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Short Communication

Determination of acidic saponins in crude drugs by high-performance liquid chromatography on octadecylsilyl porous glass

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ABSTRACT

High-performance liquid chromatographic (HPLC) analysis on octadecylsilyl porous glass was investigated for acidic saponins in ginseng, bupleurum root and senega. The acidic saponins, malonyl-ginsenosides, malonyl-saikosaponins and senegins, as well as neutral saponins in the crude drugs were separated rapidly by HPLC on this column with aqueous acetonitrile containing KH_2PO_4 as the mobile phase at room temperature.

INTRODUCTION

In previous work, an octadecylsilyl porous glass (MPG-ODS) was prepared and it was found to be a useful packing material for reversed-phase high-performance liquid chromatography (HPLC) [1,2]. Saponins of ginseng, the root of *Panax ginseng,* and other crude drugs were determined by HPLC on MPG-ODS [3–5].

Neutral dammarane saponins (ginsenosides-Rb_1, -Rb_2, -Rc and -Rd) and an acidic saponin (ginsenoside-Ro, a glucuronide saponin of oleanolic acid) isolated from ginseng have been extensively studied from the chemical, pharmacognostic and pharmacological view points [6–8]. Kitagawa et *al.* [9] reported that white ginseng contains a considerable amount of acidic malonate of the dammarane saponins, malonyl-ginsenosides-Rb,, -Rb_2, -Rc and -Rd. These malonyl-ginsenosides are unstable and readily demalonylated by heating. Recently, acidic malonate of saikosaponins, malonyl-saikosaponin a and d, were also isolated from bupleurum root [10]. Other well known acidic saponins are those contained in senega. Senega is the root of ***Polygala senega*** and has been used as an expectorant. Acidic saponins of Senega, senegins II, II', III and IV, are the glycosides of pentacyclic triterpenes and the sugar moieties contain glucose, fucose, rhamnose, xylose, galactose, etc. The structures of the acidic saponins and related netural saponins are shown in Fig. 1.

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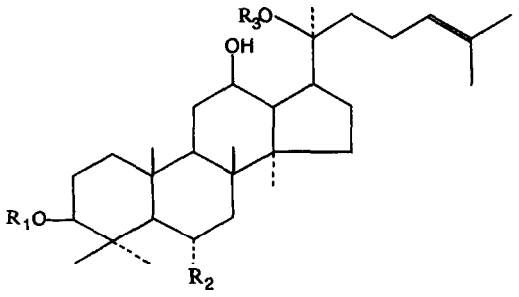
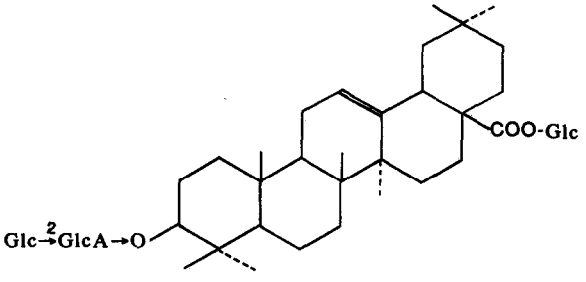
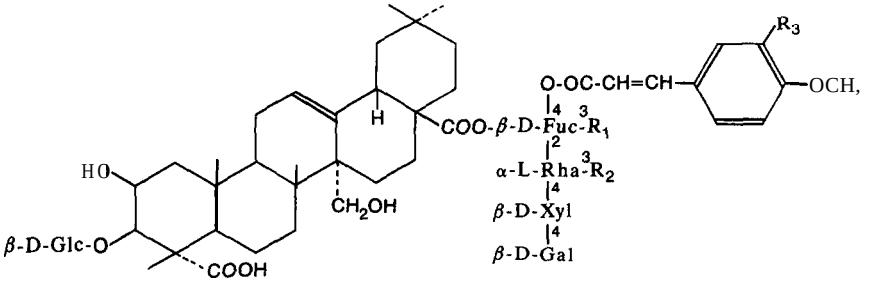


		R_1	R_2	R_3
malonyl-ginsenoside	Rb_1	Ma→^{6}Glc→^{2}Glc→	H	Glc→^{6}Glc→
malonyl-ginsenoside	Rb_2	Ma→^{6}Glc→^{2}Glc→	H	Ara*p*→^{6}Glc→
malonyl-ginsenoside	Rc	Ma→^{6}Glc→^{2}Glc→	H	Ara*f*→^{6}Glc→
malonyl-ginsenoside	Rd	Ma→^{6}Glc→^{2}Glc→	H	Glc→
ginsenoside	Rb_1	Glc→^{2}Glc→	H	Glc→^{6}Glc→
ginsenoside	Rb_2	Glc→^{2}Glc→	H	Ara*p*→^{6}Glc→
ginsenoside	Rc	Glc→^{2}Glc→	H	Ara*f*→^{6}Glc→
ginsenoside	Rd	Glc→^{2}Glc→	H	Glc→
ginsenoside	Re	H	Rha→^{2}Glc→O	Glc→
ginsenoside	Rf	H	Glc→^{2}Glc→O	H
ginsenoside	Rg,	H	Glc→O	Glc→
ginsenoside	Rg_2	H	Rha→^{2}Glc→O	H

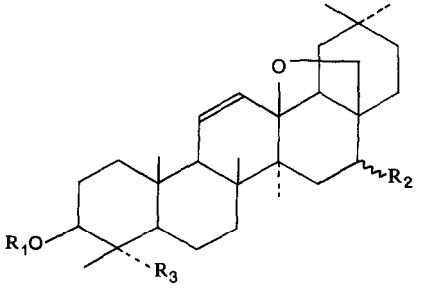


ginsenoside Ro (chikusetsusaponin-V)

Fig. 1.



	R_1	R_2	R_3
senegin II	H	H	OCH,
senegin II	H	H	H
senegin III	a-L-Rha	H	H
senegin IV	a-L-Rha	a-L-Rha	H



	R_1	R_2	R_3
malonyl-saikosaponin a	Ma→6Glc→3Fuc→	β-OH	CH_2OH
malonyl-saikosaponin d	Ma→6Glc→3Fuc→	a-OH	CH_2OH
saikosaponin a	Glc→3Fuc→	β-OH	CH_2OH
saikosaponin c	Rha→4Glc→ (Glc→6)	β-OH	CH_3
saikosaponin d	Glc→3Fuc→	α-OH	CH_2OH

Fig. 1. Structures of saponins. Ma = malonyl; Glc, β-D-glucopyranosyl; Rha = α-L-rhamnopyranosyl; Ara, = a-L-arabinopyranosyl; Ara, = a-L-arabinofuranosyl; GlcA = β-D-glucuropyranosyl; Xyl = xylopyranosyl; Gal = galactopyranosyl; Fuc = β-D-fucopyranosyl.

In traditional oriental medicine, several crude drugs are generally prescribed in a single formula. Ginseng is one of the most important crude drugs in such medicine. Bupleurum root is often prescribed with ginseng.

In this paper, we report the HPLC analysis on an MPG-ODS column of the acidic saponins in the crude drugs. Simultaneous determinations of the acidic and neutral saponins were successful at room temperature.

EXPERIMENTAL

Materials

Acetonitrile was of HPLC grade (Wako, Tokyo, Japan). Water for use as a mobile phase constituent was prepared by passage through a Milli-Q water purification unit (Millipore, Bedford, MA, USA). Other chemicals were of analytical-reagent grade. A Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) was used for pretreatment of samples.

The standard samples of ginsenosides-Rb_1, -Rb_2, -Rc, -Rd, -Rf, -Rg_2 and -Ro, and senegins II, II', III and IV were kindly supplied by Professor J. Shoji (Showa University, Tokyo), those of malonyl-ginsenosides-Rb_1, -Rb_2, -Rc and -Rd by Professor I. Kitagawa (Osaka University, Osaka) and those of malonyl-saikosaponins a and d by Dr. H. Taguchi (Tsumura, Ibaragi, Japan). Saikosaponins a, c and d were purchased from Wako.

HPLC conditions

Octadecylsilyl porous glass (MPG-ODS) was supplied by Ise Chemical Industrial (Tokyo, Japan). Its pore size was 550 Å and its particle size distribution was 8–10 μm. MPG-ODS was packed into stainless-steel columns (150 × 4.0 mm I.D. and 250 × 4.0 mm I.D.) by the high-pressure slurry technique. The effective theoretical plate numbers were 6100 and 7070, respectively. The HPLC system used consisted of a Tosoh Model CCPM multipump, 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.

Sample preparation from crude drugs

A 1.0-g mass of crude drugs was pulverized and extracted with 20 ml of 70% methanol at room temperature (20°C) for 30 min and the extract was filtered. After the procedure had been repeated five times, the extracts were combined and evaporated. The residue was dissolved in water and applied to a Sep-Pak C_{18} cartridge pretreated with 5 ml of water and 2 ml of methanol. After washing the cartridge with 10 ml of water and 15 ml of 30% methanol, the sample was eluted with 5 ml of methanol and the eluate was evaporated to dryness under reduced pressure. The residue was dissolved in 2 ml of the eluent and a 1–10-μl portion was injected into the HPLC system.

RESULTS

For the HPLC of neutral saponins on the MPG-ODS columns, a mixture of acetonitrile and water was used as the mobile phase [3–5]. A mobile phase containing 15–50% of acetonitrile in water did not give satisfactory results for the separation of the acidic saponins.

With aqueous acetonitrile containing KH_2PO_4, excellent HPLC separation of the acidic saponins and also the neutral saponins was achieved. Fig. 2 shows the correlation of the concentration of KH_2PO_4 with the k' values of the saponins.

Isocratic elution was examined for quantitative analysis. As shown in Fig. 3, ginsenosides-Rb_1,

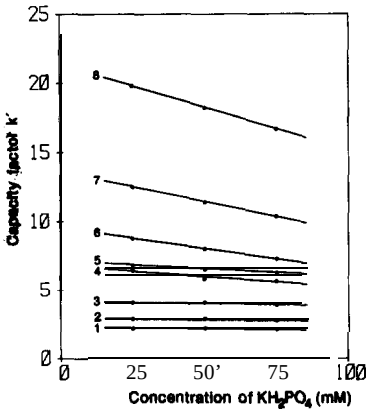


Fig. 2. Effect of concentration of KH_2PO_4 on the capacity factors (k') of the saponins: 1 = malonyl-ginsenoside-Rb,; 2 = malonyl-ginsenoside-Rc; 3 = malonyl-ginsenoside-Rb,; 4 = ginsenoside-Rb,; 5 = malonyl-ginsenoside-Rd; 6 = ginsenoside-Rc; 7 = ginsenoside-Rb,; 8 = ginsenoside-Rd. Concentration of acetonitrile, 25%.

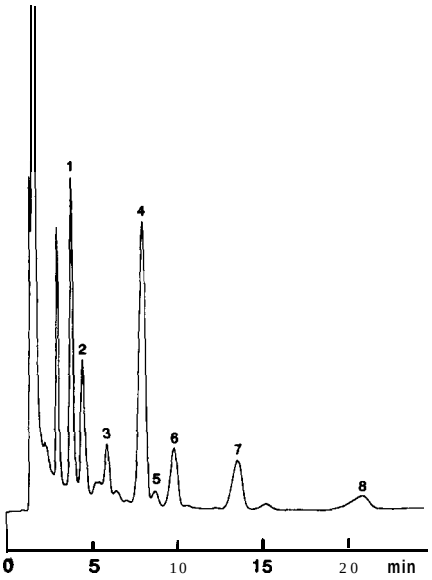


Fig. 3. Chromatogram of ginsenosides and malonyl-ginsenosides in an extract of ginseng (sample 1). Saponins as in Fig. 2. Column, MPG-ODS (150 × 4 mm I.D.); eluent, acetonitrile-50 m*M* KH_2PO_4 (25.5:74.5); flow-rate, 1.0 ml/min; detection at 203 nm.

-Rb_2, -Rc and -Rd and malonyl-ginsenosides-Rb_1, -Rb_2, -Rc and -Rd in an extract of ginseng were well separated on the 150 mm × 4 mm I.D. MPG-ODS column with acetonitrile-50 m*M* KH_2PO_4 (25.5:74.5). The flow-rate was 1.0 ml/min and the peaks were monitored at 203 nm. With this eluent, linear responses between peak areas and amounts injected were obtained from five replicate injections of standard solutions of malonyl-ginsenosides-Rb_1, -Rc and -Rb_2 in the range of O-5 μg, as indicated by following equations:

$y = 1.70x - 0.01$; $r = 0.999$ (malonyl-ginsenoside-Rb_1)

$y = 1.17x - 0.01$; $r = 0.999$ (malonyl-ginsenoside-Rc)

$y = 0.83.x - 0.02$; $r = 0.997$ (malonyl-ginsenoside-Rb_2)

where y is the peak area and x the amount injected (μg). For the least-squares regression the correlation coefficients were very close to 1.

The mean recovery of known amounts of malonyl-ginsenosides-Rb_1, -Rc and -Rb_2 in ginseng extracts was 96% (R.S.D. = 2.1%; n = 5).

From the results of quantitative analysis (n = 4), the contents of malonyl-ginsenosides-Rb,, -Rc and -Rb_2 were 0.40% (S.D. = 0.003%), 0.23% (S.D. = 0.04%) and 0.13% (SD. = 0.006%) respectively, in the extract of ginseng in Fig. 3.

For the separation of other ginsenosides such as ginsenosides-Rf, -Rg_2 and -Ro, the 250 × 4.0 mm I.D. MPG-ODS column was used. Simultaneous analysis of eleven acidic and neutral saponins was achieved in 25 min with acetonitrile-50 m*M* KH_2PO_4 (25.5:74.5), as shown in Fig. 4. The eleven saponins simultaneously analysed were malonyl-ginsenosides-Rb_1, -Rb_2, -Rc and -Rd and ginsenosides-Ro, -Rf, -Rg_2, -Rb_1, -Rb_2, -Rc and -Rd. The flow-rate was 2.0 ml/min.

The HPLC of malonyl-saikosaponins a and d, acidic saponins in bupleurum root, was also possible by addition of 50 m*M* KH_2PO_4 to the mobile

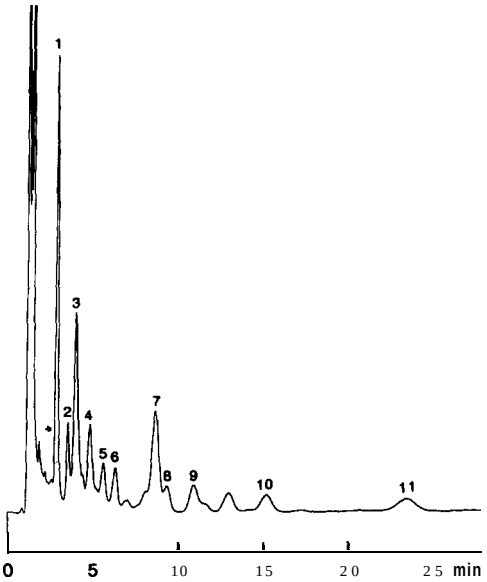


Fig. 4. Chromatogram of ginsenosides and malonyl-ginsenosides in an extract of ginseng (sample 2). Saponins: 1 = ginsenoside-Ro; 2 = ginsenoside-Rf; 3 = malonyl-ginsenoside-Rb,; 4 = malonyl-ginsenoside-Rc; 5 = ginsenoside-Rg,; 6 = malonyl-ginsenoside-Rb,; 7 = ginsenoside-Rb,; 8 = malonyl-ginsenoside-Rd; 9 = ginsenoside-Rc; 10 = ginsenoside-Rb,; 11 = ginsenoside-Rd. Column, MPG-ODS (250 × 4 mm I.D.); eluent, acetonitrile-50 m*M* KH_2PO_4 (25.5:74.5); flow-rate, 2.0 ml/min; detection at 203 nm.

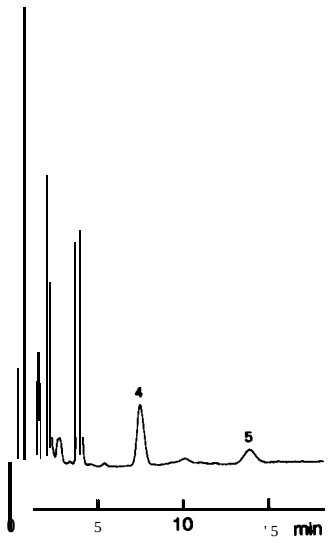


Fig. 5. Chromatogram of saikosaponins and malonyl-saikosaponins in an extract of bupleurum root. Saponins: 1 = saikosaponin c; 2 = malonyl-saikosaponin a; 3 = saikosaponin a; 4 = malonyl-saikosaponin d; 5 = saikosaponin d. Column, MPG-ODS (150 × 4 mm I.D.); eluent, acetonitrile-50 m*M* KH_2PO_4 (27.5:72.5); flow-rate, 2.0 ml/min; detection at 203 nm.

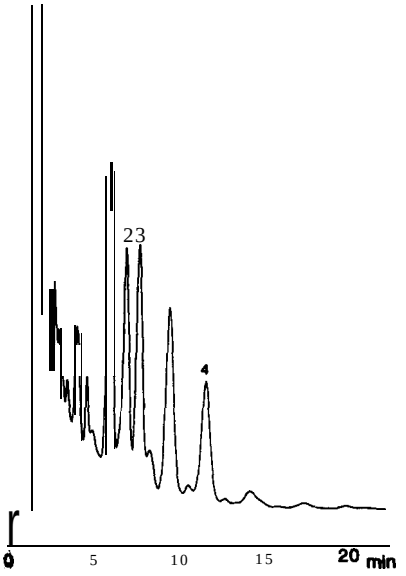


Fig. 6. Chromatogram of senegins in an extract of senega. Saponins: 1 = senegin IV; 2 = senegin III; 3 = senegin II'; 4 = senegin II. Column, MPG-ODS (150 × 4 mm I.D.); eluent, 30% acetonitrile in phosphate buffer (pH 6.8); flow-rate, 1.0 ml/min; detection at 315 nm.

phase. Malonyl-saikosaponins a and d and saikosaponins a, c and d were separated on the MPG-ODS column (150 mm × 4.0 mm I.D.) in 20 min with acetonitrile–50 m*M* KH_2PO_4 (27.5:72.5), as shown in Fig. 5. The flow-rate was 2.0 ml/min and the peaks were monitored at 203 nm.

Separation of senegins II' and III by HPLC was found to be difficult with aqueous acetonitrile containing KH_2PO_4. For their rapid separation, a mixture of acetonitrile and phosphate buffer (containing 25 m*M* KH_2PO_4 and 25 m*M* K_2HPO_4; pH 6.8) was used as the mobile phase. On the 150 mm × 4 mm I.D. MPG-ODS column, an excellent separation of senegins II, II', III and IV was obtained in 15 min with 30% acetonitrile in phosphate buffer as the mobile phase, as shown in Fig. 6. The flow-rate was 1.0 ml/min and the peaks were monitored at 315 nm.

. DISCUSSION

HPLC of acidic saponins of ginseng and other crude drugs has been reported by Yamaguchi and co-workers [1 1,121. The reported method took about 40 min for the separation of a mixture of ginsenosides and the column temperature was 40°C. The present method was more rapid: the eleven ginsenosides were separated within 25 min at room temperature. Rapid and excellent separation of acidic saikosaponins was also achieved with this method. The method was suitable for separation of these acidic saponins, which are unstable towards heat.

Nakajima *et al.* [13] reported the separation of senegins II, II', III and IV by HPLC on a TSK gel LS-410K (ODS silica gel) column using acetonitrike-water-acetic acid-triethylamine as the mobile phase. Much more rapid separation of the saponins was possible by using the MPG-ODS columns described here.

It is concluded that the rapid separation of the acidic saponins and also neutral saponins of ginseng, bupleurum root and senega is possible by HPLC on the MPG-ODS column. The column was found to be suitable for preparative purposes. We have previously reported a preparative separation

of the saponins from the root of *Panax ginseng* using this column [14,151. Preparative applications to other saponins are in progress.

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