Diol column. Contrary to the results of using a Diol column, we have found that the separation efficiency and resolution of ginsenosides with C_{18} bonded silica stationary phases becomes better when the temperature was increased from 25 to 60°C. The effect of temperature on the efficiency of ginsenoside separations is shown in Figure 1. It has been found that the separation efficiency of selected ginsenosides increases as a function of temperature. It is also clear that the temperature affects the separation efficiency of all studied ginsenosides to varying degrees.

At lower temperatures, Re overlapped with Rg1 and Rf with Rb1 (Figure 2A). Raising the temperature increased the resolution of the peaks and a partial separation of Rf and Rb1 began at 30°C and complete resolution was achieved at 35°C. A further increase in temperature improved the resolution of Re and Rg1 as well. A beginning of resolution of these compounds was noted at 40°C and complete resolution at 60°C (Figure 2B). Therefore, further experiments in this work used elevated 60°C as an optimum temperature. Figure 2 illustrates the temperature influence on the separation of ginsenosides at 25°C (A) and 60°C (B).

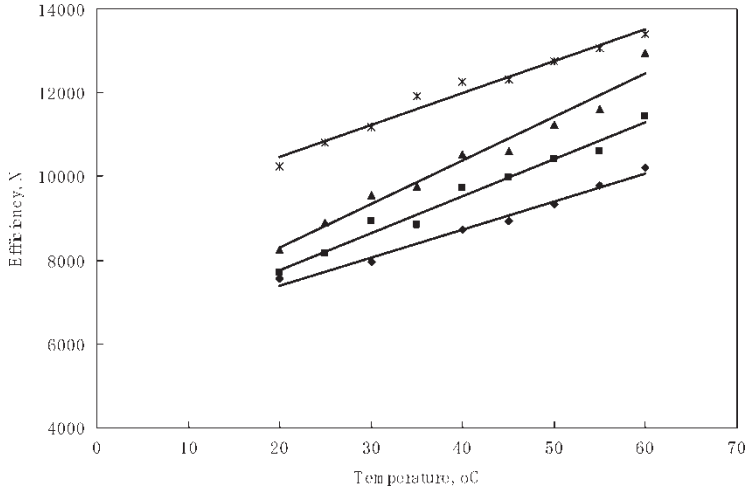

Figure 1. Effect of temperature on the separation efficiency of ginsenosides Rb1 (◆), Rc(■), Rb2(▲), Rd(*). Chromatographic conditions: Columns: X-PressPak C18S 2.0 μm, 2.1 mm I.D. × 50 mm L and ODS C18, 1.8 μm, 2.1 mm i.d. × 50 mm L; Mobile phase: Acetonitrile/Water – 35 : 65 v/v; Detection: 205 nm; Flow rate 0.2 mL/min.

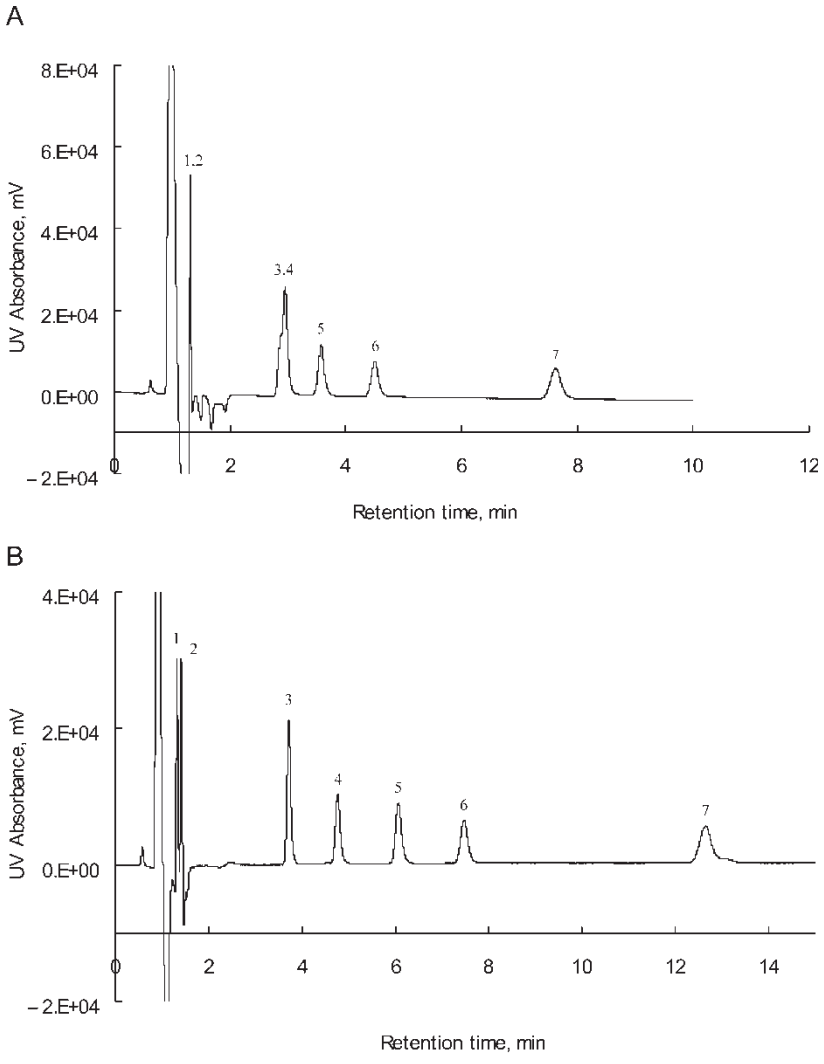

Figure 2. An example of the effect of temperature on the separation of ginsenosides. Chromatogram A is at 25°C, chromatogram B is at 60°C. Peaks: 1-Re; 2-Rg1; 3-Rf; 4-Rb1; 5-Rc; 6-Rb2; 7-Rd. Chromatographic conditions the same as in Figure 1.

Effect of Mobile Phase Velocity

A thorough study of the full van Deemter curve across a range of temperatures in reversed phase HPLC reported by F.V. Warren et al.[36] showed that an increase in temperature led to a higher height equivalent to the theoretical plates (HETP) value at all flow rates. That was the eddy diffusion contribution

(A term) reflecting the effect upon an overall upward shift in the van Deemter curve.

In our investigation, we have also studied the influence of the mobile phase velocity to the efficiency of the ginsenosides separation. The dependence of HETP on the mobile phase linear velocity (u) at the elevated temperature 60°C was plotted as shown in Figure 3. Efficiency as high as 14000 plates/meter was obtained at the optimum linear velocity at 60°C.

It should be noted that the increase in flow rate improved the chromatographic performance. According to the van Deemter plot we have found the optimum flow rate for the separation of ginsenosides around 0.20 mL/min, giving high separation efficiency of all studied seven ginsenoside compounds, with decreasing both duration of analysis and the solvent consumption. Therefore, for further experiments we chose 0.2 mL/min as the optimum flow rate.

The increase in linear velocity was reflected in an increase in the inlet column backpressure as well. It should be noted that the mobile phase linear velocity affected the chromatographic performance rather than the pressure itself. Figure 4 shows the relationship between the column inlet pressure and the mobile phase linear velocity at the elevated temperature of 60°C for 2.0 and 1.8 μm C_{18} bonded silica stationary phases.

Our group previously reported that ginsenosides separation using gradient elution mode with C_{18} bonded silica stationary phases on a conventional HPLC system was around 62 min.[34] Using the developed

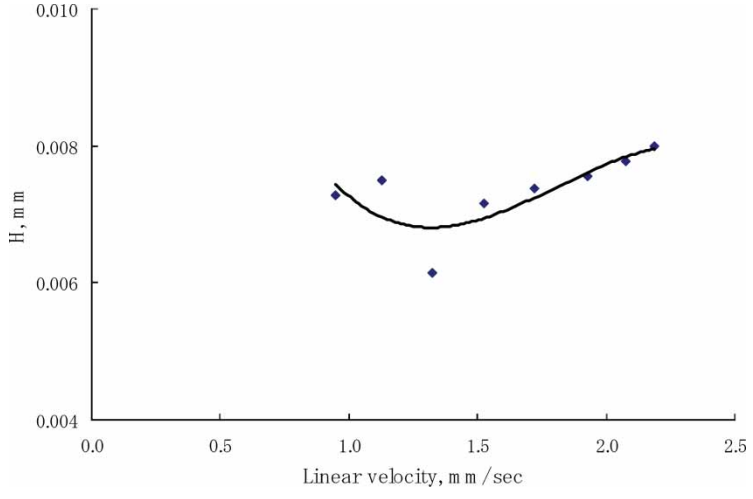

Figure 3. van Deemter plot of Rd at 60°C. Chromatographic condition: Columns: X-PressPak C18S 2.0 μm, 2.1 mm i.d. × 50 mm L and ODS C18, 1.8 μm, 2.1 mm I.D. × 50 mm L; Mobile phase: Acetonitrile/Water – 35:65 v/v; Detection: 205 nm; Column temperature 60°C.

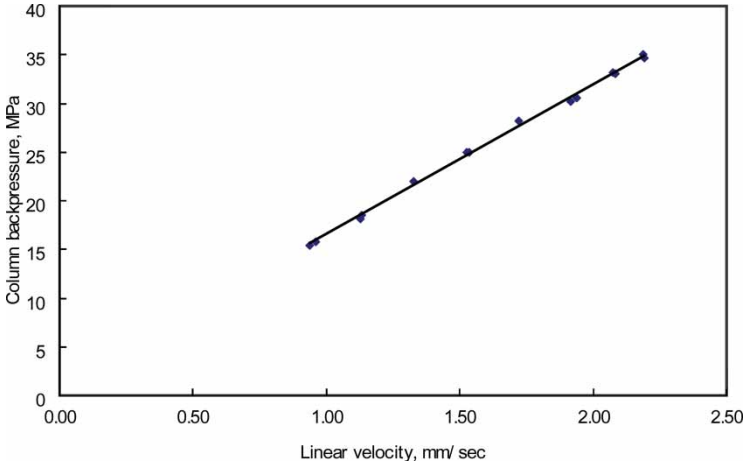

Figure 4. Effect of mobile phase linear velocities on the column backpressure. Chromatographic conditions are the same as in Figure 3.

optimum condition in UHPLC system allows us to separate all the seven ginsenosides within 13.0 min, resulting in a decrease in both separation time and solvent consumption. It was interesting to note that the elution order of ginsenosides Re and Rg1 was reversed as compared to the result in the previous study,[34] although in both cases C_{18} bonded silica stationary phase was used. Preliminary results showed that this may be caused by elevated temperature.

van't Hoff Plot

The retention and the reproducibility can be controlled by means of column temperature.[37] The logarithm of retention factor (ln k) on the reciprocal of absolute temperature (1/T) is a fundamental relationship known as a van't Hoff plot. According to the van't Hoff equation, in general, retention time of analytes decreases with increasing the column temperature. However, in the present case unusual retention behavior was observed with the ginsenosides separation when the column temperature was increased from 25 to 60°C. It can be seen from Figure 2 that with increasing column temperature the separation time of ginsenosides increased. To explain the specific phenomenon, the retention of steroids and cardiac glycosides were investigated. The reason for choosing these compounds is the similarity of their chemical structure with the ginsenosides. All these compounds have a common characteristic carbon skeleton with four fused rings (steroid or aglycone) structure. Our purpose was to find which part of the molecule, i.e., sugar or aglycone is responsible for the delayed retention at elevated temperatures. The results

showed that in contrast to ginsenosides, the retention time of steroids (Hydrocortisone, Cortisone, β-Estradiol and Estrone) decreased linearly with increasing column temperature (Figure 5A). On the other hand, the retention time of cardiac glycoside (Digitoxin) was increased up till 55°C, but a further increase in temperature resulted in a decrease in retention time as can be seen in Figure 5B. It is suggested, that the increase in retention time of both ginsenosides and digitoxin at elevated temperatures is affected mainly by the sugar part of these molecules, although further investigations concerning the retention mechanism of ginsenosides with C_{18} bonded stationary phase is needed. This might be because intramolecular interactions of bulky compounds become more effective with increasing the column temperature. According to the previously published investigation of our group on the computer based molecular-dynamics simulation, the mobility of ODS ligands increases with the increasing column temperature.[38] At low temperature range, these ligands form more ordered conformation and becomes rigid. Upon increasing the temperature, the C_{18} ligands can move freely,[39] and thus the interaction between the stationary phase and analyte molecules might be stronger, resulting in an increase retardation of eluting molecules. At 25°C all seven standard ginsenosides were eluted within 7.9 min, while at 60°C it was 12.9 min (Figure 2). Moreover, the resulting van't Hoff plots (Figure 5B) of selected ginsenosides over the temperature range from 25 to 60°C in which retention increases with increasing temperature has two linear relationships. As the temperature is increased from 25 to 35°C a large increase in retention of ginsenosides was observed. Further increasing of the temperature from 40 to 60°C led to a slow increase in retention, although resolution between peaks improved. We suggest that this is due to the phase change of C_{18} bonded silica, which occurs at around 35°C,[38] In reversed phase HPLC using bonded phases, the explanation of curvature has sometimes been that there was a phase transition to a more ordered and extended phase.[40] The fact is that the temperature at around 35°C corresponds to the melting point of ODS stationary phase. Above this temperature, these ligands show the liquid like behavior.[38,39] Generally, when non-linear van't Hoff plots are observed, it is assumed that the enthalpy and entropy changes with temperature. But, as reported by Chester and Coym,[41] if changes in the phase ratio are considered, a non linear behavior may or may not be due to changes in enthalpy or entropy.

Detailed investigations on temperature dependence separation of ginsenosides are continuining and results will be published elsewhere.

Application

Using the UHPLC system we obtained high efficiency of ginsenosides separation with a shorter analysis time and decreased solvent consumption, compared to conventional HPLC for the same ginsenosides samples.[12,34] The optimized conditions (temperature and mobile phase velocity) were applied to the separation of

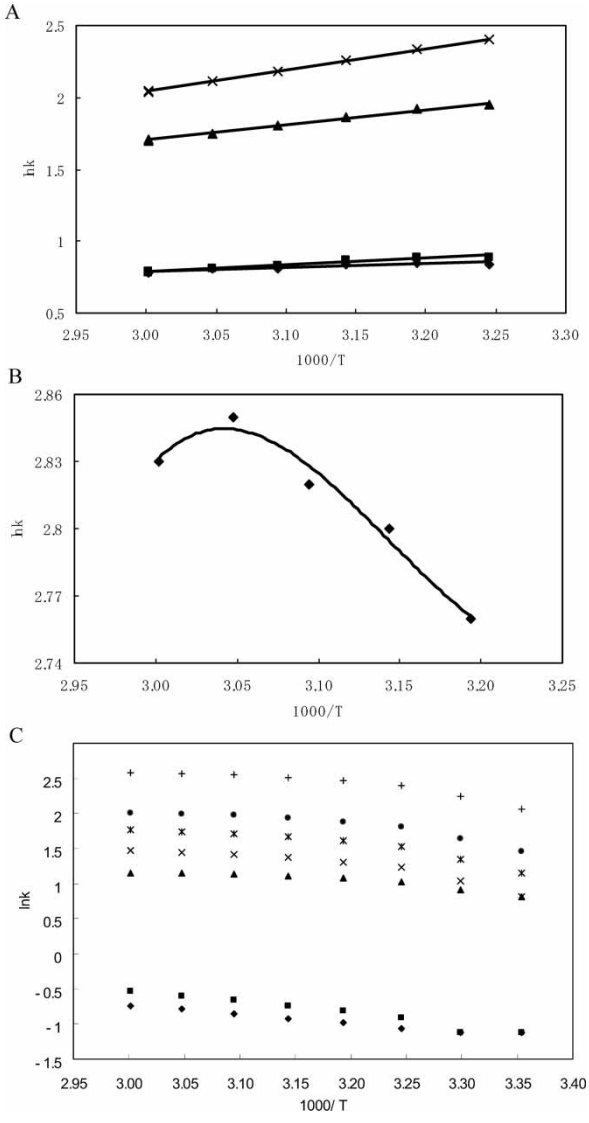

Figure 5. A. van't Hoff plot of Steroids (Hydrocortisone (◆), Cortisone (■), β-Estradiol (▲) and Estrone (x)) over the temperature range 35 to 60°C. Chromatographic conditions: Columns: X-PressPak C18S 2.0 μm, 2.1 mm i.d × 50 mm L and ODS C18, 1.8 μm, 2.1 mm i.d × 50 mm L; Mobile phase: Acetonitrile/Water – 40 : 60 v/v; Detection: 240 nm; Flow rate 0.2 mL/min. B. van't Hoff plot of Digitoxin from 40 to 60°C. Chromatographic conditions: Columns: X-PressPak C18S 2.0 μm, 2.1 mm i.d × 50 mm L and ODS C18, 1.8 μm, 2.1 mm i.d × 50 mm L; Mobile phase: Acetonitrile/Water – 40 : 60 v/v; Detection: 220 nm; Flow rate 0.2 mL/min. C. van't Hoff plot of Ginsenosides from 25 to 60°C. Ginsenosides: Re (◆), Rg1 (■), Rf (▲), Rb1 (x), Rc (*), Rb2 (•) and Rd (+). Chromatographic conditions are the same as in Figure 1.

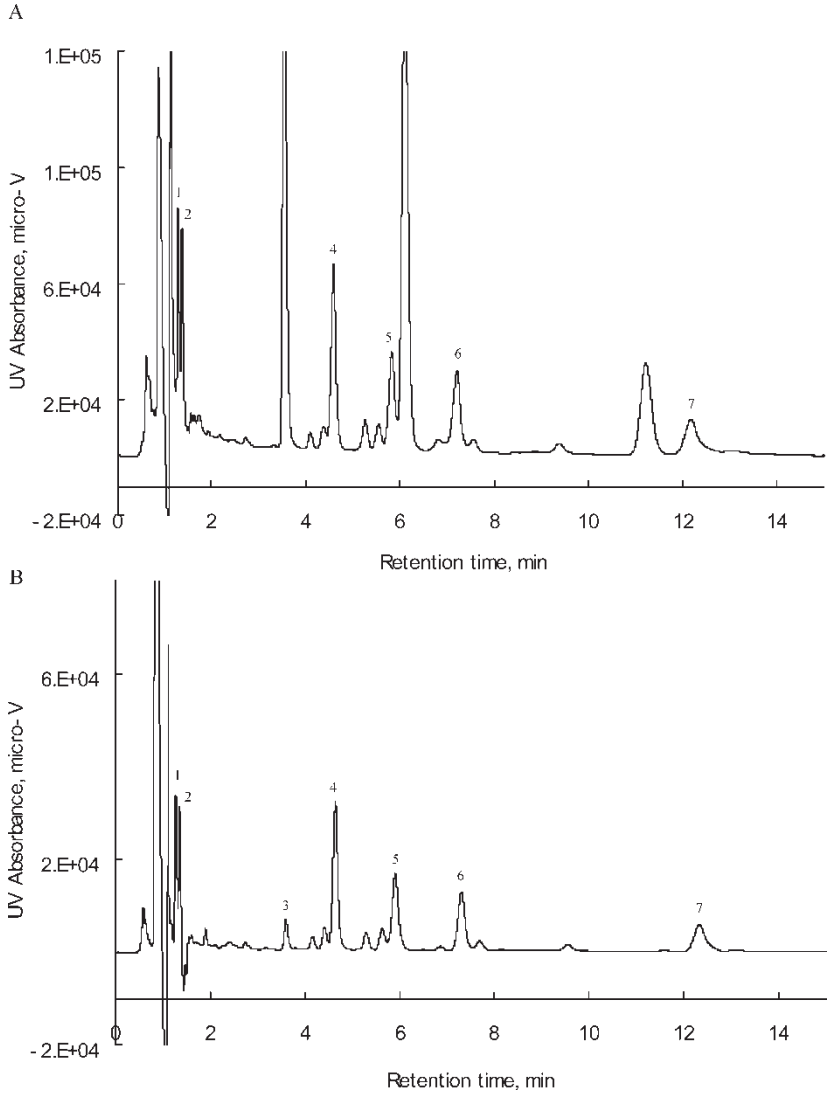

Figure 6. A and B. Chromatograms of two different commercial ginseng real samples. Chromatographic conditions are the same as in Figure 1.

ginsenoside in real samples. The samples were injected into the UHPLC system after solid phase extraction.[34] Figure 6 shows the chromatograms of the separation of ginsenosides in commercial ginseng samples, using the optimized conditions. The identification of the peaks was carried out based on the reference standard sample. Investigations on quantitative analysis of ginsenosides in real samples are still ongoing and results will be published elsewhere.

CONCLUSION

This work demonstrates the high separation power of UHPLC at elevated temperatures with the octadecyl silane bonded silica stationary phase columns for the analysis of ginsenosides with isocratic elution mode. The results obtained suggest that this chromatographic system can be useful for the separation of ginsenoside samples, giving good resolution with short analysis time and decreased solvent consumption.

The effect of temperature on the retention behavior of the ginsenoside was found to be significant. Generally, chromatographic separation of the ginsenosides was enhanced as the temperature was increased. Furthermore, an increase in retention times and improved resolutions of the analytes were observed. Two linear relationships in the van't Hoff plots were observed with all selected ginsenosides over the studied temperatures from 25 to 60°C. These observations can be attributed to the phase change of the stationary phase occurring at around 35°C.

The high resolution power of UHPLC at elevated temperatures was achieved by optimizing the chromatographic conditions. It was found that effective separation should be possible using the selected elevated temperature. The best separation of seven ginsenosides was observed at 60°C using acetonitrile/water – 35:65 v/v as the mobile phase within 13.0 min. This result was never before observed with ODS stationary phases using isocratic elution mode. These results indicate that increasing the temperature may increase the ability to resolve difficult to separate substances by UHPLC.

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REFERENCES

1. Harding, A.R. *Ginseng and Other Medicinal Plants*; A.R. Harding Publishing Co.: Columbus, Ohio, 1931.
2. Hu, S.Y. The genus Panax (Ginseng) in Chinese medicine. Econ. Bot. **1976**, *30*, 11.
3. Dharmananda, S. HerbalGram **2002**, *54*, 34.
4. Fuzzati, N. Analysis methods of ginsenosides. J. Chromatogr. B **2004**, *812* (1–2), 119–133.
5. Bonfill, M.; Casals, I.; Palazon, J.; Mallol, A. Improved high performance liquid chromatographic determination of ginsenosides in Panax ginseng-based pharmaceuticals using a diol column. Biomed. Chromatogr. **2002**, *16* (1), 68–72.
6. Lou, D.-W.; Saito, Y.; Zarzycki, P.K.; Ogawa, M.; Jinno, K. Isocratic separation of ginsenosides by high-performance liquid chromatography on a diol column at subambient temperatures. Anal. Bioanal. Chem. **2006**, *385* (1), 96–104.

7. Lou, D.-W.; Saito, Y.; Jinno, K. Solid-Phase Extraction and High-Performance Liquid Chromatography for Simultaneous determination of Important Bioactive Ginsenosides in Pharmaceutical Preparations. Chromatographia **2005**, *62* (7–8), 349–354.
8. Corthout, J.; Naessens, T.; Apers, S.; Vlietinck, A.J. Quantitative determination of ginsenosides from Panax ginseng roots and ginseng preparations by thin layer chromatography–densitometry. J. Pharm. Biomed. Anal. **1999**, *21* (1), 187–192.
9. Vanhaelen-Fastre, R.J.; Faes, M.L.; Vanhaelen, M.H. High-performance thin-layer chromatographic determination of six major ginsenosides in Panax ginseng. J. Chromatogr. A. **2000**, *868* (2), 269–276.
10. Cui, J.-F.; Bjorkhem, I.; Eneroth, P. Gas chromatographic-mass spectrometric determination of 20(S)-protopanaxadiol and 20(S)-protopanaxatriol for study on human urinary excretion of ginsenosides after ingestion of ginseng preparations. J. Chromatogr. B Biomed. Sci. Appl. **1997**, *689* (2), 349–355.
11. Zhu, S.; Zou, K.; Cai, S.; Meselhy, M.R.; Komatsu, K. Simultaneous determination of triterpene saponins in ginseng drugs by high-performance liquid chromatography. Chem. Pharm. Bull. **2004**, *52* (8), 995–998.
12. Pietta, P.; Mauri, P.; Rava, A. Improved high-performance liquid chromatographic method for the analysis of ginsenosides in Panax ginseng extracts and products. J. Chromatogr. **1986**, *356* (1), 212–9.
13. Kanazawa, H.; Nagata, Y.; Kurosaki, E.; Matsushima, Y.; Takai, N. Comparison of columns of chemically modified porous glass and silica in reversed-phase high-performance liquid chromatography of ginsenosides. J. Chromatogr. **1993**, *632* (1–2), 79–85.
14. Park, M.K.; Park, J.H.; Han, S.B.; Shin, Y.G.; Park, I.H. High-performance liquid chromatographic analysis of ginseng saponins using evaporative light scattering detection. J. Chromatogr. A. **1996**, *736* (1–2), 77–81.
15. Kwon, S.W.; Han, S.B.; Park, I.H.; Kim, J.M.; Park, M.K.; Park, J.H. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. J. Chromatogr. A. **2001**, *921* (2), 335–339.
16. Shangguan, D.; Han, H.; Zhao, R.; Zhao, Y.; Xiong, S.; Liu, G. New method for high-performance liquid chromatographic separation and fluorescence detection of ginsenosides. J. Chromatogr. A **2001**, *910* (2), 367–372.
17. Liu, S.; Cui, M.; Liu, Z.; Song, F.; Mo, W. Structural analysis of saponins from medicinal herbs using electrospray ionization tandem mass spectrometry. J. Am. Soc. Mass. Spectrom. **2004**, *15* (2), 133–141.
18. Majors, R.E. Recent Developments in LC Column Technology. LC•GC, Europe **2003**, *16* (6a).
19. MacNair, J.E.; Lewis, K.C.; Jorgenson, J.W. Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns. Anal. Chem. **1997**, *69* (6), 983–989.
20. MacNair, J.E.; Patel, K.D.; Jorgenson, J.W. Ultrahigh-pressure reversed-phase capillary liquid chromatography: isocratic and gradient elution using columns packed with 1.0-micron particles. Anal. Chem. **1999**, *71* (3), 700–708.
21. Tolley, L.T.; Jorgenson, J.W.; Moseley, M.A. Very high pressure gradient LC/MS/MS. Anal. Chem. **2001**, *73* (13), 2985–2991.
22. Lippert, J.A.; Xin, B.; Wu, N.; Lee, M.L. Fast ultrahigh-pressure liquid chromatography: On-column UV and time-of-flight mass spectrometric detection. J. Microcolumn Sep. **1999**, *11* (9), 631–643.
23. Wu, N.; Lippert, J.A.; Lee, M.L. Practical aspects of ultrahigh pressure capillary liquid chromatography. J. Chromatogr. A **2001**, *911* (1), 1–12.

24. Lan, K.; Jorgenson, J.W. Automated measurement of peak widths for the determination of peak capacity in complex chromatograms. Anal. Chem. **1999**, *71* (3), 709–714.
25. Xiang, Y.; Yan, B.; McNeff, C.V.; Carr, P.W.; Lee, M.L. Synthesis of micron diameter polybutadiene-encapsulated non-porous zirconia particles for ultrahigh pressure liquid chromatography. J. Chromatogr. A **2003**, *1002* (1–2), 71–78.
26. Jerkovich, A.D.; Mellors, J.S.; Jorgenson, J.W. *The Use of Micron-Sized Particles in Ultrahigh-Pressure Liquid Chromatography*; Recent Developments in LC Column Technology, June 2003.
27. Vanhoenacker, G.; Sandra, P. Elevated temperature and temperature programming in conventional liquid chromatography-fundamentals and applications. J. Sep. Sci. **2006**, *29* (12), 1822–1835.
28. Antia, F.; Horváth, Cs.J. High-performance liquid chromatography at elevated temperatures: examination of conditions for the rapid separation of large molecules. Chromatogr. A **1988**, *435*, 1–15.
29. Edge, A.M.; Shillingford, S.; Smith, C.; Payne, R.; Wilson, I.D. Temperature as a variable in liquid chromatography: development and application of a model for the separation of model drugs using water as the eluent. J. Chromatogr. A **2006**, *1132* (1–2), 206–210.
30. Greibrokk, T.; Andersen, T.J. High-temperature liquid chromatography. J. Chromatogr. A **2003**, *1000* (1–2), 743–755.
31. Wang, X.; Barber, W.E.; Carr, P.W. A practical approach to maximizing peak capacity by using long columns packed with pellicular stationary phases for proteomic research. J. Chromatogr. A **2006**, *1107* (1–2), 139–151.
32. Xiang, Y.; Yan, B.; Yue, B.; McNeff, C.V.; Carr, P.V.; Lee, M.L. Elevated-temperature ultrahigh-pressure liquid chromatography using very small polybutadiene-coated nonporous zirconia particles. J. Chromatogr. A **2003**, *983* (1–2), 83–89.
33. Sheng, G.; Sheng, Y.; Lee, M.L. Elevated temperature liquid chromatography using reversed-phase packed capillary columns. J. Microcol. Sep. **1997**, *9* (2), 63–72.
34. Lou, D.-W.; Saito, Y.; Jinno, K. Simultaneous LC determination of ginsenosides using a modified extraction procedure and an improved step gradient program. Chromatographia. **2006**, *63* (1–2), 31–37.
35. Bidlingmeyer, B.A. Trends in Reversed-Phase HPLC Column Practices. J. Chromatogr. Sci. **1997**, *35* (8), 392–401.
36. Warren, F.V.; Bidlingmeyer, B.A. Influence of temperature on column efficiency in reversed phase liquid chromatography. Anal. Chem. **1988**, *60* (24), 2821–2824.
37. Zarzycki, P.K.; Kulhanek, K.M.; Smith, R.; Bartoszuk, M.A.; Lamparczyk, H. Planar chromatography versus column chromatography: A performance comparison. LCGC North America **2005**, *23*, 286–300.
38. Ban, K.; Saito, Y.; Jinno, K. Molecular-dynamics simulation for the characterization of liquid chromatographic stationary phase: effect of temperature. Anal. Sci. **2005**, *21* (4), 397–402.
39. Ban, K.; Saito, Y.; Jinno, K. Characterization of the microscopic surface structure of the octadecylsilica stationary phase using a molecular-dynamics simulation. Anal. Sci. **2004**, *20* (10), 1403–1408.
40. Sentel, K.B.; Henderson, A.N. Entropy dominated subambient temperature separations in reversed-phase liquid chromatography. Anal. Chem. Acta **1991**, *246* (1), 139–149.

41. Chester, T.L.; Coym, J.W. Effect of phase ratio on van't Hoff analysis in reversed-phase liquid chromatography, and phase-ratio-independent estimation of transfer enthalpy. J. Chromatogr. A **2003**, *1003* (1–2), 101–11.

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