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Comparison of columns of chemically modified porous glass and silica in reversed-phase high-performance liquid chromatography of ginsenosides

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ABSTRACT

Reversed-phase high-performance liquid chromatograms of an extract of ginseng and mixtures of ginsenosides (ginseng saponins) on a number of columns of chemically modified porous glass (MPG, pore size 550 A) and silica (pore size 80 and 300 A) were compared, Although the retention behaviour of ginsenosides was similar on the columns examined, the capacity factors of ten ginsenosides on an octadecylsilyl-MPG (MPG-ODS) column were smaller than those on silica columns. It is concluded that the MPG-ODS column has a number of advantages over conventional silica-ODS columns for the chromatography of ginsenosides. These properties are attributable to the optimum pore size for the molecular size of the saponins on the one hand and to the narrow distribution range of the pore size on the other.

INTRODUCTION

Microporous glass (MPG) is a promising material as a packing in high-performance liquid chromatography (HPLC) owing to its high chemical resistance and its homogeneous and cylindrical pores. It is stable between pH 2 and 12. The distribution of the pore size is relatively narrow compared with silica [1]. Although chemically modified silicas are one of the most commonly used packing materials, their pore size distributions are broad and sometimes bimodal.

We have prepared octadecylsilyl porous glass (MPG-ODS) and used it as a packing in reversed-phase HPCL [2]. Columns of MPG-ODS have been successfully used for the analytical and preparative HPLC of ginsenosides, saponins of ginseng [3–8]. Two water-acetonitrile mobile phases have been used for the isocratic elution of water-soluble panaxatriols and other less hydrophilic saponins. Recently, Petersen and Palmqvist [9] reported the HPLC of ginsenosides with a C_{18} silica column. They used two-step gradient elution with water–acetonitrile for the simultaneous separation of six main ginsenosides. The retention times of ginsenosides on the MPG-ODS column were shorter than those on the octadecylsilylsilica (silica-ODS) columns. We have examined the chromatographic behaviour of the saponins and related compounds on a number of columns. This paper is concerned with a comparison of columns of chemically modified MPG and silica in the reversed-phase HPLC of ginsenosides and other substances.

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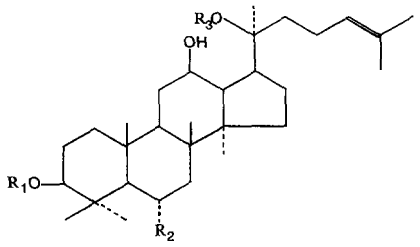
EXPERIMENTAL

Materials

The MPG-ODS, silica-ODS (pore size 80 A; particle size 5 μm) and silica-ODS (pore size 300 A; particle size 5 μm) used were Hitachi gels 3 16 I, 3 156 and 3063, respectively. Other chemically modified packing materials of MPG and silica were prepared by methods analogous to that for MPG-ODS [2]. They were packed into a stainless-steel column (150 × 4.0 mm I.D.) by the slurry method. MPG (particle size 10 μm) was supplied by Ise Chemical Industries. Silica was Hitachi gel 3041.

Malonyl-ginsenosides were kindly supplied by Professor I. Kitagawa of the University of Osaka (Osaka, Japan). Standard samples of ginsenosides were obtained from Wako (Osaka, Japan) and those of estrogens were from Sigma (St. Louis, MO, USA). Acetonitrile for the mobile phase was of HPLC grade (Wako). Water was distilled and passed through a Milli-Q purification system (Millipore, Bedford, MA, USA). Other chemicals were of analytical-reagent grade.



		R_1	R_2	R_3
malonyl-ginsenoside	Rb_1	Glc^{2}-Glc^{6}-Ma	H	Glc^{6}-Glc
malonyl-ginsenoside	Rb_2	Glc^{2}-Glc^{6}-Ma	H	Glc^{6}-Ara(p)
malonyl-ginsenoside	Rc	Glc^{2}-Glc^{6}-Ma	H	Glc^{6}-Ara(f)
malonyl-ginsenoside	Rd	Glc^{2}-Glc^{6}-Ma	H	Glc
ginsenoside	Rb_1	Glc^{2}-Glc	H	Glc^{6}-Glc
ginsenoside	Rb_2	Glc^{2}-Glc	H	Glc^{6}-Ara(p)
ginsenoside	Rc	Glc^{2}-Glc	H	Glc^{6}-Ara(f)
ginsenoside	Rd	Glc^{2}-Glc	H	Glc
ginsenoside	Re	H	O-Glc^{2}-Rha	Glc
ginsenoside	Rg_1	H	0-Glc	Glc

HPLC conditions

The HPLC system consisted of a Tosoh Model CCPM prep pump, a UV-8010 monitor, an SC-8010 system controller and data processor and a PP-8010 recorder. The system was operated at room temperature. Predetermined volumes of an extract of ginseng, mixture of ginsenosides and estrogens were injected into the chromatograph. The flow-rate was 1 ml/min and the peaks were monitored at 203 nm.

For isocratic elution, mixtures of acetonitrile-50 m*M* KH_2PO_4 at several concentrations were used as the mobile phases. For the one-step separation of both panaxatriol- and panaxadiol-ginsenosides, a 30-min linear gradient elution from acetonitrile-50 m*M* KH_2PO_4 (15:85) to acetonitrile-50 m*M* KH_2PO_4 (50:50) was employed. The column void time was determined with sodium nitrite.

Sample preparation from ginseng

Ginseng was pulverized and extracted with 70% methanol at room temperature (20°C) for 30 min and the extract was filtered and evaporated. The residue was dissolved in water and applied to a Sep-Pak C_{18} cartridge. After washing the column 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 HPLC system.

RESULTS AND DISCUSSION

The chromatograms of a ginseng extract on the columns of MPG-ODS, silica-ODS (pore size 80 A) and silica-ODS (pore size 300 A) with linear gradient elution are shown in Fig. 1. The content of malonyl-ginsenoside-Rd in the extract was too low to be detected in the chromatograms. Although the retention behaviours of the ginsenosides on both the MPG and silica columns were similar, the capacity factors of ten saponins were smaller on MPG than on silica columns. As shown in Fig. 2, the resolution between ginsenoside-Rg_1 and -Re on the MPG-ODS column was better than that on the silica-ODS column. At concentrations of acetonitrile lower than 30%, the two ginsenosides were so retained on the silica-ODS column that they were not eluted in 30 min.

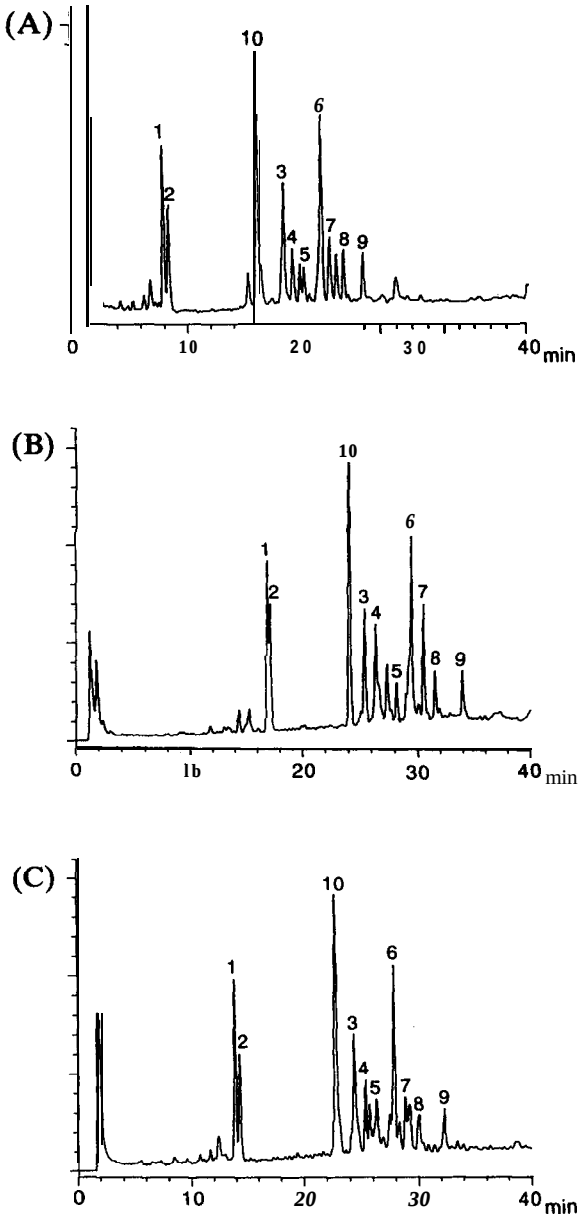


Fig. 1. Chromatograms of a ginseng extract on (A) MPG-ODS, (B) silica-ODS (80 A) and (C) silica-ODS (300 A) columns. Peaks: 1 = ginsenoside-Rg, 2 = ginsenoside-Re; 3 = malonyl-ginsenoside-Rb,; 4 = malonyl-ginsenoside-Rc; 5 = malonyl-ginsenoside-Rb,; 6 = ginsenoside-Rb,; 7 = ginsenoside-Rc; 8 = ginsenoside-Rb,; 9 = ginsenoside-Rd; 10 = ginsenoside-Ro (a pentacyclic terpenoid). Eluent, linear gradient from acetonitrile–50 m*M* KH_2PO_4 (15:85) to acetonitrile-50 m*M* KH_2PO_4 (50:50); flow-rate, 1.0 ml/min; detection, 203 nm.

The correlations of the capacity factors (k') of ginsenosides and estrogens on the MPG-ODS and silica-ODS (80 A) columns were fairly good, as shown in Fig. 3. To obtain similar ***k'*** values for ginsenosides under isocratic conditions, the content of the organic solvent in the mobile phases was lower on the MPG-ODS than on the silica-ODS column. Under the common gradient conditions, ten ginsenosides and six estrogens were retained on silica-ODS to a greater extent than on MPG-ODS. These greater retentions may be attributed to the smaller size and ink-bottle shape of the pores of the silica gel.

The order of the elution of malonyl-ginsenoside-Rd and ginsenoside-Rb_1 was reversed on MPG-ODS and silica-ODS (80 A). Under isocratic conditions, the same elution order of the two saponins was observed on MPG-ODS and silica-ODS (300 A) (Fig. 4). The correlation of log ***k'*** of the ginsenosides and the estrogens on the MPG-ODS column were larger with silica-ODS (300 A) than with silica-ODS (80 A). These results suggest that a dominant factor of the retention order is the pore size. The log ***k'*** values of eight panaxadiol ginsenosides on the MPG-ODS column were plotted against the content of acetonitrile in the mobile phase, as shown in Fig. 5. Similar relationships were obtained with silica-ODS (300 A), silica-ODS (80 A) and silica-phenyl (80 A) in the acetonitrile concentration range 27-30%. The order of the elution of the eight saponins did not change with variation in the concentration of acetonitrile in the range examined for the four columns.

MPG and silica (80 A) were chemically modified with alkyl groups of chain lengths C_4, C_8 and C_{12} and with a phenyl group. The chemically modified MPGs and silicas were packed into a stainless-steel column (150 × 4.0 mm I.D.). Mixtures of ginsenosides were chromatographed on the columns with gradient elution. The results are summarized in Fig. 6.

The elution order of malonyl-ginsenoside-Rd and ginsenoside-Rb_1 on MPG-phenyl and -C_4 and the silica (80 A) columns was reversed on MPG-C_8, -C_{12} and -ODS and silica-ODS (300 A). Among the silica columns, the silica-ODS (300 A) column achieved the shortest retention times of the ten saponins. The best resolution of ginsenoside-Rg_1 and

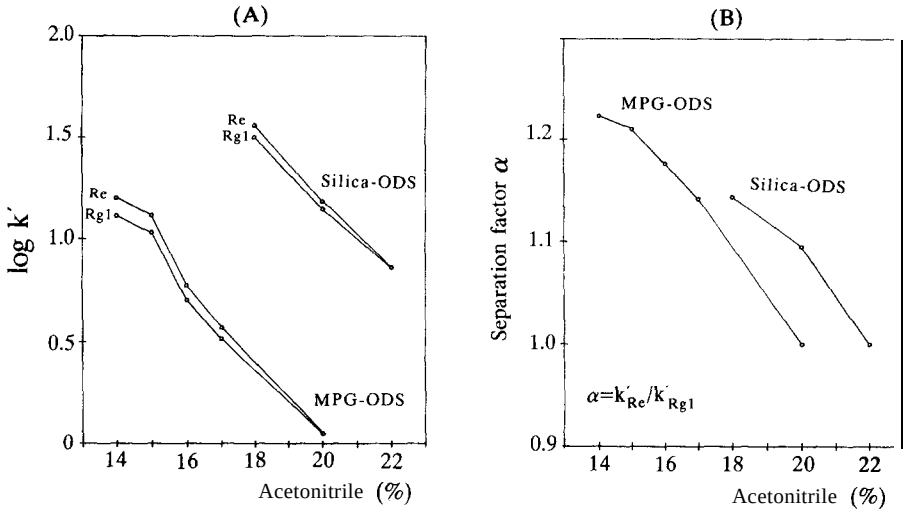


Fig. 2. Separation of ginsenoside-Rg, and -Re on MPG-ODS and silica-ODS (80 Å) columns with isocratic elution. Mobile phase. acetonitrile-water.

-Re was obtained on phenyl-modified MPG and silica.

Table I gives separation factors (α) of panaxadiol ginsenosides with isocratic elution on the MPG-ODS, silica-ODS (80 Å), silica-ODS (300 Å) and silica-phenyl (80 Å) columns. The resolutions between malonyl-ginsenoside-Rc and -Rb_2 (2-3) and between ginsenoside-Rc and -Rb_2 (6-7) were better on the MPG-ODS column than on the silica-ODS columns. Ginsenoside-Rc and -Rb_2 are isomers at the C-20-arabinose moiety, *i.e.*, the former is α-L-arabinofuranose whereas the latter is α-L-arabino-

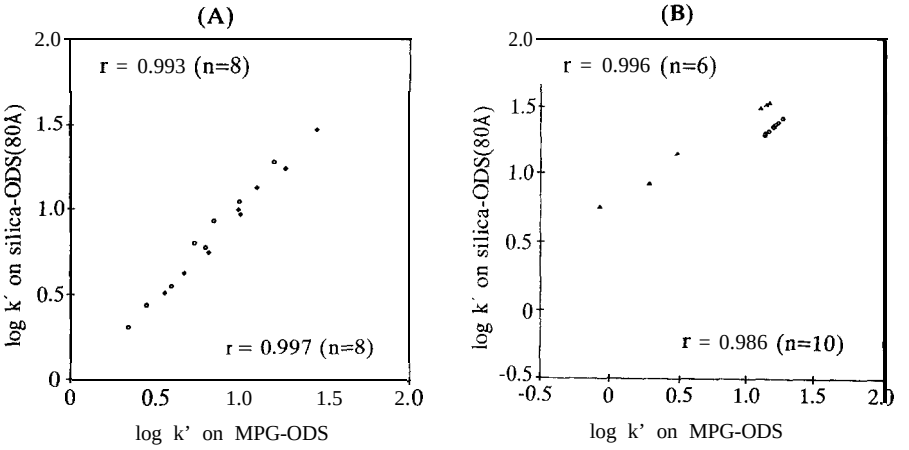


Fig. 3. Correlation of log k' values of ginsenosides and estrogens between MPG-ODS and silica-ODS (80 Å). (A) Isocratic elution; (B) gradient elution. Under isocratic conditions, the ratios of acetonitrile and 50 mM KH_2PO_4 for the MPG-ODS and silica-ODS (80 Å) columns were 25:75 and 30:70, respectively, for points (0), and 24:76 and 29:71, respectively, for points (0). Ginsenoside-Rg, and -Re were not separated under these conditions. Gradient conditions were the same as shown in Fig. I. Symbol ○ represents the ten ginsenosides as numbered in Fig. 1 and △ six estrogens, *viz.* estrone, estradiol, estriol, equiline, 17β-estradiol-3-(β-D-glucuronide) and estriol-16α(β-D-glucuronide).

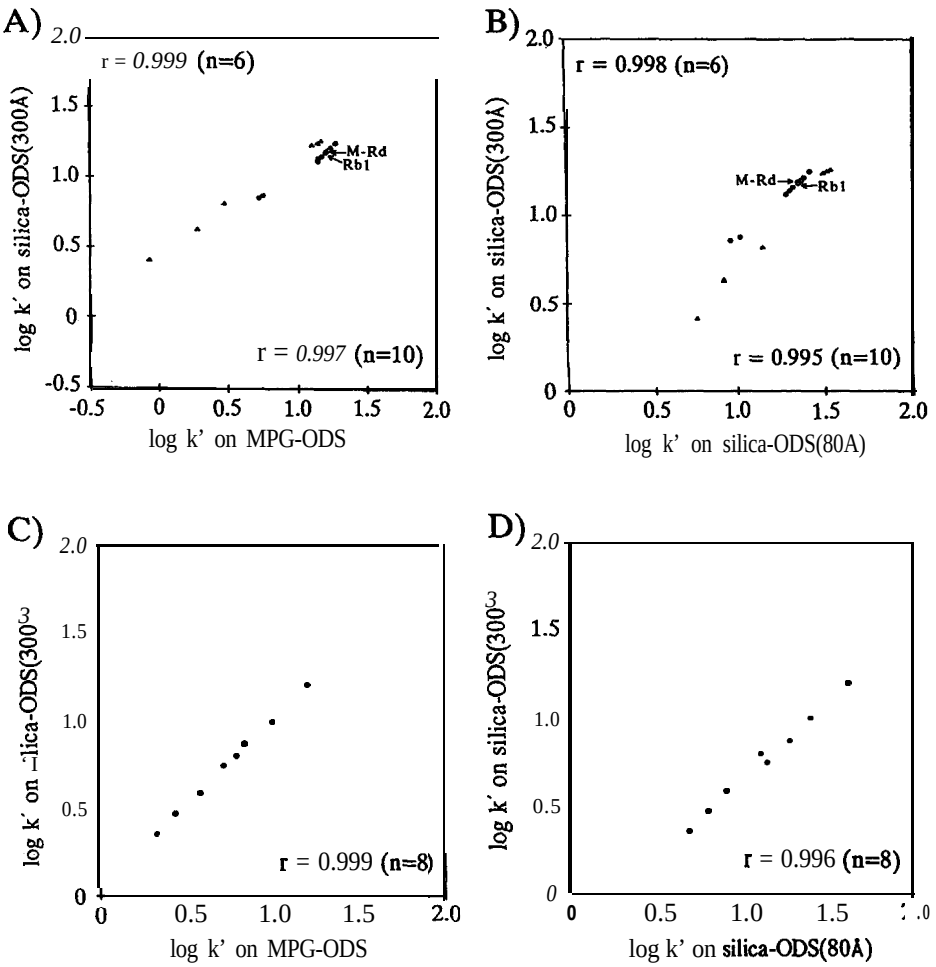


Fig. 4. Correlation of log k' values of ginsenosides and estrogens between MPG-ODS and silica-ODS (300 A) columns and between silica-ODS (80 A) and silica-ODS (300 A) columns. (A) Correlations between MPG-ODS and silica-ODS (300 A) with gradient elution; (B) correlation between silica-ODS (80 A) and silica-ODS (300 A) with gradient elution; (C) correlation between MPG-ODS and silica-ODS (300 A) with isocratic elution; (D) correlation between silica-ODS (80 A) and silica-ODS (300 A) with isocratic elution. Under isocratic conditions, the ratios of acetonitrile and SO mM KH_2PO_4 for MPG-ODS and silica-ODS columns were 25:75 and 28:72, respectively. Gradient conditions were the same as shown in Fig. 1. The symbol 0 respresent the ten ginsenosides as numbered in Fig. 1 and △ the six estrogens listed in Fig. 3. M-Rd = malonyl-ginsenoside-Rd; Rbl = ginsenoside-Rb,.

pyranose. MPG-ODS achieved a better separation of the isomeric glycosides at C-20. The fact that the separation factors of the isomeric glycosides on silica-ODS columns (80 and 300 Å) were similar suggests that the pore size did not affect the separation of these isomeric glycosides.

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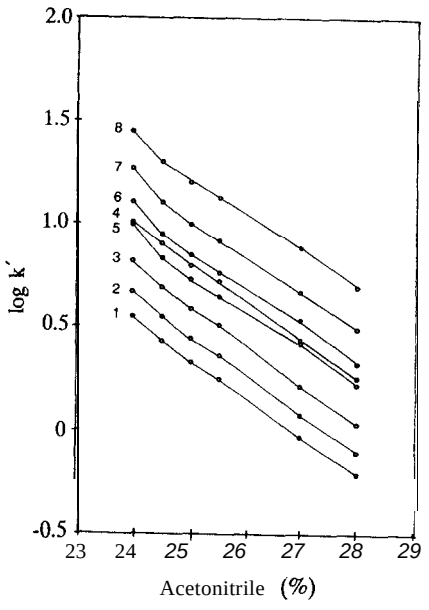

Fig. 5. Plots of log k' against the content of acetonitrile in the mobile phase. 1 = Malonyl-ginsenoside-Rb,; 2 = malonyl-ginsenoside-Rc; 3 = malonyl-ginsenoside-Rb,; 4 = malonyl-ginsenoside-Rd; 5 = ginsenoside-Rb,; 6 = ginsenoside-Rc; 7 = ginsenoside-Rb,; 8 = ginsenoside-Rd.

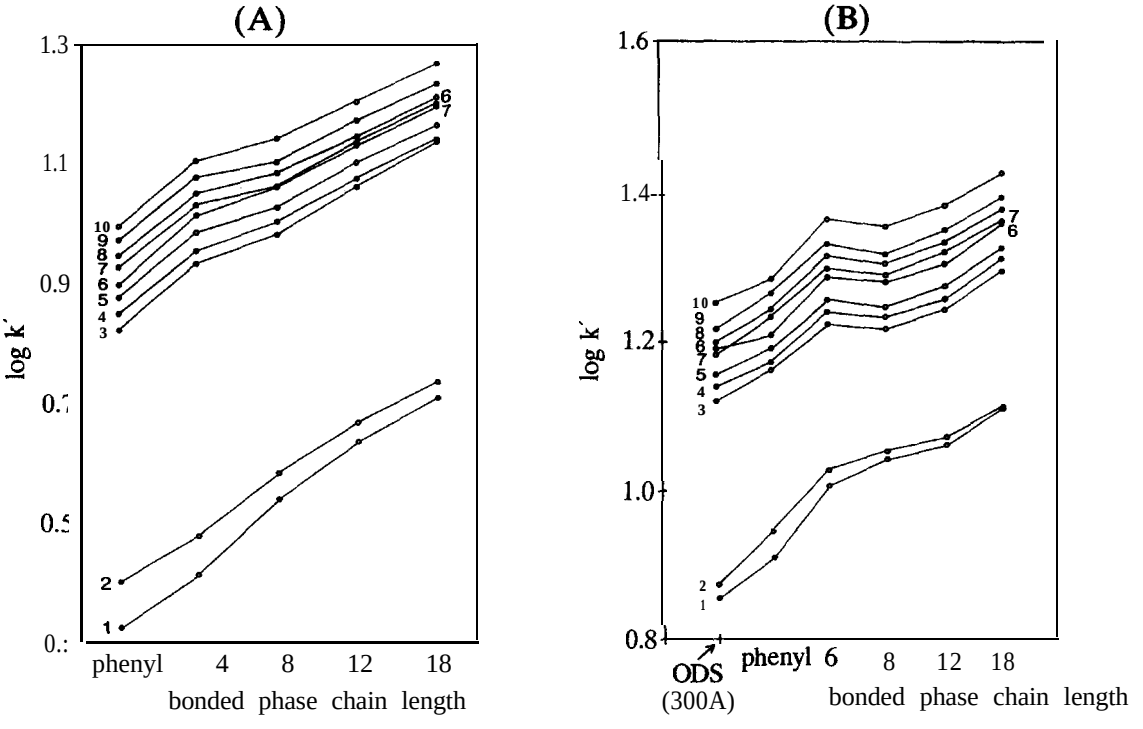

Fig. 6. Comparison of capacity factors of ginsenosides on chemically modified (A) MPG and (B) silica columns. 1 = Ginsenoside-Rg,; 2 = ginsenoside-Re; 3 = malonyl-ginsenoside-Rb,; 4 = malonyl-ginsenoside-Rc: 5 = malonyl-ginsenoside-Rb_2; 6 = malonyl-ginsenoside-Rd; 7 = ginsenoside-Rb,; 8 = ginsenoside-Rc; 9 = ginsenoside-Rb,; 10 = ginsenoside-Rd.

TABLE I

COMPARISON OF SEPARATION FACTORS (a)

HPLC conditions: eluent, for MPG-ODS, acetonitrile-50 m*M* KH_2PO_4 (25:75), for silica-ODS (80 A), acetonitrile-50 m*M* KH_2PO_4 (30:70), for silica-ODS (300 A), acetonitrile-50 m*M* KH_2PO_4 (28:72) and for silica-phenyl, acetonitrile-50 m*M* KH_2PO_4 (27:73); flow-rate, 1 .O ml/min; detection, 203 nm.

Compounds separated"	Separation factor (a)			
	MPG-ODS	Silica-ODS (80 A)	Silica-ODS (300 A)	Silica-phenyl
1-2	1.29	1.32	1.31	1.21
2-3	1.40	1.31	1.30	1.19
3–4	1.61	1.66	1.63	1.34
5-6	1.31	1.35	1.32	1.22
6–7	1.41	1.30	1.34	1.20
7-8	1.60	1.70	1.62	1.34

[a] 1 =Malonyl-ginsenoside-Rb,; 2 =malonyl-ginsenoside-Rc; 3 = malonyl-ginsenoside-Rb,; 4 = malonyl-ginsenoside-Rd; 5 = ginsenoside-Rb,; 6 = ginsenoside-Rc; 7 = ginsenoside-Rb,; 8 = ginsenoside-Rd.

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