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Simultaneous determination of ginsenosides and saikosaponins by high-performance liquid chromatography

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SUMMARY

Octadecyl porous glass was used as the packing for reversed-phase high-performance liquid chromatography. A mixture of ginsenosides and saikosaponins (saponins of ginseng and bupleurum root, respectively) were analysed with detection at 203 nm. A well resolved chromatogram of ginsenoside Rb_1, Rc, Rb_2 and Rd and saikosaponin a, b_2 and c was obtained with acetonitrile–water (25.5:74.5) as the mobile phase. The whole separation was achieved in 20 min with a flow-rate of 1.5 ml/min. Calibration graphs for ginsenoside Rb_1, Rc, Rb_2 and Rd and saikosaponin a and c were linear up to 5 μg. Rapid and accurate simultaneous determinations of the saponins are possible by the described method.

INTRODUCTION

Octadecyl porous glass (IPG-ODS) was previously prepared as a packing for reversed-phase high-performance liquid chromatography (HPLC)[1] and rapid and excellent separations and determinations of ginsenosides were achieved with the column[2,3]. Ginsenosides are saponins of ginseng, the roots of *Panax ginseng*, which has log been used in traditional oriental medicine.

In medicine, several crude drugs are generally prescribed in a single formula. Among the important crude drugs often prescribed with ginseng are bupleurum root (the roots of *Bupleurum falcatum*) and glycyrrhiza. Pharmacological studies on these crude drugs have centred on the saponins. Ginsenosides, saikosaponins and glycyrrhizin were isolated from ginseng, bupleurum root and glycyrrhiza, respectively. They are the glycosides of tetracyclic and pentacyclic triterpenes and the sugar moieties contain glucose, fucose, rhamnose, glucuronic acid, arabinose, xylose, etc. Structures are shown in Fig. 1.

As the crude drugs are used in the same pharmaceutical preparations, simultaneous determinations of the saponins are often required. This paper describes

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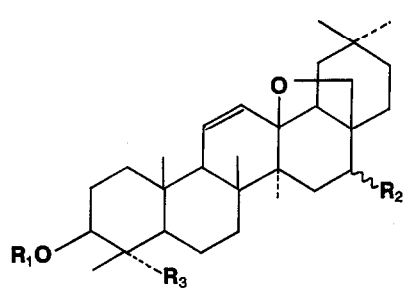
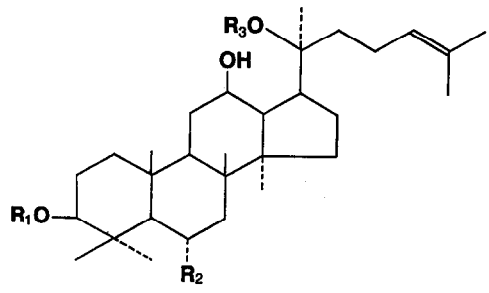


	R_1	R_2	R_3
Saikosaponin a	Fuc^{3}Glc	β-OH	CH_2OH
c	Glc^{4}(Glc^{6})Rha	β-OH	CH_3
d	Fuc^{3}Glc	α-OH	CH_2OH



	R_1	R_2	R_3
Ginsenoside Rb_1	Glc^{2}Glc-	H	Glc^{6}Glc-
Rb_2	Glc^{2}Glc-	H	Ara(p)^{6}Glc-
Rc	Glc^{2}Glc-	H	Ara(f)^{6}Glc-
Rd	Glc^{2}Glc-	H	Glc-

Fig. 1. Structures of saikosaponins and ginsenosides.

the simultaneous HPLC determination of the saponins of ginseng and bupleurum root using an IPG-ODS column. The determination of acidic saponins such as glycyrrhizin, ginsenoside Ro and malonyl derivatives of ginsenosides and saikosaponin was also possible by HPLC with an IPG-ODS column, details of which were reported separately[4].

EXPERIMENTAL

The HPLC system consisted of a Tosoh Model CCPM multi-pump, a Rheodyne Model 7125 valve, a Tosoh Model UV-8000 monitor and a Hitachi Model 833A data processor. The system was operated at room temperature.

Octadecylsilyl porous glass (IPG-ODS) was supplied by Ise Chemical Industries (Tokyo, Japan) with a pore size of 550 Å and a particle size distribution of 8–10 μm. IPG-ODS was packed into a stainless-steel column (150 × 4.0 mm I.D.) by the high-pressure slurry technique.

Acetonitrile used as the mobile phase was of HPLC grade (Wako, Tokyo, Japan). Water was deionized and distilled. Other chemicals were of analytical-reagent grade.

Standard samples of ginsenosides were kindly supplied by Prof. J. Shoji of Showa University, Tokyo. The diene-saikosaponins were prepared by treating the parent saponins with 0.1 *M* hydrochloric acid. Standard samples of other saponins, crude drugs and pharmaceutical preparations were obtained from commercial sources.

RESULTS AND DISCUSSION

Chromatograms of a mixture of saikosaponins monitored at 203 and 254 nm are shown in Fig. 2. The C_{13}–C_{28} cyclic ether moieties of saikosaponin a, c and d are labile under acidic conditions and the diene products, saikosaponin b_1, b_2 and h, are derived

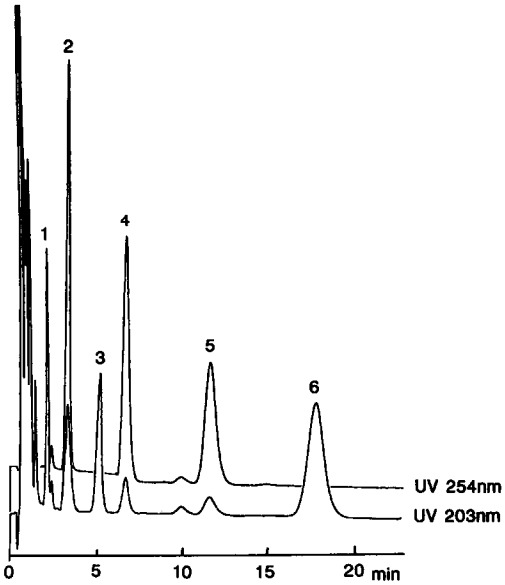


Fig. 2. HPLC of a mixture of saikosaponins. 1 = Saikosaponin c; 2 = saikosaponin h; 3 = saikosaponin a; 4 = saikosaponin b_2; 5 = saikosaponin b_1; 6 = saikosaponin d. Column, IPG-ODS (150 × 4 mm I.D.); eluent, acetonitrile–water (28.0:72.0); flow-rate, 2.0 ml/min; detection, 254 and 203 nm.

from saikosaponin a, d and c, respectively. The diene-saponins have a strong absorption band at *ca.* 250 nm, which is lacking in the parent saponins. Previously reported HPLC methods for the analysis of saikosaponins were based on the absorption at 254 nm after conversion of the ether-saponins into diene-saponins[5,6].

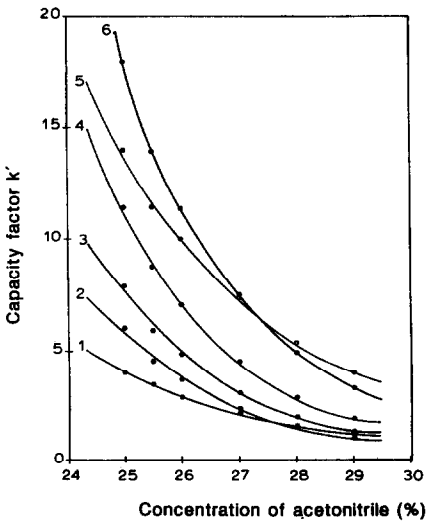


Fig. 3. Effect of acetonitrile concentration in the mobile phase on the capacity factor (k') of saponins. 1 = Saikosaponin c; 2 = ginsenoside Rb_1: 3 = ginsenoside Rc; 4 = ginsenoside Rb_2; 5 = saikosaponin a; 6 = ginsenoside Rd.

Such methods are not suitable for the analysis of samples containing the both types of saponins, such as decocted preparations. The simultaneous determination of saikosaponin a, b, c, d and h was possible by monitoring at 203 nm using the present conditions.

A mixture of ginsenoside Rb_1, Rc, Rb_2 and saikosaponin a and c was chromatographed by monitoring at 203 nm with a flow-rate of 1.5 ml/min with varying concentrations of acetonitrile–water as the mobile phase. The results in Fig. 3 indicate that the six saponins were separated with a mobile phase containing 25% of acetonitrile. A well resolved chromatogram of the six saponins and two diene-saikosaponins was obtained with acetonitrile–water (25.5:74.5) in 20 min, as shown in Fig. 4.

Solutions containing 0–5 μg of the saponin were injected and the corresponding peak areas integrated. The calibration graphs, shown in Fig. 5, were linear and reproducible. Results of the regression analysis and the correlation coefficients (r) were as follows: saikosaponin a, $y = 3.54x - 0.19$ ($r = 0.999$); saikosaponin c, $y = 2.33x - 0.32$ ($r = 0.999$); ginsenoside Rb_1, $y = 3.09x - 0.45$ ($r = 0.999$); ginsenoside Rc, $y = 1.35x - 0.09$ ($r = 0.999$); ginsenoside Rb_2, $y = 0.92x - 0.05$ ($r = 0.999$); and ginsenoside Rd, $y = 1.11x - 0.08$ ($r = 0.999$).

Extracts of bupleurum root and ginseng and of a mixture of the two drugs were analysed using the above procedure. Ginseng was extracted with methanol as described previously[2,3]. Bupleurum root was extracted with weakly alkaline methanol. The extracts were applied to a Sep-Pak C_{18} cartridge, dissolved in the mobile phase and injected into the HPLC system. Separation of the saponin peaks was satisfactory.

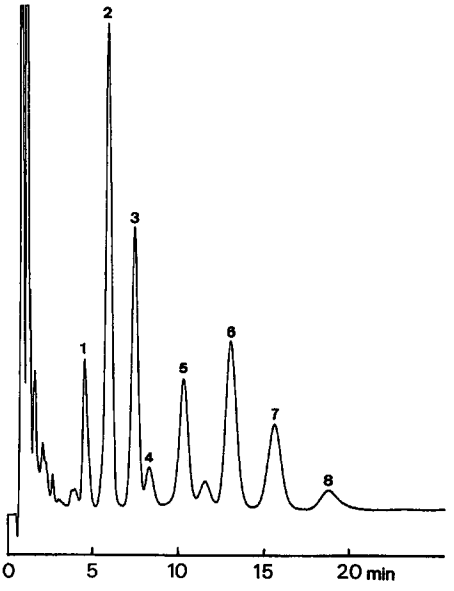


Fig. 4. HPLC of a mixture of saponins. 1 = Saikosaponin c; 2 = ginsenoside Rb_1; 3 = ginsenoside Rc; 4 = saikosaponin h; 5 = ginsenoside Rb_2; 6 = saikosaponin a; 7 = ginsenoside Rd; 8 = saikosaponin b_2. Column, IPG-ODS (150 × 4 mm I.D.); eluent, acetonitrile–water (25.5:74.5); flow-rate, 1.5 ml/min; detection, 203 nm.

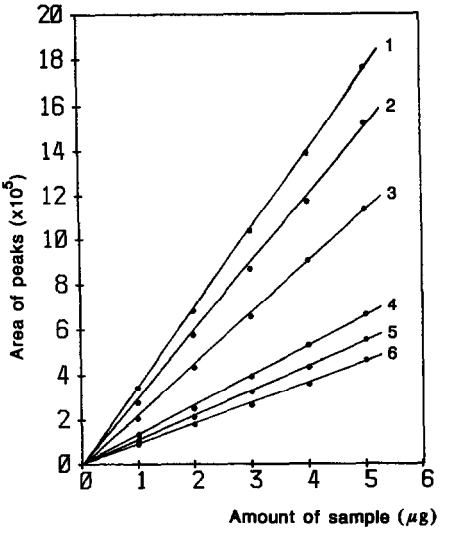


Fig. 5. Calibration graphs for saponins. 1 = Saikosaponin a; 2 = saikosaponin c; 3 = ginsenoside Rb_1; 4 = ginsenoside Rc; 5 = ginsenoside Rb_2; 6 = ginsenoside Rd. Column, IPG-ODS (150 × 4 mm I.D.); eluent, acetonitrile–water (25.5:74.5); flow-rate, 1.5 ml/min; detection, 203 nm.

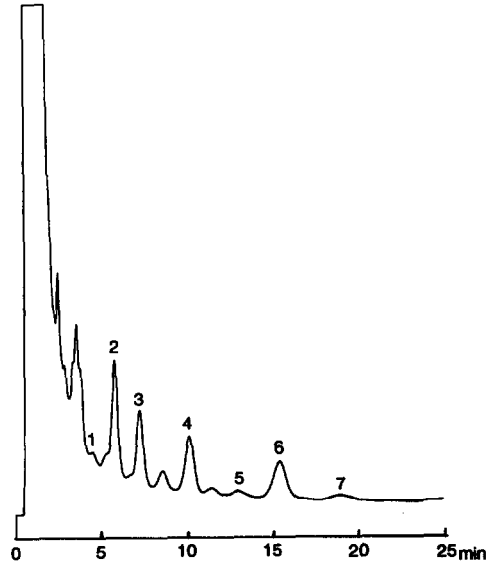


Fig. 6. HPLC of saponins from a commercial product (Shosaiko-to). 1 = Saikosaponin c; 2 = ginsenoside Rb_1; 3 = ginsenoside Rc; 4 = ginsenoside Rb_2; 5 = saikosaponins a; 6 = ginsenoside Rd; 7 = saikosaponin b_2. Column, IPG-ODS (150 × 4 mm I.D.); eluent, acetonitrile–water (25.5:74.5); flow-rate, 1.5 ml/min; detection, 203 nm.

Known amounts of saikosaponins were added to bupleurum root, the crude drug was extracted and saikosaponins in the extracts were determined. The recoveries of the amounts added were in the range 97–101%. The recovery test for ginsenosides has been described previously[2].

A pharmaceutical preparation of commercial origin, Shosaiko-to, was analysed by the above procedure. The analytical sample was prepared in the same way as for the crude drugs. The chromatogram (Fig. 6) indicated the presence of saikosaponin b_2, a diene-saponin. The content of the saponins in the preparation were as follows: ginsenoside Rb_1, 0.11; ginsenoside Rc, 0.13; ginsenoside Rb_2, 0.12; saikosaponin a, 0.03; and ginsenoside Rd, 0.19%. The contents of saikosaponin c and b_2 were too small to be determined exactly.

For the analysis of pharmaceutical preparations containing crude drugs other than ginseng and bupleurum root, the methanol extract was further extracted with butanol. This procedure did not affect the determinations of saikosaponins and ginsenosides. The following crude drugs did not give interfering chromatographic peaks: glycyrrhiza, ginger, atractylodes rhizome, hoelen, jujuba, pinellia tuber, scutellaria root, citrus unshu peel, evodia fruit, coptis rhizome, ophiopogon tuber, non-glutinous rice, cinnamon bark, peony root, Japanese angelica root, cimicifuga rhizome, astragalus root, cnidium rhizome, rehmannia root and magnolia bark.

It is concluded that the simultaneous determination of saikosaponins and ginsenosides is possible by the present method. The determination of the saponins in crude drugs and various pharmaceutical preparations is in progress.

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