Preparative High-Performance Liquid Chromatography on Chemically Modified Porous Glass. Isolation of Saponins from Ginseng

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Preparative high-performance liquid chromatography on octadecylsilyl porous glass (pore size, 550 Å) with acetonitrile–water as the mobile phase was applied for the isolation of saponins from *Panax ginseng*. In a single run, several milligrams of pure ginsenosides were obtained from 10 g of roots of *Panax ginseng*. The method was simple, rapid and convenient and should be applicable to isolation of other saponins of crude drugs.

Keywords preparative high-performance liquid chromatography; octadecylsilyl porous glass; saponin; ginsenoside; *Panax ginseng*

We prepared octadecylsilyl porous glass (MPG-ODS) as the packing material for reversed phase high-performance liquid chromatography (HPLC).[1] Rapid, simple and accurate determinations of ginsenosides and other saponins in crude drugs and pharmaceutical preparations were achieved by the HPLC on MPG-ODS.[2–5] As a part of our interest in the HPLC on MPG-ODS, we applied the method for preparative purposes. This paper describes the preparative separation of ginsenoside-Rb_1, Rc, Rb_2, Rd, Rg_1 and Re from *Panax ginseng*. An automatic fraction collection system was used.

Experimental

Materials MPG-ODS was supplied from Ise Chemical Industries Co., Ltd.[6] The particle size of the packing material was 10 μm for the analytical and 20 μm for the preparative columns. Acetonitrile used as the mobile phase of analytical HPLC was of HPLC grade (Wako, Tokyo, Japan). Water was deionized and distilled. Other chemicals were of reagent grade.

Analytical HPLC The analytical HPLC system was composed of a Tosoh Model CCPM multi pump, a Rheodyne Model 7125 valve, a Tosoh Model UV-8000 monitor and a Hitachi Model 833A dataprocessor. The system was operated at room temperature. A stainless-steel column (150 × 4 mm i.d.) packed with MPG-ODS was used. The mobile phases were mixtures of acetonitrile and water. The flow-rate was 1 ml/min and the peaks were monitored at 203 nm.

Preparative HPLC The preparative HPLC systems used consisted of a Tosoh Model CCPM prep pump, a UV-8100 monitor, an SC-8010 system controller and data processor, and an FC-8000 fraction collector and a PP-8010 recorder. MPG-ODS was packed into stainless-steel tubings of 500 × 20 mm i.d. and of 500 × 50 mm i.d. The system was operated at room temperature.

Sample Preparation from the Crude Drug Roots of *Panax ginseng* were pulverized and extracted with methanol. The extract was evaporated and dissolved in water. The aqueous solution was passed through a solid phase, Mega Bond Elute (Analytichem International), pretreated with water and methanol. After the solid phase was washed with water and 30% methanol, the sample was eluted with methanol and the eluate was evaporated to dryness under reduced pressure. The residue was dissolved in the eluent and injected into the preparative HPLC system.

Results

Octadecylsilyl porous glass (MPG-ODS) used in the preparative HPLC was the same as used in the analytical HPLC in its composition and pore size (550 Å). MPG-ODS with a particle size of 20 μm was packed in the columns for preparative HPLC, though that with the 10-μm size was used in the HPLC analysis of saponins reported previously.[2–5] Chromatograms from the analytical column packed with the 20-μm size MPG-ODS are shown in Fig. 1. They were similar to those obtained with the 10-μm size MPG-ODS under the same conditions, though the peaks were somewhat broader.

The mobile phases used in the preparative HPLC were mixtures of acetonitrile and water. The compositions of the mobile phase for the 500 × 20 mm i.d. column were 16% acetonitrile for the separation of ginsenoside Rg_1 and Re and 28% acetonitrile for that of ginsenoside Rb_1, Rc, Rb_2

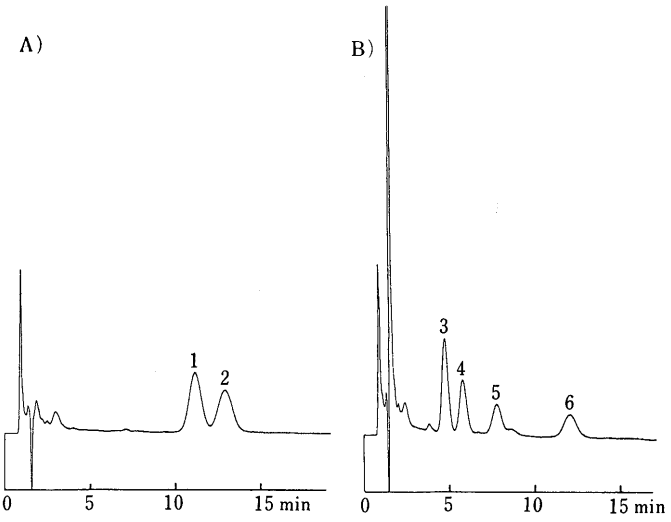

Fig. 1. Analytical Chromatograms of a Ginseng Extract

Peak No.: 1, ginsenoside-Rg_1; 2, ginsenoside-Re; 3, ginsenoside-Rb_1; 4, ginsenoside-Rc; 5, ginsenoside-Rb_2; 6, ginsenoside-Rd. Column: MPG-ODS (150 × 4 mm i.d., particle size, 20 μm). Eluent: A, acetonitrile–water (16 : 84); B, acetonitrile–water (27.5 : 72.5). Flow-rate: 1.0 ml/min. Detection: 203 nm.

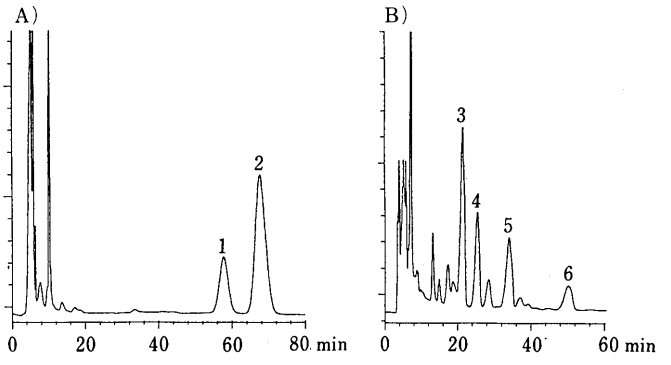

Fig. 2. Preparative Chromatograms of a Ginseng Extract

Peak numbers are the same as Fig. 1. Column: MPG-ODS (500 × 20 mm i.d., particle size, 20 μm). Eluent: A, acetonitrile–water (16 : 84); B, acetonitrile–water (28 : 72). Flow-rate: 15.0 ml/min. Detection: 203 nm.

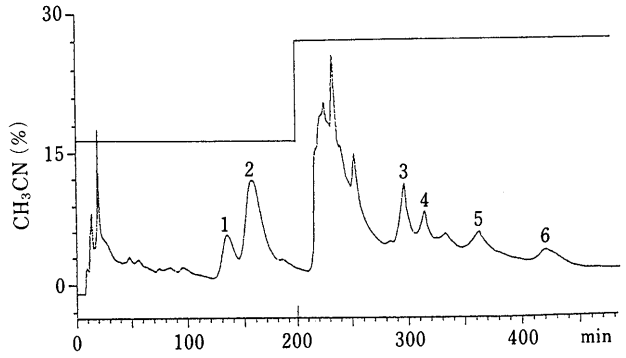

Fig. 3. Preparative Chromatogram of a Ginseng Extract

Peak numbers are the same as Fig. 1. The acetonitrile concentration in the mobile phase is plotted as ordinate. Column: MPG-ODS (500 × 50 mm i.d., particle size, 20 μm). Flow-rate: 35.0 ml/min. Detection: 203 nm.

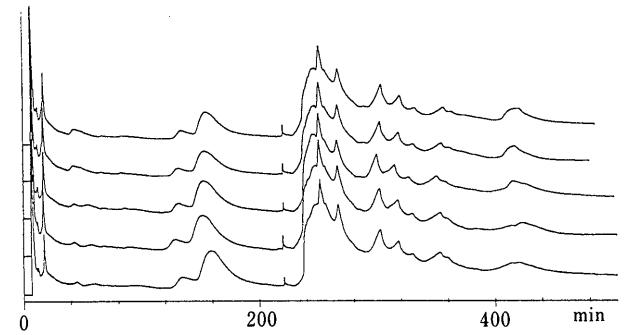

Fig. 4. The Reproducibility of the Preparative Chromatograms

Column: MPG-ODS (500 × 20 mm i.d., particle size, 20 μm). Eluent: 0—220 min, acetonitrile–water (16:84); 220—450 min, acetonitrile–water (27:73). Flow-rate: 39.0 ml/min. Detection: 203 nm.

and Rd. The flow-rate was 15 ml/min and the pressure was 55 kgf/cm^2. The peaks were monitored at 203 nm. In a run, an extract from 0.3 to 0.6 g of ginseng root was loaded on the HPLC. The chromatograms are shown in Fig. 2. The six ginsenosides were separated within 70 min and their yields were in the range of 0.1 to 1 mg in a single run.

In preparative HPLC a column of 500 × 50 mm i.d. was used, to which the extracts from 10 g of ginseng were applied. In order to isolate major ginsenosides by a single run, a step gradient elution was used. The mobile phase compositions were 16% acetonitrile at 0—200 min, and 28% at 200—450 min with a flow-rate of 35 ml/min and a pressure of 38 kgf/cm^2. The chromatogram is shown in Fig. 3. Several repeated runs gave almost the same chromatograms as shown in Fig. 4. The reproducibility enabled us to collect fractions with an automatic system.

In a single preparative HPLC run, 4—5 mg of ginsenoside-Rg_1, 7—8 mg of ginsenoside-Re and 7—10 mg of ginsenoside-Rb_1 were separated from 10 g of ginseng roots. The purity of the ginsenosides was confirmed by the analytical chromatograms as described below. The average recoveries in four runs were 59.5, 56.0 and 63.6% for ginsenoside-Rg_1, Re and Rb_1, respectively. Ginsenoside-Rc, Rb_2, Rd obtained in the run were contaminated with minor components and they were rechromatographed with the 500 × 20 mm i.d. column. About 2—3 mg of pure ginsenoside-Rc and 1.5—3 mg of ginsenoside-Rb_2 and 0.4—1.4 mg of ginsenoside-Rd were separated by the procedures. The recoveries were 53.0, 41.5 and 41.0 for ginsenoside-Rc, Rb_2 and Rd, respectively.

The analytical HPLC system was used to monitor the purity of the collected fractions. Each fraction obtained gave a single chromatographic peak and the impurities contained were less than 1%. The fractions were also analyzed by the thin layer chromatography (TLC) with the lower layer of a mixture of chloroform, methanol and water (13:7:2) as the solvent. A single TLC spot with the same *Rf* value as the standard sample of the assigned peak was observed for each fraction. The fractions were evaporated and freeze dried. White crystalline products were obtained. The ^{13}C nuclear magnetic resonance spectra of the products were exactly the same as those of authentic ginsenosides.

Discussions

There have been several reports[7-10] on preparative-scale HPLC of ginsenosides. Before 1981,[7-8] the preparative HPLC was used for the final purification of crude saponin fractions obtained by other means. Kaizuka and Takahashi[9] reported the separation by normal phase HPLC on PrepPAK-500 silica gel column using chloroform–methanol–ethanol–water. Water-soluble compounds are most conveniently separated by reversed phase (RP) columns and it is undesirable to use halogen containing solvents on a preparative scale. In these works, saponin fractions were monitored by refractive index detectors.

Recently, sensitive analyses of ginsenoside have been developed on RP-HPLC with ultraviolet detection at short wavelength. It is usually easy to scale up to preparative separation under similar chromatographic conditions. Yamaguchi and coworkers[10] separated ginsenosides on a preparative scale as borate complexes on a basic ion-exchange column. Before obtaining pure saponins, borate had to be removed as volatile methyl borate by repeated co-distillation of each eluate with methanol and the procedure seems to be somewhat tedious.

We separated ginsenosides by preparative HPLC with RP column of chemically modified porous glass, MPG-ODS. The excellent separation may be partly due to the optimal pore size of 550 Å and the narrow distribution range of the size.[1,6] The composition of organic solvent in the mobile phase was much smaller in HPLC with MPG-ODS than with silica-ODS columns. The savings in organic solvents are advantageous for both economical and environmental reasons. The chromatographic conditions were almost identical for analytical and preparative HPLC. The conditions in preparative scales can be readily estimated from the analytical conditions and this provides another advantage for MPG-ODS. The present method does not require any special equipment such as a pump capable of producing high flow rates. The HPLC pump used was operated at flow-rates of 0.01—40 ml/min.

In conclusion, the method is simple, rapid and convenient and is suited for the laboratory-scale purification of ginsenosides. It should be applicable to the isolation of other saponins of crude drugs. Preparative separations of malonylginsenosides and the other saponins on MPG-ODS column are in progress in our laboratories.

Acknowledgement This work was supported in part by The Science Research Promotion Fund of the Japan Private School Promotion

Foundation and by Ise Chemical Industries Co., Ltd. Technical assistances of Yasushi Asano, Akinori Sato and Emi Kurosaki are gratefully acknowledged.

References and Notes

1) Y. Matsushima, Y. Nagata, K. Takakusagi, M. Niyomura and N. Takai, *J. Chromatogr.*, **332** 265 (1985).
2) H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *Chromatographia*, **24**, 517 (1987).
3) H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *Shoyakugaku Zasshi*, **43**, 121 (1989).
4) N. Takai, H. Kanazawa, Y. Nagata, Y. Matsushima and M. Tomoda, *Seisan Kenkyu*, **41**, 773 (1989).
5) H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *J. Chromatogr.*, **507**, 327 (1990).
6) The packed columns of MPG-ODS are now commercially available from Wako (IPG-55-ODS-10H) and Hitachi (Hitachi gel #3161).
7) T. Nagasawa, T. Yokozawa, Y. Nishino and H. Oura, *Chem. Pharm. Bull.*, **28**, 2059 (1980); *idem*, *ibid.*, **28**, 3701 (1980).
8) J. Zhou, M. Z. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari and T. Fuwa, *Chem. Pharm. Bull.*, **29**, 2844 (1981).
9) H. Kaizuka and K. Takahashi, *J. Chromatogr.*, **258**, 235 (1983).
10) H. Yamaguchi, H. Matsuura, R. Kasai, K. Mizutani, H. Fujino, K. Ohtani, T. Fuwa and O. Tanaka, *Chem. Pharm. Bull.*, **34**, 2856 (1986).