Journal of Chromatography A, 1217 (2010) 1995–2001

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Preparative isolation and purification of ginsenosides Rf, Re, Rd and Rb1 from the roots of *Panax ginseng* with a salt/containing solvent system and flow step-gradient by high performance counter-current chromatography coupled with an evaporative light scattering detector

Xiaocheng Qi[a,b], Svetlana Ignatova[c], Guoan Luo[a,b,*], Qionglin Liang[b], Frank Wu Jun[d], Yiming Wang[a,b], Ian Sutherland[c,**]

[a] *Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China*
[b] *Department of Chemistry, Tsinghua University, Beijing 100084, China*
[c] *Brunel Institute for Bioengineering, Brunel University, Kingston Lane, Uxbridge, Middlesex UB8 3PH, United Kingdom*
[d] *XiangCam TCM Research Centre Ltd. Unit 16, Meditrina, Babraham Research Campus, Cambridge CB22 3AT, United Kingdom*

ARTICLE INFO

Article history:
Received 28 November 2009
Received in revised form 14 January 2010
Accepted 18 January 2010
Available online 25 January 2010

Keywords:
High performance counter-current chromatography
Ginsenosides
Panax ginseng
Salt/containing solvent system
Evaporative light scattering detection

ABSTRACT

Ginseng is a popular herb worldwide and has had varied uses in traditional Asian medicine for thousands of years. There are several different species of the herb, but all share the same constituents. Ginsenosides, the most extensively studied chemical components of ginseng, are generally considered to be one of the most important active ingredients of the plant. In this study, we have developed fast and efficient methodology for isolation of four known ginsenosides Rf, Rd, Re and Rb1 from Ginseng by high performance counter-current chromatography (HPCCC) coupled with evaporative light scattering detection (ELSD). The crude sample for HPCCC was purified firstly from a ginseng extraction using macroporous resin. The enriched saponin fraction (480 mg) was separated by using methylene chloride–methanol–5 mM aqueous ammonium acetate–isopropanol (6:2:4:3, v/v,) as the two-phase solvent system and yielded 10.7 mg of Rf, 11.0 mg of Rd, 13.4 mg of Re and 13.9 mg of Rb1. The purity of these ginsenosides was 99.2%, 88.3%, 93.7% and 91.8%, respectively assessed by HPLC-DAD-ELSD, and their structures were characterized by electrospray ionization mass spectrometry (ESI-MS) and compared with standards. Ammonium acetate was used to shorten the separation time and eliminate emulsification together with a flow step-gradient. The salt can be removed by re-dissolving the sample using acetone.

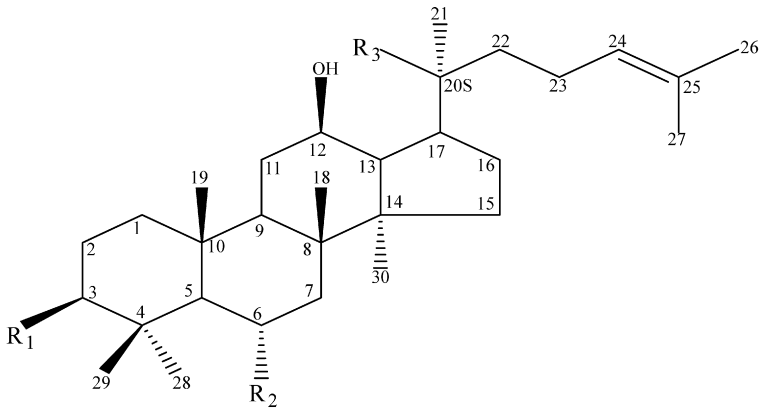
1. Introduction

Ginseng is a popular herb worldwide and has had varied uses in traditional Asian medicine for thousands of years. There are several different species of the herb, but all species share the same constituents that include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids [1,2]. Ginsenosides, the most extensively studied chemical components of ginseng, are generally considered to be one of the most important active ingredients of the plant [3,4]. Ginsenosides Rf, Rb1, Rb2, Rc, Rd, Rg1 and Re are major constituents of white and red ginseng. The chemical structures of the four ginsenosides studied are given in Fig. 1.

Du et al. [5] successfully separated ginsenoside Rb1, ginsenoside Re and ginsenoside Rg1 from the Chinese phytomedicinal formulation *Sanqi Zongdai Pian*, traditionally made from Ginseng roots, by using high-speed counter-current chromatography (HSCCC). Ginsenosides, being dammarane saponins, have very pure UV absorbance due to their lack of chromophores. Therefore, all HSCCC fractions were analyzed by TLC, which is quite a tedious process. Later HSCCC coupled with an evaporative light scattering detector (ELSD) was introduced for the separation of ginsenoside Re, ginsenoside Rg1, ginsenoside Rd and ginsenoside Rb1 [6]. However, the separation of ginsenoside Rf by HSCCC has not been reported yet. In this paper, macroporous resin was first used for the preparation of the crude sample. Then, a salt/containing solvent system

* Corresponding author at: Department of Chemistry, Tsinghua University, Beijing 100084, China. Tel.: +86 10 62781688; fax: +86 10 62781688.
** Corresponding author at: Brunel Institute for Bioengineering, Brunel University, Kingston Lane, Uxbridge, Middlesex UB8 3PH, UK. Tel.: +44 1895 266920; fax: +44 1895 274 608.
E-mail addresses: luoga@mail.tsinghua.edu.cn (G. Luo), ian.sutherland@brunel.ac.uk (I. Sutherland).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.01.057



	R_1	R_2	R_3
Ginsenoside-Rf	OH	-O-Glc^{2}—^{1}Glc	H
Ginsenoside-Rd	-O-Glc^{2}—^{1}Glc	H	-O-Glc
Ginsenoside-Re	OH	-O-Glc^{2}—^{1}Rha	-O-Glc
Ginsenoside-Rb1	-O-Glc^{2}—^{1}Glc	H	-O-Glc^{6}—^{1}Glc

Fig. 1. Structures of four ginsenosides from ginseng extraction.

for HPCCC separation of ginsenosides was developed for the first time (Fig. 2).

2. Experimental

2.1. Apparatus

Two different scales of HPCCC instruments were used in this study. An analytical Milli-CCC and a preparative Midi-CCC were both from Dynamic Extractions (Slough, UK). The analytical Milli-CCC had a coil of 20.4 mL and 1.6 mm bore tubing. It worked at a rotation speed of 1800 rpm. The preparative Midi-CCC had a coil of 912 mL and 4.0 mm bore tubing and worked at a rotational speed of 1250 rpm. Both of them were coupled to an evaporative light scattering detector (ELSD) PL-ELS-1000 (Polymer Laboratories, USA).

Samples were analyzed by a Waters 2695 high performance liquid chromatography (HPLC) instrument equipped with 2996 photodiode array detector (Waters, USA). Chromatography data were collected using Empower Pro workstation (Waters, USA). An Alltima C18 column (250 × 4.6 mm i.d., 5 μm, Alltech, USA) and a Phenomenex Luna C18(2) (150 × 4.6 mm i.d., 5 μm, Phenomenex, USA) were supplied for the HPLC analysis. An ion trap tandem mass spectrometer (Agilent Corp., USA) was employed to analyze and identify fractions from the HPCCC separation.

2.2. Materials and reagents

Panax ginseng (*C.A. Meyer*) was purchase from Changbai Mountain specialty cooperatives of Jilin Baigang (Jilin, China). The ginsenosides Rf, Re, Rd and Rb1 standards were from Shanghai Tauto Biotech Co. Ltd. (Shanghai, China). All analytical grade solvents (methylene chloride, methanol, and isopropanol), ammonium acetate for the HPCCC separation and HPLC grade acetonitrile for HPLC analysis were supplied by Fisher Chemicals (Loughborough, UK). Deionised water was prepared by a Millipore water purification system (Watford, UK).

2.3. Preparation of crude sample

The dried roots of *P. ginseng* (*C.A. Meyer*) (0.9 kg) were ground to a coarse powder (in accordance with the provisions of the Chinese Pharmacopoeia) and extracted three times with 7.2 L of 90% aqueous ethanol for 45 min. The extract solution was filtered and concentrated. This concentrated sample was diluted by deionised water with the relative density 1.06 g/mL and then separated using a 0.6 L macroporous resin column. Seven bed volumes of deionised water were flashed through the column to remove the contaminants. Afterwards, four bed volumes of 70% aqueous ethanol were used to elute ginsenosides in isocratic mode with a flow rate of 10 mL/min. All fractions containing ginsenosides were collected at 2 min intervals and dried under vacuum at 60 °C. The concentration of ginsenosides was determined to be 76% pure by UV.

2.4. Solvent system preparation

All solvent systems used in this paper were made by mixing organic solvents in a separatory funnel. After vigorous shaking, solvents were left to settle till both phases would become transparent and, therefore, equilibrated. Then the two phases were separated shortly before HPCCC separation. The upper aqueous phase (more polar) was used as the stationary phase and the lower organic (less polar) as the mobile phase, which corresponds to normal phase mode.

Table 1
The partition coefficients (K_D) of four ginsenosides for methylene chloride–methanol–water–isopropanol solvent systems.

No.	Ratio	Settling time (s)	K_D			
			Rf	Re	Rd	Rb1
1	5:2:4:4	55	0.60	1.44	0.81	2.42
2	4:2:4:4	35	0.62	1.36	0.88	2.02
3	5:2:4:3	35	0.41	1.81	0.85	3.52
4	6:2:4:4	40	0.47	1.82	0.86	3.98
5	6:2:4:3	29	0.78	2.82	1.29	5.44
6	6:2:4:3*	29	0.59	2.20	1.13	5.28

Note: K_D was calculated as peak area percentage in the lower phase divided by that in the upper phase.

* System No. 6 had the same ratio as system No. 5, but contained an additional 5 mg ammonium acetate per milliliter solvent.

2.5. Measurement of partition coefficient and settling time

In the present study, the partition coefficients (K_D) for the target compounds were measured by HPLC as follows. Two-phase solvent systems with different ratios of methylene chloride, methanol, water, isopropanol were prepared in a test tube. Approximately 6 mg of crude sample was dissolved in equal volumes of aqueous and organic phases of the thoroughly equilibrated two-phase solvent system. After the distribution equilibrium was established, an equal volume of upper and lower phases (400 μL) each was transferred into a separate vial and solvents removed at 60 °C under vacuum conditions. Then 1 mL of 50% aqueous methanol was added to solubilise the remaining contents of the vial. Afterwards, both upper (aqueous phase) and lower (organic phase) were analyzed separately by HPLC at 203 nm. The partition coefficient was defined as $K_D = A_{upper}/A_{lower}$, where A_{upper} and A_{lower} were ginsenoside HPLC peak areas in the upper and lower phases respectively.

The settling time, which is highly correlated with the retention of stationary phase, was expressed as the time for a clear layer between the two phases to be formed when each phase was mixed in the ratios given in Table 1.

2.6. HPCCC separation

The solvent system, containing the chosen ratios of methylene chloride, methanol, water and isopropanol was prepared, equilibrated and separated shortly before CCC separation. 480 mg of crude sample was dissolved in 20 mL of stationary phase (upper phase).

In each HPCCC preparative separation run, the coil was filled with the stationary (upper) phase in the head to tail mode. Then the mobile (lower) phase was pumped into the coil at a flow rate of 20 mL/min with a centrifuge rotational speed of 1250 rpm at 25 °C. When hydrodynamic equilibrium was established, the sample solution was injected into the coil through a 20 mL sample loop. The eluent was continuously monitored by connecting the tail outlet of the coiled column with the ELSD through a split valve. The ELSD system was set to a nebulizer temperature of 90 °C, evaporation temperature of 120 °C, and nitrogen (as the nebulizer gas) flow rate adjusted to 1.7 L/min. Each peak fraction was collected according to the elution profile and analyzed by HPLC. After the separation was completed, retention of the stationary phase was measured by collecting the column contents by forcing them out of the column with pressurized nitrogen gas.

2.7. HPLC analysis and identification of HPCCC fractions

Two HPLC methods were developed for this study. The first method was used for the analysis of crude sample and pooled fractions. HPLC separation was carried out on an Alltima C18 column (250 × 4.6 mm i.d., 5 μm) at 25 °C with a binary mobile phase consisting of 5 mM aqueous ammonium acetate (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 mL/min. The gradient elution program was as follows: 0–15 min, hold 21% B; 15–69 min, 21–38.1% B. For rapid screening of HPCCC fractions the first HPLC method was shortened from 69 to 40 min by using a Phenomenex Luna C18(2) (150 × 4.6 mm i.d., 5 μm) column and adjusting the gradient to: 0–12 min, hold 30% B, 12–40 min, 30–60% B. Temperature and flow rate were kept the same as for the first HPLC method. All HPLC analysis was done with DAD detection at 203 nm.

Identification of the purified compounds was performed by LC–ESI-MS. The split rate was 1:3. The ion trap MS analysis was carried out in negative mode using the following operation parameters: negative mode, capillary voltage: +3500 V; capillary exit voltage: 94 V; skimmer voltage: 40 V; target mass: 622.03 *m/z*; threshold: 100,000 cps; drying gas: 9 L/min; nebulizer pressure: 35 psig; gas temperature: 350 °C; mass scanning range: 100–2200 *m/z*.

3. Results and discussion

3.1. Selection of solvent system

A successful separation by HPCCC depends on the selection of a suitable solvent system, which provides an ideal range of the partition coefficient (K_D) for target compounds and a short settling time (less than 20 s) [7]. Furthermore, for any preparative separation a normal phase mode should be a priority, as fractions will then elute with the organic phase, making it much easier to remove the solvent and recover the target compounds. Besides, the organic mobile phase can be recycled and used again reducing environmental impact [8].

As most of the ginsenosides were medium polar or polar compounds, they were hard to dissolve in non-polar solvents but dissolved easily in water or other appropriate solvents. Therefore, solvent systems based on n-heptane or n-hexane could not be used for the separation of ginsenosides as they have large K_D values in normal-phase mode and take a long time to elute. In contrast, low K_D values can be obtained in normal-phase mode if a strong polar solvent system is used. Their K_D values will then be quite low and compounds will elute early and close to each other. While solvent systems containing chloroform have been widely used in China for natural product separations in conventional high speed CCC (HSCCC), since 2004 they have been being replaced by other chlorinated solvent systems such as methylene chloride–methanol–water [9]. This type of system is particularly good for the fractionation of root extracts as it provides a good solubility for saponin-like compounds and a gradual polarity change between phases by varying the methanol ratio in the system or by adding another alcohol like isopropanol [10]. The results given in Table 1 show that the methylene chloride–methanol–water-isopropanol (6:2:4:3, v/v) system can provide a separation of the target compounds. When this system was used for HPCCC separation, the K_D values could be reduced to some degree by increasing the volume ratio of isopropanol. For example, increasing isopropanol could improve the K_D value of ginsenoside Rb1, but the separation of ginsenosides Rf and Rd remain unsatisfactory.

Varying ratios of methylene chloride–methanol–water–isopropanol did not improve the situation (Table 1). Besides, the addition of crude sample into the solvent system caused emulsification, which potentially leads to stripping of the stationary phase from the column and, therefore, loss of separation efficiency.

It was established that the emulsification could be suppressed by the addition of acid or salt. But an acidic environment would lead to decomposition of ginsenosides. Instead, ammonium acetate

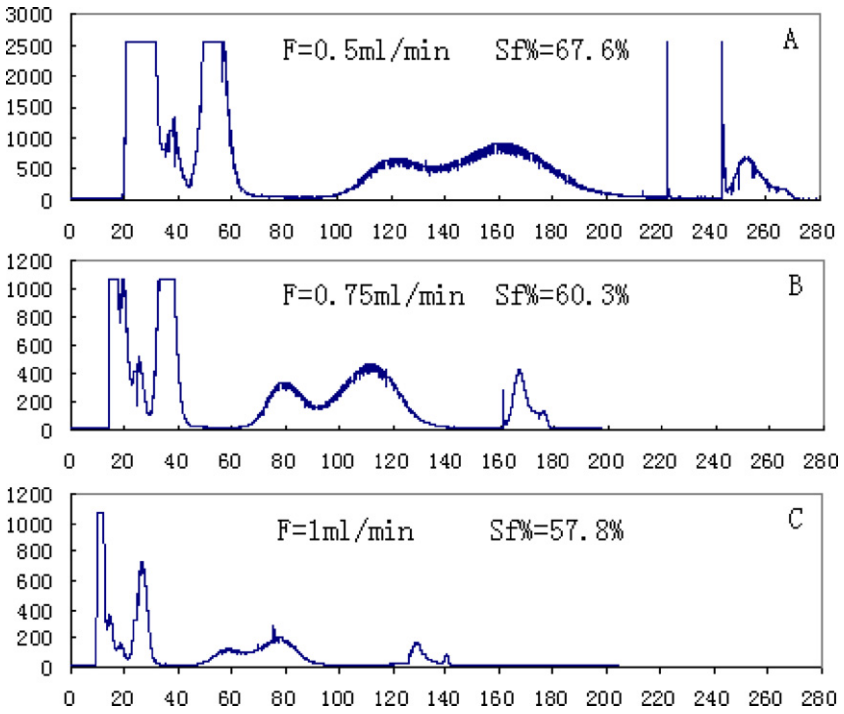


Fig. 2. Milli-HPCCC chromatograms in normal phase mode at different flow rates. Experimental conditions: solvent system was methylene chloride–methanol–5 mM aqueous ammonium acetate–isopropanol (6:2:4:3, v/v); the sample—10 mg of the crude was dissolved in a 1 mL of the upper phase; ELSD conditions were as follows: nitrogen flow rate was 1.2 L/min, nebulizer temperature was 65 °C and evaporative temperature was 80 °C, 1/10 signal output.

was chosen, as it was part of the HPLC mobile phase, therefore not causing problems during HPCCC fraction analysis, and can be easily removed by re-suspending fractions in acetone. Interestingly, the addition of salt resulted in a very slight decrease in K_D values (see Table 1, systems 5 and 6), which means shorter separation time.

3.2. Optimization of operational parameters on analytical Milli-HPCCC

The solvent system methylene chloride–methanol–5 mg/mL aqueous ammonium acetate–isopropanol (6:2:4:3, v/v,) provides a suitable K_D for the targets as well as a good solubility for the crude sample and was therefore chosen for further experiments. The HPCCC was operated in normal-phase mode, when the upper aqueous phase was the stationary phase and the retention was more than 50%. It is well known in CCC [7] that reducing the flow rate improves the retention of stationary phase, but the total time for separation is increased. The results of Ha et al. [10] using HSCCC indicated that reducing the flow rate could improve the resolution. In our case, increasing the flow rate led to an improvement of the resolution even with loss of stationary phase from 67.6% at 0.5 mL/min to 57.8% at 1 mL/min (Fig. 2A–C). It is still not clear what caused this unexpected behavior of the methylene chloride–methanol–5 mM aqueous ammonium acetate–isopropanol (6:2:4:3, v/v,) system. It might be due to the combination of a relatively small column bore of 1.6 mm, where capillary forces still apply, and emulsification caused by the crude. Therefore, by increasing flow rate, we were helping the system remove all the emulsification contaminants quicker with improved mixing and therefore a higher efficiency separation.

Loading studies involving sample concentration and loading volume in order to maximize process throughput and efficiency were also conducted on the analytical Milli-HPCCC. Varying sample concentration at a fixed sample loading volume of 0.56 mL (2.7% of column volume) showed that the detection of ginsenosides by ELSD was limited to a concentration of not more than 10 mg/mL. Optimising sample volume at a fixed sample concentration of 5.6 mg/mL showed that injecting too large a sample volume would result in loss of stationary phase. Hence, 2.7% of coil volume was chosen as the optimal value for sample loading.

3.3. Scale-up to preparative Midi-HPCCC

As there is approximately a 45× capacity between the analytical (20.4 mL coil volume) and preparative (912 mL coil volume) HPCCC columns, a linear scale-up would result in a 20 mL sample volume, loading of 250 mg of crude material and a 20 mL/min mobile phase flow rate. However, as the Midi column (72.5 m) is much longer than the Milli one (10 m), a better resolution can be achieved [11]. Therefore, the loading of crude material was increased up to 480 mg. When this purification procedure was transferred to the preparative Midi-HPCCC instrument, the separation at 20 mL/min took nearly 3.5 h (results are not shown). Two groups of peaks eluted with 40 min interval between them. In order to shorten the separation, the flow rate was increased up to 50 mL/min. This led to a 70 min run with better resolution between peaks in the second group (results are not shown). This is typical for wide bore columns when higher flow rate in HPCCC can lead to better mixing and better separation [11]. However, the peaks from the first

Table 2
Summary of scale-up parameters from analytical to preparative HPCCC.

Instrument type	Crude sample mass (mg)	Flow rate (mL/min)	"*g*" field	Rotational speed (rpm)
Analytical Milli-CCC HPCCC	5.6	0.5	181	1800
Preparative Midi-DE HPCCC	480	20–50	192	1250

Table 3
The value of efficiency factors for the four targets [12].

Targets	Technical indicators			
	Pt (g/h)	*Pe* (mg/h)	*Er* (L/mg)	*Ge* ($mg^2\ h^{-1}\ L^{-1}$)
Ginsenosides Rf	0.23	14.59	0.06	243.17
Ginsenosides Rd	0.23	12.69	0.08	158.63
Ginsenosides Re	0.23	9.35	0.24	38.96
Ginsenosides Rb1	0.23	6.73	0.71	9.48

group with $K_D \leq 1$, more non-polar ginsenosides, including R_f and R_d were poorly separated. Hence, the combination of both flow rates as a step-gradient was applied to achieve a reasonable purification (Fig. 3). At the beginning of the separation, the Midi-HPCCC column was equilibrated at 20 mL/min to maintain as high as possible stationary phase retention. After 67 min of separation when the first group of peaks eluted from the column, the flow rate was ramped up to 50 mL/min to elute the second group of ginsenosides. The rotor was stopped at 120 min and the column contents were pumped out with organic mobile phase at 200 mL/min. All HPCCC fractions were collected and analyzed as described below in Section 3.4. A summary of scale-up conditions from the analytical to preparative CCC is listed in Table 2.

According to Table 2, with the same '*g*' level maintained, the crude sample mass was scaled up 85 times, which is greater than simple volumetric scale-up due to the fact that the Midi column is 7.25-fold longer and 2.5-fold wider than the Milli column. Scale-up from an analytical to a preparative process proved to be successful.

Zhang et al. [12] have developed several factors to assess the efficiency of CCC separation. The same approach has been applied in this study and shown in Table 3.

3.4. Analysis and identification of HPCCC fractions

To remove the ammonium acetate from the HPCCC fractions, they were dried down and then re-dissolved in warm acetone. As a result, the salt was precipitated and then removed, while aliquots were dried down to recover the target compounds.

Fractions of target peaks (marked red or grey) in Fig. 3 were collected and combined after HPLC analysis. 10.7 mg of Rf, 11.0 mg of Rd, 13.4 mg of Re and 13.9 mg of Rb1 (Fig. 4) were obtained, with purities of 99.2%, 88.3%, 93.7% and 91.8%, respectively. The HPLC chromatogram of the crude sample, standards and the purified target peaks is shown in Fig. 4. The targets have the same HPLC retention time as the standards (Fig. 4). The ESI-MS/MS experiments were performed in the negative ion mode for the determination of molecular weight and MS/MS production of each target fraction by HSCCC separation. ESI-MS/MS spectra of ginsenosides Rf, Rd, Re and Rb1 are shown in Fig. 5 and listed in Table 4.

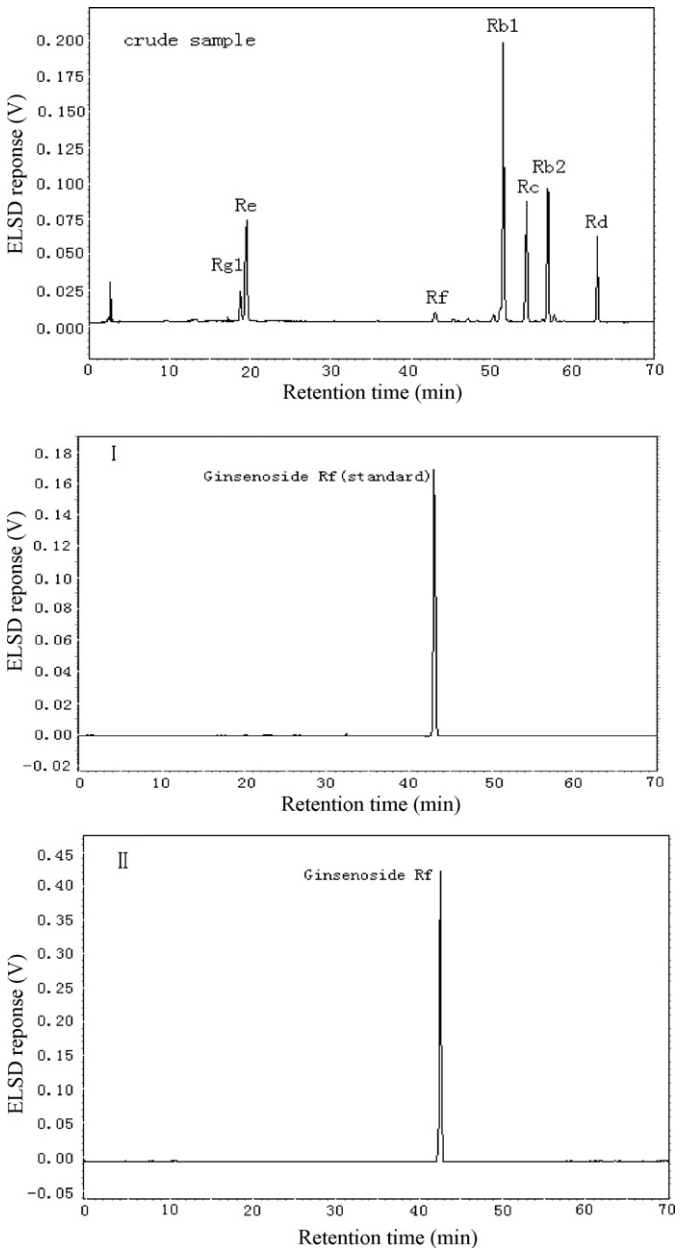


Fig. 4. HPLC/ELSD chromatogram of crude sample, standards and targets (I–VIII). The column was an Alltima C18 column (250 mm × 4.6 mm i.d., 5.0 μm). Column temperature was 25 °C; solvent A: 5 mM aqueous ammonium acetate; solvent B: acetonitrile; elution gradient were as follows: 0–15 min, hold 21% B; 15–69 min, 21–38.1% B; flow rate was 1.0 mL/min.

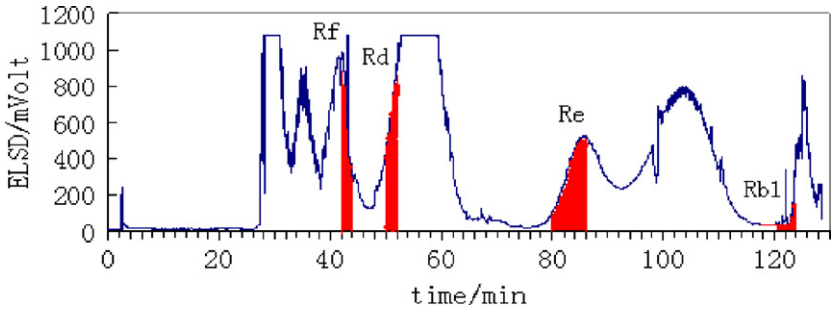


Fig. 3. Purification of four known ginsenosides by Midi-HPCCC using methylene chloride–methanol–5 mM aqueous ammonium acetate–isopropanol (6:2:4:3, v/v); loading 480 mg of crude sample in 20 mL lower phase; step flow gradient: 1–67 min at 20 mL/min, 67–120 min at 50 mL/min. Pump out the column content with the flow rate of 200 mL/min; rotation speed 1250 rpm, stationary phase retention 50%; ELSD condition: gas flow −1.7 L/min, nebulizer temperature −90 °C, evaporative temperature −120 °C with 1/10 signal output.

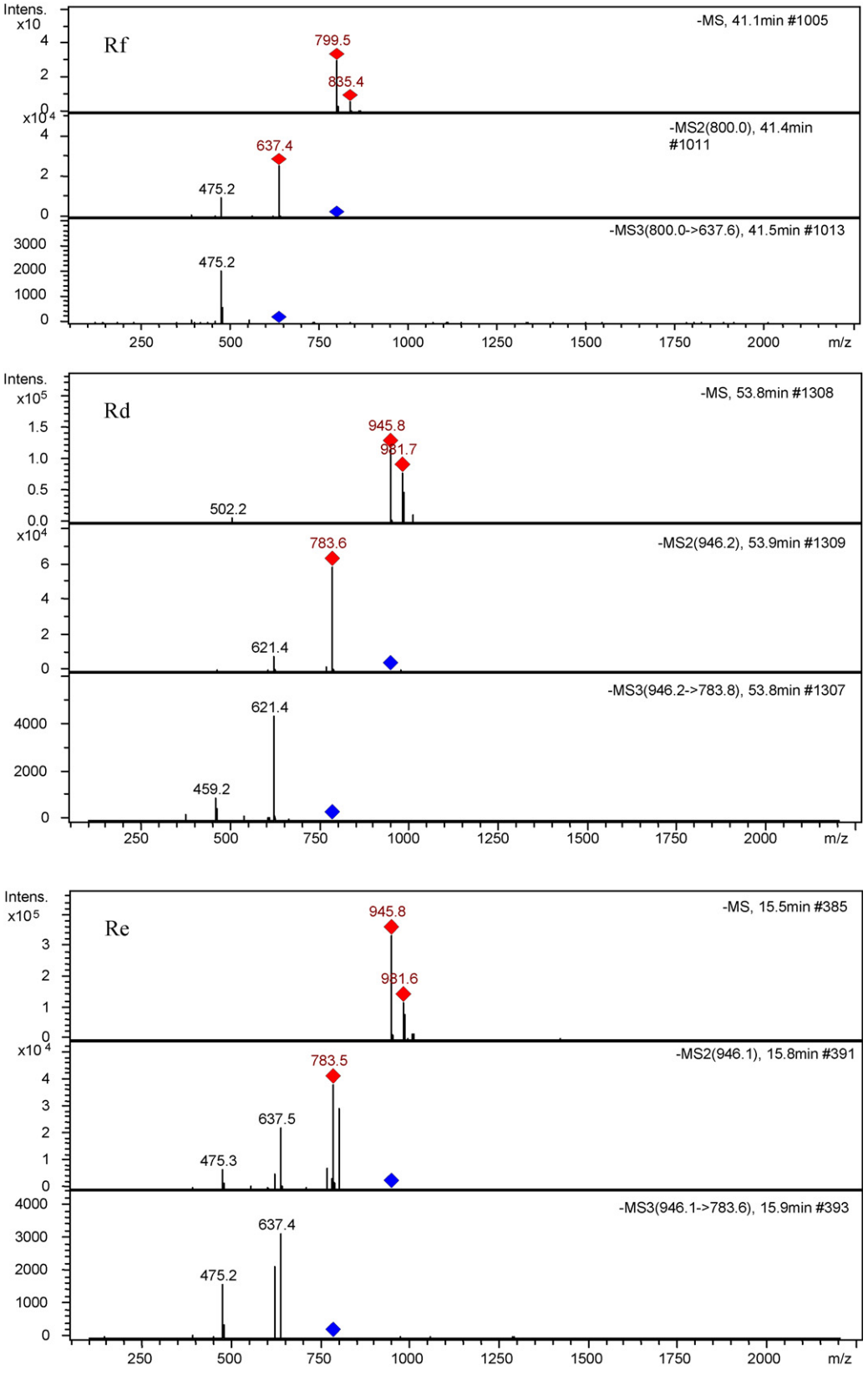


Fig. 5. ESI-MS/MS spectra of the targets.

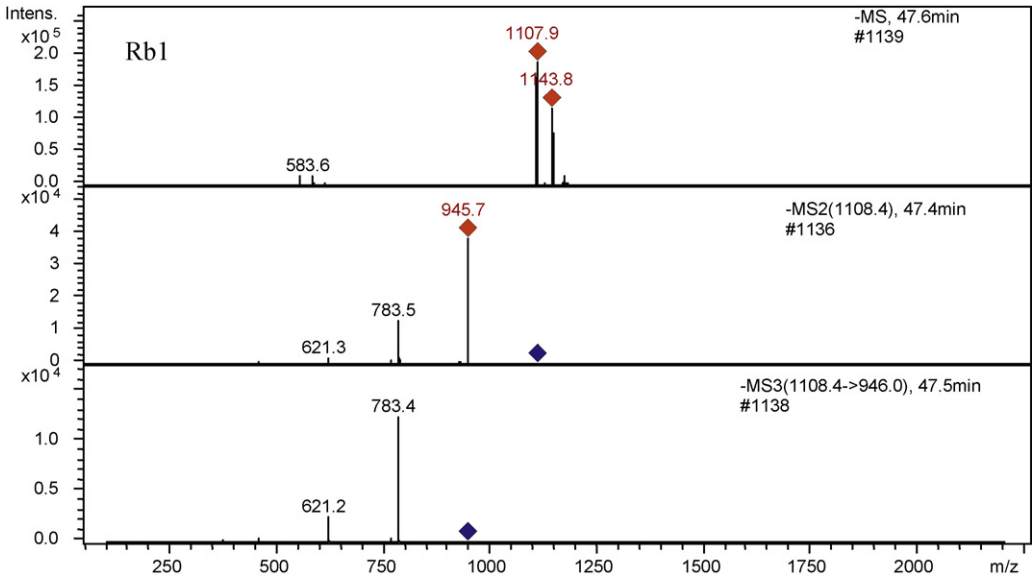


Fig. 5. (*Continued*).

Table 4
ESI-MS/MS analysis results of targets.

	MS	MS^2	MS^3
Ginsenosides Rf	799.5$[M-H]^-$	637.4$[M-H(Glc\text{-}H_2O)]^-$	475.2$[M-H\text{-}2(Glc\text{-}H_2O)]^-$
Ginsenosides Rd	945.8$[M-H]^-$	783.6$[M-H(Glc\text{-}H_2O)]^-$	621.4$[M-H\text{-}2(Glc\text{-}H_2O)]^-$, 459.2$[M-H\text{-}3(Glc\text{-}H_2O)]^-$
Ginsenosides Re	945.8$[M-H]^-$	783.5$[M-H\text{-}(Rha\text{-}H_2O)\text{-}O]^-$	637.5$[M-H\text{-}(Rha\text{-}H_2O)\text{-}(Glc\text{-}H_2O)]^-$; 475.2$[M-H\text{-}(Rha\text{-}H_2O)\text{-}2(Glc\text{-}H_2O)]^-$
Ginsenosides Rb1	1107.9$[M-H]^-$	945.7$[M-H\text{-}(Glc\text{-}H_2O)]^-$	783.5$[M-H\text{-}2(Glc\text{-}H_2O)]^-$; 621.2$[M-H\text{-}3(Glc\text{-}H_2O)]^-$

4. Conclusion

As ginsenosides easily emulsify as they are dissolved in organic solvents, a new solvent system has been successfully developed and applied for the first time using an inorganic salt as ginsenosides are unstable in an acidic environment. The salt chosen was ammonium acetate which is easily volatile and can be precipitated in warm acetone for pure sample recovery.

A rapid and high-throughput HPCCC purification method has been developed for the large-scale preparation of the water-soluble constituent ginsenosides Rf, Re, Rd and Rb1 from *P. ginseng* (*C.A. Meyer*). Scale-up from an analytical Milli-HPCCC to a preparative Midi-HPCCC was successfully achieved using step-flow gradient CCC.

Acknowledgements

The authors would like to acknowledge the generosity of Xiangxue Pharmaceuticals Group, China in funding the project at Tsinghua University and for Xiaocheng Qi to undertake a working visit to the Advanced Bioprocessing Centre at Brunel University for training. They would also like to acknowledge the financial support provided by HEIF3 and West Focus to fund the supervision of Xiaocheng Qi while working at Brunel. The authors would also like to thank colleagues at Brunel Institute for Bioengineering, UK – Dr Hugh Guan, Mr Peter Hewitson, Dr. Ian Garrard and Prof Derek Fisher, and at Tsinghua University—Mr. Yijun Cheng, Dr. Min Zhang, for their help and support during the experimental work.

References

[1] F.C. Lee, Facts about Ginseng the Elixir of Life, Hollyn Int. Corp., Elizabeth, NJ, 1992, p. 37.
[2] A.S. Attele, J.A. Wu, C.S. Yuan, Biochem. Pharmacol. 58 (1999) 1685.
[3] S. Shibata, O. Tanaka, J. Shoji, H. Saito, in: H. Wagner, H. Hikino, N.R. Farnworth (Eds.), Economic and Medicinal Plant Research, vol. 1, Academic Press, London, 1985, p. 217.
[4] K.C. Huang, The Pharmacology of Chinese Herbs, CRC Press, Boca Raton, FL, 1993, p. 21.
[5] Q. Du, G. Jerz, R. Waibel, P. Winterhalter, J. Chromatogr. A 1008 (2003) 173.
[6] X.-L. Cao, Y. Tian, T.-Y. Zhang, Q.-H. Liu, L.-J. Jia, Y. Ito, J. Liquid Chromatogr. Relat. Technol. 26 (2003) 1579.
[7] Y. Ito, J. Chromatogr. A 1065 (2005) 145.
[8] I.J. Garrard, L. Janaway, D. Fisher, J. Liquid Chromatogr. Relat. Technol. 30 (2007) 151.
[9] I.A. Sutherland, D. Fisher, J. Chromatogr. A 1216 (2009) 740.
[10] Y.W. Ha, S.S. Lim, I.J. Ha, Y.-C. Na, J.-J. Seo, H. Shin, S.H. Son, Y.S. Kim, J. Chromatogr. A 1151 (2007) 37.
[11] I.A. Sutherland, J. Chromatogr. A 1151 (2007) 6.
[12] M. Zhang, S. Ignatova, Q. Liang, F. Wu Jun, I. Sutherland, Y. Wang, G. Luo, J. Chromatogr. A 1216 (2009) 3869.