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Development of a strategy and process parameters for a *green* process in counter-current chromatography: Purification of tanshinone IIA and cryptotanshinone from *Salvia miltiorrhiza* Bunge as a case study

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ABSTRACT

A strategy for the development of a *green* process using counter-current chromatography technology is presented in this paper. The strategy began with solvent system selection, followed by linear scale-up from an analytical to a preparative process with optimized operating parameters. A two-stage separation using a multi-injection method was performed with a solvent system of hexane–dichloromethane–methanol–water (4:0.75:4:1) for the 1st stage and a hexane–ethanol–water (4:2:2) for the 2nd stage. A 191.8 mg of tanshinone IIA was purified, with a 97% purity and 34.4% recovery and a 276.7 mg of cryptotanshinone was separated, with a 95% purity and 31.8% recovery from 2.1 g of crude extract. Process parameters (throughput, efficiency, environmental risk factor and general process evaluation) and mass factors (mass intensity, separation mass efficiency and *greenness*) of a target were developed for monitoring of the counter-current chromatography process.

1. Introduction

Counter-current chromatography (CCC) technology has huge potential as a green technology due to its reduced solvent consumption and ability to substitute for a number of different processing steps, but, in its 40 year history and application in various fields [1,2], it has mainly been evaluated for its ability to purify and recover target compounds. How to minimize waste, recycle solvents and improve efficiency have not been seriously considered. The CCC separation process gives a number of advantages than compared to classical column liquid chromatography (LC), such as (1) no solid matrix for the stationary phase, (2) higher loading capacity, (3) more choices of separation solvents and (4) ease of scale-up to the kilogram scale [3].

Salvia miltiorrhiza Bunge, Chinese name – Danshen, is a well-known herbal medicine for the treatment of cardiovascular disease and inflammation [4]. Tanshinones and salvianolic acids are the main groups of effective constituents in the root of this plant [4]. It was reported that tanshinone IIA and cryptotanshinone (their structures are given in Fig. 1) could protect against liver damage [5]. Several scientific articles have been published on the separation of tanshinone IIA and cryptotanshinone using CCC [6–12] and LC technologies [13]. Satisfactory target purifications were obtained using these methods, but they needed long processing times and purity of the target compound was the only goal pursued.

In recent years, books, scientific articles and international conferences have more often drawn attention to the negative aspects of the chemical industry on human life and the planet's environment [14,15]. Resource recycling and waste minimizing have been the common view for approaching sustainability. Now the 12 principles of green chemistry [16] and 12 principles of green chemical engineering [17] are becoming the accepted norm. Although CCC technology has been used in various fields, purity and recovery of targets have been the only criteria used in evaluation of the success of a particular CCC method. How to minimize waste, recycle solvents and improve process efficiency have been seldom discussed in CCC technology publications. While pilot and process scales CCC

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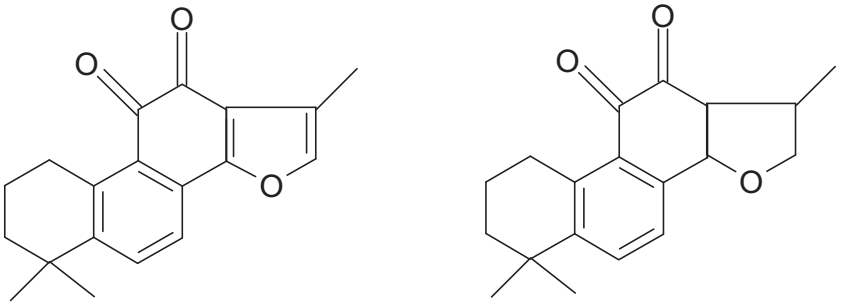

Fig. 1. The chemical structures of tanshinone IIA and cryptotanshinone.

have already been set up successfully and separations at the kilogram scale have been easily realized [18] the above issues have so far not been considered by scientists.

This paper proposes a strategy for how to develop a green CCC purification process. A case study on the separation of tanshinone IIA and cryptotanshinone is presented to illustrate this approach. Mass factors have been introduced and together with the process parameters developed previously [19] have been used to evaluate the separation process.

2. Experimental

2.1. Apparatus

Separation of tanshinones was performed on two different scales of high-performance counter-current chromatography (HPCCC) both supplied by Dynamic Extractions (Slough, UK). The analytical HPCCC machine was equipped with a 17.2 mL coil of 0.8 mm bore tubing while the preparative one was equipped with a 950 mL coil of 4.0 mm bore tubing. The high performance liquid chromatography (HPLC) used was a Waters 2695 system (Waters, USA) equipped with 2996 photodiode array detector and Empower Pro workstation. An analytical C_{18} HPLC column was from Alltech (250 mm × 4.6 mm i.d., 5 μm, USA) for HPCCC fraction analysis. An ion trap mass spectrometer (Agilent Corp., USA) was used to identify the isolated compounds. Extraction of tanshinones from the herb was performed in an ultrasonic cleaner (KQ500E, 40 kHz, 500 W, Kunshan Ultrasonic Instruments, Kunshan, China).

2.2. Materials and reagents

Dried roots of *Salvia miltiorrhiza* Bunge were purchased from China national group corporation of traditional and herbal medicine (Beijing, China). Standards of tanshinone IIA and cryptotanshinone were from the National Institute of China for the Control of Pharmaceutical and Biological Products (Beijing, China). All analytical grade of solvents used for HPCCC separation and chromatographic grade of solvents for HPLC were from Fisher Chemicals (Loughborough, UK). A deionised water used was purified by a Millipore water purification system (Watford, UK).

2.3. Preparation of crude extract

1 kg of dried roots were powdered and extracted twice ultrasonically with the mixture of methanol and dichloromethane (4:1, v/v; 5 L) at room temperature for 20 min each time. The extract solution was filtrated and concentrated by rotary evaporation followed by separation on a 2.0 L of macroporous resin with 5 bed volumes of 95% aqueous ethanol. Fractions of tanshinones were collected and dried at 50 °C. The content of cryptotanshinone in fractions was 41.4% measured by HPLC and tanshinone IIA −17.0%.

2.4. Solvent system selection

The solvent systems were selected based on the partition coefficient (K_D) values of tanshinone IIA and cryptotanshinone in the crude extract. Two types of solvent systems with different solvent ratios of hexane–dichloromethane–methanol–water and hexane–ethanol–water were tested. The procedure was as follows: 3 mg of the crude sample was added into each vial with different solvent systems. Then vials were shaken to mix the sample with the solvents. After settling equal volumes (500 μL) of upper and lower phase were transferred into new vials and evaporated at 35 °C until dryness. The residues were re-dissolved in equal volumes (1 mL) of methanol and analysed by HPLC at 280 nm to calculate the K_D value, which was defined as the peak area of sample in the upper phase divided by that in the lower phase.

2.5. HPCCC separation

The two-stage separations were performed on both analytical and preparative CCC instruments. First the analytical scale was used for method development and optimisation of operating parameters followed by scale up to a preparative coil. In the 1st separation stage, the column was filled with the stationary phase (the upper phase of 1st solvent system). Then the mobile phase (the lower phase of 1st solvent system) was pumped into the column from centre to periphery at a flow rate of 45 mL/min while the column was rotating at 1250 rpm at 25 °C. After hydrodynamic equilibrium was established, 50 mL of sample solution with 0.7 g of crude was injected into the column. Those fractions eluting between 38 min and 54 min from seven different runs were collected, combined and evaporated to dryness.

In the 2nd separation stage, the column was filled with stationary phase (the lower phase of the 2nd solvent system). Afterwards, the mobile phase (the upper phase of 2nd solvent system) was pumped into the column from the periphery to the centre direction at a flow rate of 25 mL/min. The combined residue after the 1st separation was resolubilised in 350 mL of the mobile phase (the upper phase of 2nd solvent system) and used for seven injections. All the fractions were collected according to the UV trace at 280 nm and analysed by HPLC.

2.6. HPLC analysis and identification of HPCCC fractions

The crude sample and purified fractions were analysed by HPLC at 280 nm. Separation was carried out at 25 °C with a binary mobile phase consisting of 0.05% aqueous trifluoroacetic acid (solvent A)

Table 1
Process parameters for the CCC purification process.

Factor name	Definition	Unit
Pt	$\frac{\text{Mass of crude sample processed (g)}}{\text{Time per run (h)}}$	g/h
Pe	$\frac{\text{Mass of the target compound (g)}}{\text{Time consumption (h)}}$	g/h
Er	$\frac{\text{Volume of solvent consumption (L)}}{\text{Mass of the target compound (g)}}$	L/g
Ge	$\frac{Pe}{Er}$	$g^2\ h^{-1}\ L^{-1}$

Table 2
Mass factor of CCC purification process.

Factor name	Definition	Unit
MI	$\frac{\text{Total mass of solvent and crude sample loaded (g)}}{\text{Mass of the target compound produced (g)}}$	–
SME	$\frac{\text{Mass of the target compound produced (g)}}{\text{Total mass of solvent and crude sample loaded (g)}} \times 100\,(\%)$	–
G	$SME \times Ge$	$g^2\ h^{-1}\ L^{-1}$

and acetonitrile (solvent B) using a linear gradient of solvent B from 20 to 70% at a flow rate of 1.0 mL/min. Identification of the purified compound was performed by LC–ESI–MS. The ion trap MS analysis was carried out in positive mode using the following operation parameters: capillary voltage: 3000 V; capillary exit voltage: 94 V; skimmer voltage: 40 V; drying gas: 9 L/min; nebulizer pressure: 30 psig; gas temperature: 350 °C; mass scanning range: 50–1000 *m*/*z*.

3. *Green* process strategy in CCC purification

Most chemical scientists, who are working on CCC separation of natural products, devote themselves into looking for suitable solvent systems to give a K_D value in the range of 0.5–2.0 and a good separation factor (α) [20]. The conventional strategy for development of a CCC separation process involves: (1) solvent system selection optimised by K_D values and α factor; (2) analytical scale separation evaluated by purity and recovery; and (3) scale-up to preparative scale, if step (2) successful, or return to step (1) if the purity and/or recovery are not satisfactory, then another solvent system will be tried.

Issues of separation time, solvent consumption and efficiency have been rarely discussed by CCC chemical scientists. The purification process of natural products by CCC has not generally been considered *green* as it uses organic solvents. CCC, however, can be a much more efficient technology compared to LC from the solvent consumption point of view [21]. In this paper, a green strategy for CCC purification processes is presented. Two groups of variables, process parameters [19] (purity, recovery, throughput, process efficiency, environmental risk, and general process evaluation) and mass factors (mass intensity, separation mass efficiency and greenness) were used to promote the *green* CCC purification process.

3.1. Process parameters

Process parameters such as throughput (*Pt*), efficiency (*Pe*), environmental risk factor (*Er*) and general process evaluation factor (*Ge*) were developed in a previous article [19] and are listed again in Table 1.

Pt and *Pe* have been defined to optimize the CCC purification process together with target purity. These two parameters are used to estimate the capacity of the CCC process. *Er* evaluates the environmental impact of the CCC process giving the waste solvent produced per unit mass of target compound separated. *Ge* combines the *Pe* and *Er* to calculate the general productivity of the CCC process relative to its environmental impact. These four parame-

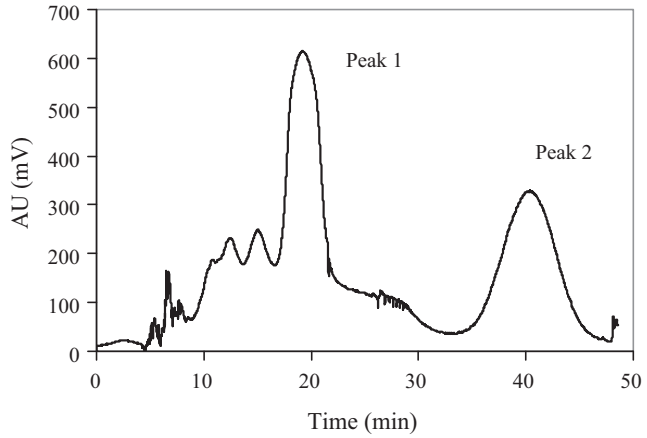

Fig. 2. First separation stage of cryptotanshinone and tanshinone IIA on Mini HPCCC using hexane–dichloromethane–methanol–water (4:0.75:4:1) solvent system; loading 20 mg in 0.8 mL upper phase; flow rate 1.0 mL/min; rotation peed 2100 rpm (Peak 1, Cryptotanshinone with contaminant; Peak 2, Tanshinone IIA). Sf = 68.6%.

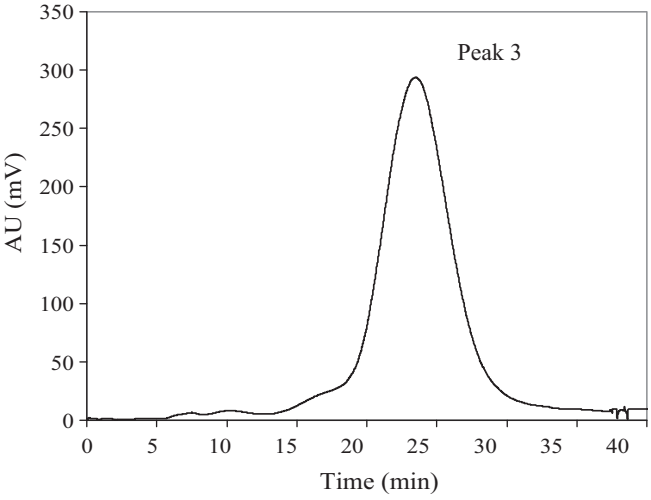

Fig. 3. Second purification stage of cryptotanshinone by Mini HPCCC using hexane–ethanol–water (4:2:2) solvent system, flow rate 0.5 mL/min; rotation speed 2100 rpm (Peak 3, cryptotanshinone). Sf = 79.1%.

Table 3
The partition coefficient (K_D) of Cryptotanshinone and Tanshinone IIA for different solvent systems.

Stage	Solvent system	Ratio	Mode	K_{Dc}	K_{Dt}	K_{Dcc}	$\alpha_{c\text{-}t}$	$\alpha_{c\text{-}c}$
1st	Hexane–dichloromethane–methanol–water	4:0.5:4:1	RP	1.40	3.05	1.49	2.18	1.06
		4:0.75:4:1	RP	1.19	2.78	1.58	2.34	1.33
		4:1:4:1	RP	0.85	2.25	0.90	2.65	1.06
2nd	Hexane–ethanol–water	4:1:2	NP	0.14	0.05	0.41	2.80	2.93
		4:2:2	NP	0.59	0.35	1.00	1.69	1.69
		4:2.3:2	NP	1.24	0.39	1.24	3.18	1.17

Note: K_D was calculated from the ratio of HPLC peak area in the upper phase to that in the lower phase for cryptotanshinone (K_{Dc}), tanshinone IIA (K_{Dt}) and the contaminant eluting after cryptotanshinone (K_{Dcc}); $\alpha_{c\text{-}t}$ (K_{Dt}/K_{Dc}) is the separation factor between cryptotanshinone and tanshinone IIA; $\alpha_{c\text{-}c}$ (K_{Dcc}/K_{Dc}) is the separation factor between cryptotanshinone and the following contaminant; RP, reverse phase mode; NP, normal phase mode.

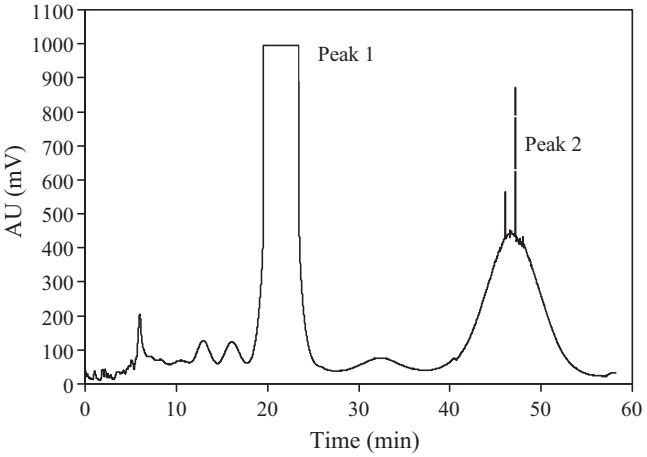

Fig. 4. First stage separation of cryptotanshinone and tanshinone IIA by Midi HPCCC using hexane–dichloromethane–methanol–water (4:0.75:4:1) solvent system; loading 700 mg in 50 mL upper phase; flow rate 45 mL/min; rotation speed 1250 rpm (Peak 1, cryptotanshinone with contaminant; Peak 2, tanshinone IIA).

ters have relationships with time describing the dynamic character of a CCC process. These process parameters were used for developing a highly efficient CCC purification process together with target purity and recovery.

3.2. Mass factors

Mass factors (mass intensity (*MI*), separation mass efficiency (*SME*) and *greenness* of a target (*G*)) are defined and listed in Table 2.

The mass intensity factor was first developed by Curzons et al. [22] to show the total mass consumption per unit of target compound produced. In this paper, *MI* was used to estimate the mass intensity of a CCC process and a further separation mass efficiency factor is the reciprocal of *MI* expressed as a percentage and is used to evaluate the product transformation capability of a CCC process. The product of *Ge* and the *SME* factor gives the *greenness* (*G*, as in Eq. (1)) of a target compound expressing the quantitative evaluation of the mass transformation relative to the environmental impact in a CCC process.

$$G = \frac{(\text{mass of the target compound produced (g)})^3}{\text{total mass of solvent and crude sample loaded (g)} \times \text{time (h)} \times \text{volume of solvent consumption (L)}} \quad (1)$$

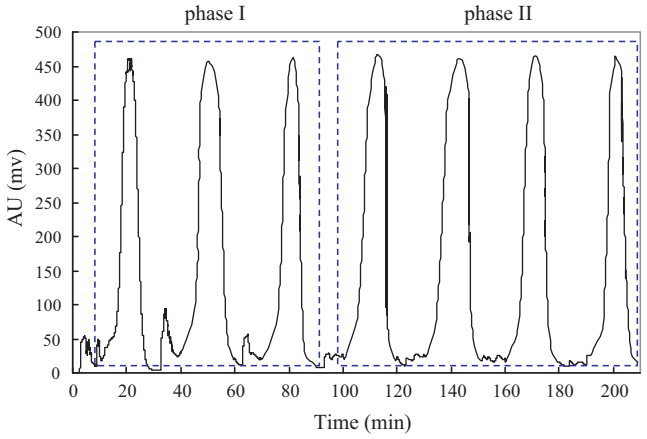

Fig. 5. Second purification stage of cryptotanshinone on Midi HPCCC by multi-injection using hexane–ethanol–water (4:2:2) solvent system, flow rate 25 mL/min; rotation speed 1250 rpm. Sf = 90.1%.

4. Results and discussion

4.1. Selection of solvent system

Tanshinone fractions from the extraction of dried roots of *Salvia miltiorrhiza* Bunge are very non-polar and contain two major target compounds with co-eluting impurities, which have very similar distribution ratios to the target compounds. Being so non-polar, tanshinones have very limited solubility in most organic solvents. Various solvent combinations of hexane–ethyl acetate–methanol–water, hexane–dichloromethane–methanol–water, hexane–ethanol–methanol–water, dichloromethane–methanol–water and non-aqueous solvent systems such as hexane–acetonitrile–methanol, hexane–acetonitrile–dichloromethane were tested to meet the sample solubility and partition coefficient criteria. However, the above solvent system tested did not give proper K_D values and $\alpha_{c\text{-}t}$ except for the system of hexane–dichloromethane–methanol–water. Table 3 shows the result of K_D values for the best solvent systems.

Although hexane–dichloromethane–methanol–water systems provided a good range of K_D values to isolate tanshinone IIA from cryptotanshinone with the separation factor $\alpha_{c\text{-}t}$ higher than 2, the latter cannot be separated from its co-eluting contaminant as the separation factor $\alpha_{c\text{-}c}$ was not more than 1.33. However, this type of solvent system gave the best solubility of the crude (10 mg/mL) and therefore, led to the highest loading even with loss in the purity of the first target. Thus, it was decided to perform a two-stage separation strategy to increase purity, throughput and process efficiency.

The aim of the 1st purification stage was to isolate the second target, tanshinone, with the minimum 95% purity and high throughput while harvesting the first target, cryptotanshinone, with as high as possible purity for this system. For this reason, the ratio of dichloromethane in the solvent system was adjusted to reduce the values of K_{Dc} and K_{Dt} and make the second target elute earlier. The hexane–dichloromethane–methanol–water (4:0.75:4:1) solvent system in RP mode was chosen for the first separation stage, as it gave the best separation factor $\alpha_{c\text{-}c}$ (1.33) between cryptotanshinone and the co-eluting contaminant and maintained a separation factor of 2.34 between cryptotanshinone and tanshinone IIA. The aim of the 2nd separation stage was to purify cryptotanshinone up to a minimum 95% purity. As the bulk of the material with contaminants has been removed in the 1st stage, solubility became less of an issue. As a result, dichloromethane was taken out of solvent system and methanol was substitute with ethanol, which is more miscible with both hexane and water. After trying different ethanol ratios (Table 3) it was established that the optimum solvent system was hexane–ethanol–water (4:2:2) run in NP mode, when cryptotanshinone was completely purified from the contaminant with an $\alpha_{c\text{-}c}$ factor of 1.69.

4.2. Optimization of operational parameters on analytical Mini-HPCCC

Optimization of separation parameters was done on the Mini HPCCC instrument. A typical chromatogram of the 1st stage on Mini is shown in Fig. 2.

The highest purities from the fractions across the cryptotanshinone peak 1 and tanshinone IIA peak 2 were 91.2% and 94.5% respectively. Three fractions across peak 1 were collected and only

Table 4
Comparative results for different CCC separation methods.

Method	Target	Purity (%)	*Pt* ($g\,h^{-1}$)	*Pe* ($g\,h^{-1}$)	*Er* ($L\,g^{-1}$)	*Ge* ($g^2\,h^{-1}\,L^{-1}$)	*MI*	*SME* (%)	*G* ($g^2\,h^{-1}\,L^{-1}$)
Method in this paper	C	95	0.750	9.88E−02	36.53	2.70E−03	25.55	3.91	1.06E−04
	T	97	0.764	6.97E−02	56.70	1.23E−03	36.36	2.75	3.38E−05
Method A [6]	C	>95	0.016	1.29E−03	182.00	7.09E−06	159.53	0.63	4.44E−08
	T	>97	0.008	1.17E−03	152.57	7.65E−06	135.41	0.74	5.65E−08
Method B [7]	C	98.8	0.086	–	–	–	–	–	–
	T	96.8	0.033	–	–	–	–	–	–
Method C [8]	C	>95	0.025	2.75E−03	76.18	3.61E−05	66.61	1.50	5.42E−07
	T	>95	0.010	1.60E−03	97.38	1.64E−05	87.49	1.14	1.88E−07
Method D [9]	C	98.7	0.043	–	–	–	–	–	–
	T	99.5	0.022	–	–	–	–	–	–
Method E [10]	T	98	4.4E−06	6.32E−07	106.10	5.95E−09	87.76	1.14	6.78E−11

Note: C is representing for cryptotanshinone, T for tanshinone IIA. Original data for calculating *Pe*, *Er*, *Ge*, *MI*, *SME* and *G* were not reported in method B and D, so a "–" represents for that in the table.

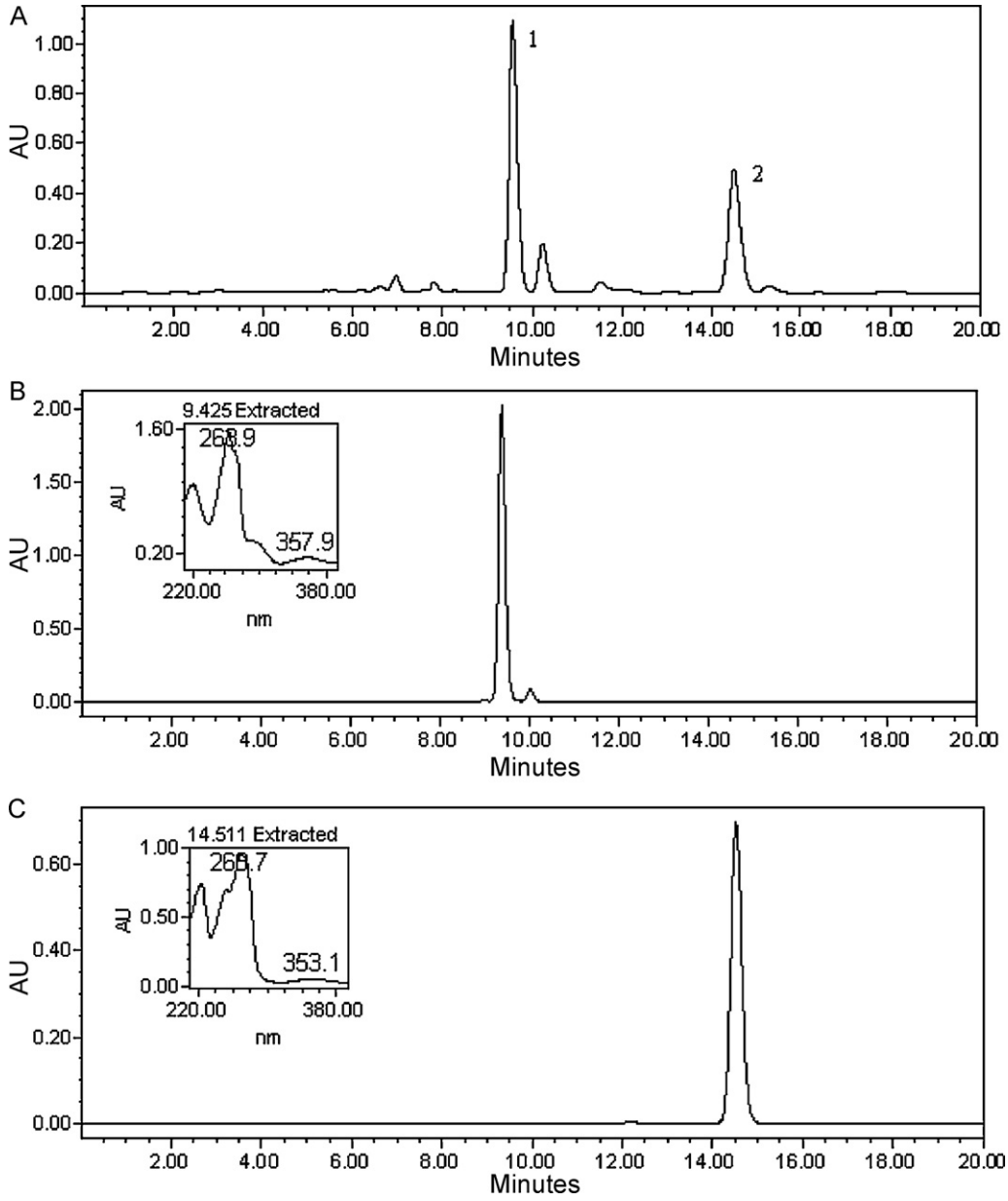

Fig. 6. HPLC chromatogram of crude A (A), purified peak 1 (B), purified peak 2 (C). Alltima C18 column (250 mm × 4.6 mm i.d., 5 μm); column temperature 27 °C. solvent A, 0.05% aqueous trifluoroacetic acid and solvent B, acetonitrile; flow rate: 1.0 mL/min; isocratic elution 0–20 min, 75% B. wavelength 280 nm. (Peak 1, Cryptotanshinone; Peak 2, Tanshinone IIA).

one of the fractions with an 85.0% purity was taken to the 2nd stage. Fig. 3 shows a chromatogram of this 2nd purification. The highest purity from fractions taken across the cryptotanshinone peak was over 98.0%.

4.3. Scale-up to preparative HPCCC

The two-stage separation was transferred to the preparative scale HPCCC – from the Mini analytical instrument to the Midi preparative one. A typical chromatogram of the first stage on Midi is shown in Fig. 4. According to volumetric scale-up theory a flow rate of 55 mL/min could have been used, but we used 45 mL/min to avoid blockage of the injection port and to increase the target purity.

The purity of cryptotanshinone in peak 1 was 86.2% and tanshinone IIA in peak 2–97.4%. Three fractions of peak 1 were collected and one of them (purity ~80.0%) was purified in the 2nd stage. Fig. 5 shows a multi-injection chromatogram of further purification. Three serial injections (Phase I) before changing out the stationary phase give a purity in the range 95–98% for cryptotanshinone while four serial injections (phase II) give a range 90–95%.

It was confirmed by HPLC analysis that the three multi-injections before re-filling the CCC column with fresh stationary phase was the optimum solution. Therefore, the throughput of the second stage is much higher than the first stage due to multiple usage of the same load of stationary phase and its high volume (Sf = 90.1%).

4.4. Process parameters and mass factors calculation

In this paper, the two stage process for separation of cryptotanshinone and tanshinone IIA from *Salvia miltiorrhiza* Bunge was developed. Comparative results, between different preparative CCC methods published and the method in this paper, are listed in Table 4. Methods A, B, C and D were developed for the separation of cryptotanshinone and tanshinone IIA, while in method E only the target tanshinone IIA could be used for comparison.

The purities of targets, both in the developed and published methods, were over 95%. Those in method D were higher than others, which gave 98.7% purity for cryptotanshinone and 99.5% for tanshinone IIA. Although the purities were quite close to each other, the *Pt* and *Pe* values in different methods differed greatly. The cryptotanshinone *Pt* value using the method in this paper was 8.7× higher than the highest one previously published (method B), while the tanshinone IIA *Pt* value was 23.2× that of method B. The *Pe* values of this paper were much higher than those in the publications cited (cryptotanshinone – 35.9× and tanshinone IIA – 43.6×). The higher *Pt* and *Pe* were a result of the method of multi-injection. As the *Er* factors shown in Table 4, the previously lowest *Er* (method C) for cryptotanshinone was 2.1× higher for tanshinone IIA 1.7× higher solvent consumption than in this paper. The highest *Er* (method A) for cryptotanshinone was 5.0× higher and for tanshinone 2.7× higher than in this paper. The general process evaluation factor, *Ge* (*Pe*/*Er*), in this paper was around 75.0× higher than method C, which was the highest found from previous literature. Factors of *Pt*, *Pe* and *Ge* express the capacity of a CCC process, which was related to process time. If a scientist pursues high process efficiency, such factors could be used to monitor a CCC process development.

Mass factors of *MI*, *SME* and *G* from different methods were also compared and listed in Table 4. The *MI* factor was to show how the total mass consumption as the per unit product produced. The value of *MI* in this paper for cryptotanshinone was 2.6× lower than the lowest from the published literature (method C) and tanshinone IIA 2.4× lower. The *SME* factor, which was to estimate the percentage of mass both crude and solvent transformed into product (the higher the value of *SME* meant the better separation method used) gave only 3.91% for cryptotanshinone and 2.75% for tanshinone IIA showing the low mass productivity character of this separation process.

An interesting question is whether a process with low *Ge* value can get a high *SME* value? The answer seems to be positive. In the example of method E, the *Ge* value 5.95E−09 was very low, while its *SME* was 1.14%, the same is true in method C. That means that it is possible for a *poor* separation to get a higher mass transformation than a *good* one. Therefore, *Ge* or *SME* are not able to evaluate both a CCC process and its product. However, *G*, the product of *Ge* and *SME*, will show the quantities of the target product produced per unit of total mass, time and solvent consumed in a the CCC process (Eq. (1)). The parameter *G* in this paper is 1.06E−4 for cryptotanshinone and 3.38E−05 for tanshinone IIA, which is 195.6× and 179.8× higher than in the closest published paper (method C).

4.5. HPL C and MS identification of targets

Purified fractions of peak 2 in Fig. 5 and phase I in Fig. 6 were collected for HPLC analysis. A 191.8-mg amount of tanshinone IIA (peak 2 in Fig. 5) was isolated with a 97% purity and 34.4% recovery. While a 276.7-mg amount of cryptotanshinone (phase I in Fig. 5) was separated with a 95% purity and 31.8% recovery. Fig. 6 shows the HPLC chromatograms and UV spectra of crude extract (Fig. 6A), purified peak 1 (Fig. 6B) and purified peak 2 (Fig. 6C).

For phase I in Fig. 6, the LC–ESI–MS mass spectrum with positive mode gave the ions of *m*/*z* 297 $(M+H)^+$, 251 $(M+H-H_2O-CO)^+$ with a molecule mass of 296. For peak 2 in Fig. 6, the LC–ESI–MS mass spectrum with positive mode gave the ions of *m*/*z* 295 $(M+H)^+$, 277 $(M+H-H_2O)^+$. The ions information agreed with cryptotanshinone and tanshinone IIA in the literature [23,24].

5. Conclusion

A strategy of how to develop a *green* process for CCC separation has been suggested in this paper. The separation of tanshinone IIA and cryptotanshinone was used as a case study. The optimization process involved (1) a solvent system selection with K_D value and α factor as the criteria, (2) optimization of the operating variables monitored by process parameters (throughput, efficiency, environmental risk and general process evaluation), with a linear scale-up from analytical to preparative CCC successfully and predictably carried out and finally (3) mass factors (intensity, separation efficiency and *greenness*) were used as production checking criteria.

A two-stage separation with a multi-injection method was performed on a preparative CCC. 2.1 g of crude extract was processed and two targets were harvested, one was 191.8 mg of tanshinone IIA with a 97% purity and 34.4% recovery, the other was 276.7 mg of cryptotanshinone with a 95% purity and 31.8% recovery. The values of *G* were 1.06E−4 for cryptotanshinone and 3.38E−05 for tanshinone IIA, which were much higher than those in previously published literature. This case study demonstrated the feasibility of a green process strategy for CCC.

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