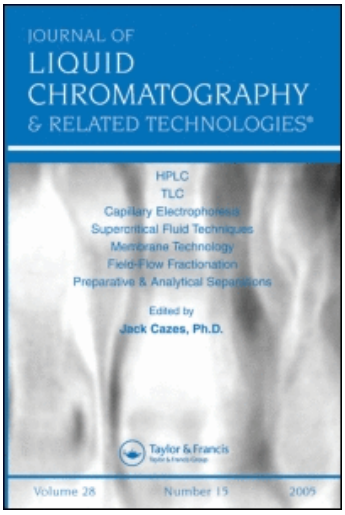

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Men-Olivier, L. Le , Renault, J. H. , Thepenier, P. , Jacquier, M. J. , Zeches-Hanrot, M. and Foucault, A. P.(1995) 'Purification of the Main Ginsenosides from a French Crop of *Panax Quinquefolium* L.', Journal of Liquid Chromatography & Related Technologies, 18: 8, 1655 — 1662
To link to this Article: DOI: 10.1080/10826079508009302
URL: http://dx.doi.org/10.1080/10826079508009302

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(8), 1655-1662 (1995)

PURIFICATION OF THE MAIN GINSENOSIDES FROM A FRENCH CROP OF *PANAX QUINQUEFOLIUM* L.

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ABSTRACT

Active components of *Panax quinquefolium* L. roots (French growing) have been separated by centrifugal partition chromatography (CPC). Five pure ginsenosides, namely Rb1, Re, Rd, Gypenoside XVII, and F11, have been isolated in one step, using a biphasic mixture of ethyl acetate / 1-butanol / water and gradient elution. Part of the effluent was monitored with an evaporative light scattering detector (ELSD), for direct control of the collected fractions, allowing an easy detection of these molecules with low or no absorbance in the UV. Isolated ginsenosides have been identified by comparison with standards and by ^{1}H and ^{13}C NMR studies.

INTRODUCTION

American ginseng *Panax quinquefolium* L. originates from the northern region of the United States and grows in several countries, *e.g.* Canada and north of China[1]. Its growth has been recently introduced in France. Tonic properties of its extracts are used in food chemistry (tonic water, dietetic..) and make it comparable to the Korean ginseng *Panax ginseng* Meyer. Ginsenosides (triterpenoic saponosides) composition for both species is close[2], but ginsenosides Rd, Re, and especially Rb (Rb1 and Rb2), are known to be more abundant in *Panax quinquefolium* L. . Rb1 could be the active compound for anti-stress properties, and has a beneficial influence against Alzheimer's disease[3]. Ginsenosides from root extracts of *Panax quinquefolium* L. have been fractionated by various chromatographic methods, including reverse phase HPLC and droplet countercurrent chromatography (DCCC)[4]; several operations were needed in HPLC and poor yields were obtained, whereas DCCC is time consuming and restricted to few biphasic systems. In both case, detection at 202-207 nm was not easy or in some case, was impossible.

Centrifugal partition chromatography (CPC), combined with evaporative light scattering detection (ELSD), appears to be a suitable method for quantitative separation of these polar molecules, and we report here a typical separation of the main ginsenosides of *Panax quinquefolium* L. which we achieved in our laboratory.

EXPERIMENTAL

Preparation of the extract

Panax quinquefolium L. roots came from a two year and three month old growing (COOPAL, Picardie, France). 466 g of ground root were heated in 6 liters of refluxing aqueous methanol (1/4) (v/v). The aqueous-alcoholic extract was concentrated under reduced pressure, then diluted with 1 liter of water and extracted 4 times with 1 liter of water saturated butanol (4 x 250 mL). The butanolic solution yielded 49.45 g (10.6%) of crude extract after evaporation of the solvent. 16 g of this extract were diluted with 150 mL of water and dialyzed against water in a

Visking PM (6000-8000) tube (Polylabo, Strasbourg, France) for 72 hours. After lyophilization, 8.6 g of purified ginsenosides were obtained, the final yield being of 5.73 %.

Apparatus

A Series 1000 HPCPC (Sanki Eng. Limited, Nagaokakyo, Kyoto, Japan) was used[5]. It is a bench top CPC (30 x 45 x 45 cm, ≈ 60 kg); the column is a stacked circular partition disk rotor which contains 2136 channels with a total internal volume of around 240 mL. The column is connected to the injector and the detector through two high pressure rotary seals. A 4-port valve included in the series 1000 allows the HPCPC to be operated in either the descending or ascending mode. The HPCPC was connected to a solvent delivery pump Techlab economy 2/ED (Techlab, D-38173 Erkerode, Germany), supplied with solvents through a gradient generator ISCO model 2360 with preparative options (ISCO, Inc., Lincoln, NB, USA). A flow splitter with a restriction was installed at the outlet of the HPCPC, the main line (90% of the flow) going to a fraction collector Pharmacia type Super Frac (Pharmacia, Uppsala, Sweden), the other line (10% of the flow) going to an evaporative light scattering detector Varex type ELSD II A (Varex, Barstonville, MD, USA). The temperature of the nebulizer was set at 118.5°C. *Panax quinquefolium* L. extracts were injected through a Rheodyne model 7125 injector with a 5 mL sample loop.

Biphasic system

The ternary system ethyl acetate (EtOAc) / 1-butanol (1-BuOH) / water (H_2O) has been used. The ternary diagram corresponding to this system is shown on Figure 1. This system is favorable for a gradient run in the normal phase mode, *i.e.* if the lower, water rich phase, is used as the stationary phase[6]. The polarity of the mobile phase can then be modified by changing the ratio of BuOH to EtOAc, which does not result in significant modification of the stationary phase composition, as shown by the orientation of the tie-lines. A similar gradient elution has been used by M. Vanhaelen *et al.*[7] with the ternary system EtOAc / 2-BuOH / H_2O, to purify several flavonoids from a commercial extract of *Ginkgo biloba* leaves. Initial composition of

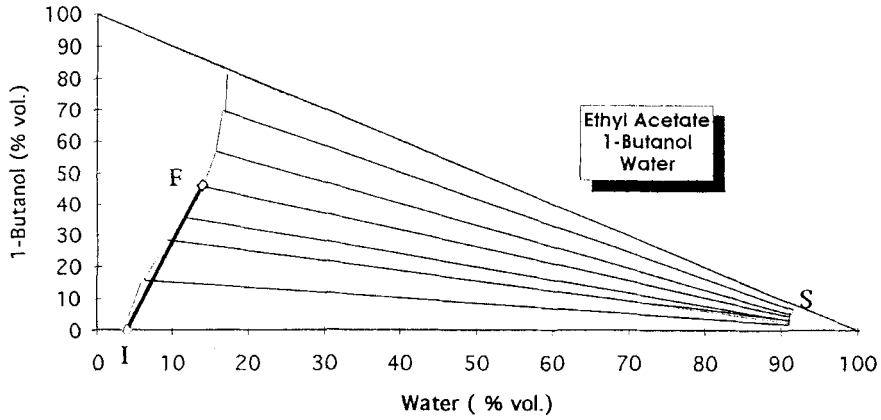


Figure 1 Ternay system ethyl acetate / 1-butanol / water. Composition of the liquid phases used to generate a gradient of butanol in ethyl acetate on a water-rich liquid stationary phase :

I : initial mobile phase
F : final mobile phase
S : stationary phase.

the mobile phase we were using was EtOAc / 1-BuOH / H_2O, 95 / 1 / 4 (v/v/v)(I on Fig. 1), and final composition was EtOAc / 1-BuOH / H_2O, 40 / 46 / 14 (F on Fig. 1) The gradient was linear with a duration of 4 hours; the flow rate was 4 mL/min and the rotational speed was 1200 rpm. With these experimental conditions, the mobile phase volume in the HPCPC was ≈ 60 mL, *i.e.* a retention of the stationary phase of about 75%. Back pressure was 29 to 34 bars,

Analysis of the collected fractions :

160 x 8 mL fractions were collected. Each fraction was analyzed by TLC on Whatman K6F plates (Whatman, Maidstone, England); the mobile phase for TLC was a mixture of EtOAc / 1-BuOH / H_2O, 1 / 4 / 3; spots were visualized by H_2SO_4 spray and heat (100°C for 10 min). Identical fractions were pooled and evaporated

to dryness. They were characterized by comparison with standards of Rg1, Re, Rc, Rd, Rb1, Rb2 and Rf (Extrasynthese, 69730 Genay, France). Fractions containing only one spot were then analyzed by NMR. Spectra were recorded in pyridine with a Bruker model AC300 (Bruker, Wissembourg, France), at 300 MHz for ^{1}H NMR, and 75 MHz for ^{13}C NMR. Spectra were compared to data found in the literature, and to spectra of standards.

Solvents

All solvents were pure grade for analysis and came from SDS (113124 Peypin, France).

RESULTS AND DISCUSSION

The polarity range of the system EtOAc / 1-BuOH / H_2O is very useful for the fractionation of ginsenosides found in *Panax quinquefolium* L.. Ginsenosides partition in favor of the lower aqueous phase (stationary) for EtOAc rich biphasic systems (*i.e.* partition coefficients are large), while they partition in favor of the organic upper phase (mobile) for BuOH rich biphasic systems (*i.e.* their partition coefficients are small). In this case, an increase of the BuOH content in the mobile phase will allow elution of ginsenosides of increasing polarities, according to a normal phase mode of chromatography. Five pure compounds have been obtained by using the gradient of Fig. 1, after injection of 210 mg of the extract (in 5 mL of stationary phase) : the major compound of *Panax quinquefolium* L., Rb1 (32 mg, 0.87 %), and four other ginsenosides, Rd (18 mg, 0.49 %), Re (13 mg, 0.35 %), gypenoside XVII (9 mg, 0.24 %)[8], and F11 (5 mg, 0.13 %)[9]. A CPC chromatogram is shown in Figure 2, and the structure of ginsenosides in Figure 3. Fraction 12-17 (9 mg) contains impure Rg1, fraction 87-93 (10 mg) is a mixture of Re and Rc, fraction 94-109 (12 mg) is a mixture of Rc and Rb2; these mixtures were identified by comparison with standards in TLC. The CPC run is fully reproducible using the same experimental settings and gradient. As can be seen in Fig. 2, elution of ginsenosides in CPC follows an order similar to that found in TLC, except for Re. Difference in polarities of ginsenosides is mainly due to their various sugar moieties : F11 has only one chain with two sugars and is eluted first, then Rd and gypenoside XVII which contain three

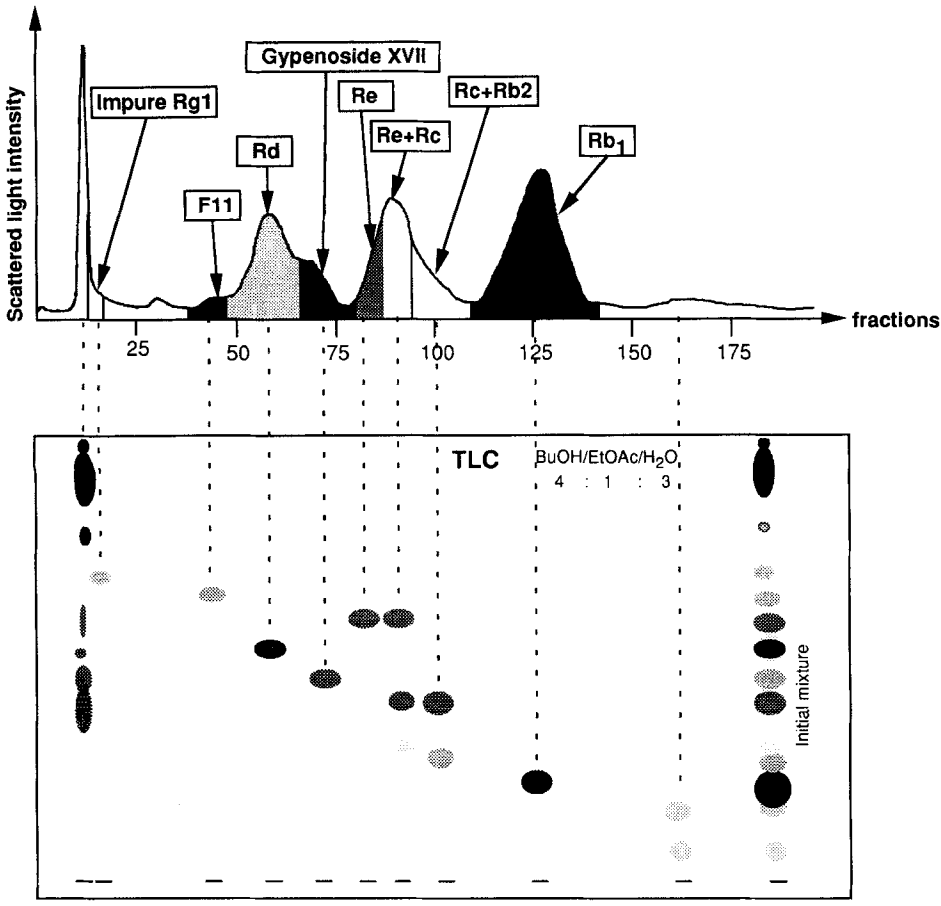
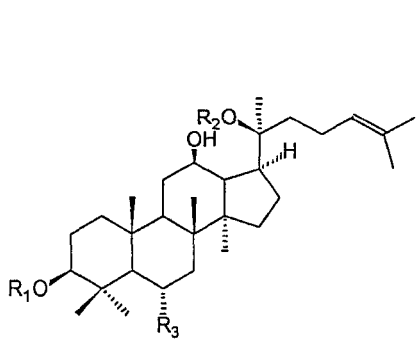
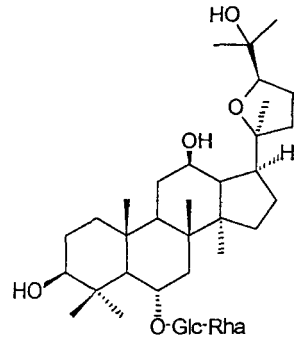


Figure 2 CPC separation of ginsenosides from *Panax quinquefolium* L. gradient elution with the ethyl acetate / 1-butanol / water system; stationary phase : water saturated with ethyl acetate and butanol (S on Fig.1). gradient duration : 4 hours. Ascending mode, flow rate : 4 ml/min rotational speed : 1200 rpm; back pressure : 29 to 34 bars volume of the mobile phase in the HPCPC : 60 ml.





F 11

	R_1	R_2	R_3
Gypenoside XVII	Glc	Glc	H
Rd	Glc-Glc	Glc-Glc	H
Rc	Glc-Glc	Ara(f)-^{6}Glc	H
Rb_1	Glc-Glc	Glc-Glc	H
Rb_2	Glc-Glc	Ara(p)-^{6}Glc	H
Re	H	Glc	O-Glc-Rha

Figure 3 Chemical structures of the ginsenosides found in *Panax quinquefolium* L.

sugars, then Rc and Rb1 which contain four sugars. Results found in the literature show that Rb1, Rd and Re are the main ginsenosides of *Panax quinquefolium* L. [2,8], which is in agreement with our results. Stated another way, F11 and gypenoside XVII, which are said to be negligible[8], are found to be up to 7% in the extract studied in our laboratory.

CONCLUSION

Centrifugal partition chromatography is well suited to the purification of polar compounds such as ginsenosides, and gradient runs allow purification of several

compounds in only one step. Scaling up our results should be easy by use of centrifugal partition chromatographs of larger capacities. *Panax quinquefolium* L. growing in France proves to be rich in ginsenosides from two year old, maximum quantities being expected for four year old growings[1]. The high content of Rb1 (>15 % of the mixture), and its fast purification by CPC makes *Panax quinquefolium* L. a valuable raw material, if its potential therapeutic interest is confirmed.

ACKNOWLEDGMENTS

We are grateful to the COOPAL for kindly providing *Panax quinquefolium* L. roots. We thank Dr. C. Lavaud for interpreting the NMR spectra. We express our gratitude to Dr. Jack Cazes for linguistic advice.

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Received: November 15, 1994
Accepted: December 1, 1994