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Effects of ionic liquid and nanogold particles on high-performance liquid chromatography-electrochemical detection and their application in highly efficient separation and sensitive analysis of five phenolic acids in Xuebijing injection

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ABSTRACT

A novel high performance liquid chromatography-electrochemical detector (HPLC-ECD) analytical system was developed in this study by integratedly utilizing ionic liquid (IL) of 1-butyl-3-methylimidazolium bromide and an additive of gold nanoparticles. The resulted pilot study was first performed to assess the effects of 1-butyl-3-methylimidazolium bromide and gold nanoparticles on the chromatographic characteristics of five phenolic acids in Xuebijing injection, including danshensu (DSS), protocatechuic acid (PA), protocatechuic aldehyde (PAH), hydroxy safflower yellow A (HSYA) and ferulic acid (FA). It was notable to observe that retainability of the phenolic acids were markly lowered by IL addition. Compared with the cases without IL addition, the retention times of DSS, PA, PAH, HSYA and FA have decreased 2.851, 1.532, 1.53, 0.818 and 0.552 min, respectively when 0.6% IL in the mobile phase. In addition, the corresponding theoretical plate numbers and peak areas for these compounds were significantly increased. Area response for DSS, PA, PAH, HSYA and FA were enhanced by 772%, 628%, 584%, 703% and 600%, respectively. It was observed that nano-gold catalysis power enabled peak areas of DSS, PAH, FA and PA to enhance 5.7, 6.2, 8.5 and 66.5 times relative to the case with addition of IL. Altogether, the optimized HPLC-ECD system was successfully applied to the pharmacokinetics study of Xuebijing injection with underlying applicability to in vivo and in vitro analysis of a variety of natural product from Chinese medicine plants, TCM formulae and associated patent TCM preparation.

1. Introduction

Many studies indicate that retaining ability of analytical compounds onto the chromatographic column is affected markly by cross-linking reaction resulting from the free silanol groups at the surface of the chromatographic stationary phase silica and its related materials [1,2]. The cross-linking reaction can be intervened by mixing ion-pair reagent into the mobile phase or adjusting its pH value. However, addition of ion-pair reagent to mobile phase often leads to higher pressure, lower tolerance and irreversible damage to stationary phase of the analytical column. Therefore, it can be predictable that the reaction of the free silanol groups poses a great challenge for obtaining ideal analytical performance by using the conventional column.

Because of their distinctive properties, ILs have been broadly applied to many fields, such as heterogeneous catalysis, synthesis, electrochemistry and separation [3–6]. Recently, ILs have also been given increasing attention by scientists of analytical chemistry, who selectively use ILs to improve chromatographic performance [7–12]. ILs are primarily used as modification agent for stationary phase or mobile phase in gas chromatography [13,14] and high performance liquid chromatography [15], for chromatographic analysis of chiral compounds [16] or others. The primary

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reaction of ILs with stationary phase is characterized by cationic precursors of ILs being firstly coated on the surface of stationary phase, where they compete with analytical basic compounds for blinding to stationary phase. After coating, ILs suppress deleterious effects of free silanols and improve chromatographic peak shapes. Then the alkyl chains of the stationary phase proceed cross-linking reaction with the imidazole cation in ILs. Finally, because of the repulsion between imidazolium cation and ionized amines, part of them move with the mobile phase, and the anionic cores of ILs form ion-pair reagent with other cationic ion of the mobile phase [17]. ILs also present a preferred electrochemical activity [18, 19]. However, there are a few cases of approach application the applicability of ILs to electrochemical detector.

In addition, nano-metal particles have received increasing attention in the fields of physics, chemistry, biomedicine, material science and related disciplines [20–25], owing to their special electronic, quantum tunnel, optical and catalytic properties. An increasing number of researchers in electrochemical field also have turned their attention to nanometer materials in attempt to make new style electrode for selective ultra-sensitive detection of target compounds [26–30]. For example, nano-gold particles have been adopted by electro-analytical chemists because of their better biocompatibility, larger specific surface area, good conduction effect and catalytic activity.

Xuebijing injection is a confidential type of species in China, and it is an intravenous preparation in Traditional Chinese Medicine (TCM). Professor Jinda Wang developed it based on the Xuefuzhuyu decoction of Qinren Wang according to the theory of 'bacteria and bacterial toxin treated simultaneously' and dialectical principle of 'Sanzhengsanfa' [31]. The efficacy includes activating blood circulation and removing blood stasis, supporting the healthy energy, strengthening the body resistance, and clearing away and relieving toxin. Its main active component is extracted from *Flos carthami*, *Radix salviae miltiorrhizae*, *Rhizoma chuanxiong*, *Radix angelicae sinensis* and *Radix paeoniae rubra*. Clinically, Xuebijing injection is used in combination with antibiotics to treat pyemia and multiple organ dysfunction syndrome. And it is the unquiet injection of TCM treatment of this disease in China, which makes the TCM become one part of the main stream of emergency medicine [32]. Xuebijing injection may regulate inflammatory reaction and anti-oxidative stress, regulate immune function, ameliorate blood coagulation function, protect endothelial cells and improve microcirculation [33–38].

Up to now, much attention has been focused on the clinical research and chemical composition analysis of Xuebijing injection [39, 40], and there are no reports on the chemical composition analysis by ECD or the pharmacokinetics of main component. Most publications described methods for the analysis of DSS, PA, PAH, HSYA or FA in biological samples by high-performance liquid chromatography with UV detector [41–45], few analyzed with ECD or mass detector [46, 47]. The poor sensitivity of the HPLC–UV method always leads to failure in the complicated sample analysis.

This work aims to characterize the effects of IL and nano-gold as additives in HPLC-ECD analytical system by comparatively assessing retention time, number of theoretical plate and corresponding peak area of five acids in Xuebijing injection. Furthermore, the optimized HPLC-ECD method was applied to profiling pharmacokinetics features Xuebijing injection.

2. Materials and methods

2.1. Apparatus

The experiments were performed on an Agilent HPLC system (Series 1100, Agilent Technologies, USA) equipped with a G1311A quaternary pump, G1379A vacuum degasser, G1316A column thermostat, G1315B DAD and G1313 auto manual sample injector. In addition, a 790 VA programmer electrochemical detector (Metrohm, Switzerland) and a G1311A quaternary pump (Series 1100, Agilent Technologies, USA) were used. The chromatography data were recorded and processed with HP chemstation software.

2.2. Chemicals and reagents

DSS (Lot no.: 110855–200506), PA (Lot no.: 101800–200205), PAH (Lot no.: 110810–200205), HSYA(Lot no.: 111637–200905) and FA (Lot no.: 11173–201012) were purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China), and their purity was over 98% by HPLC analysis. The ionic liquid, 1-butyl-3-methylimidazolium bromide, was supplied by Shanghai Chengjie Chemical Reagent Co., Ltd. (Shanghai, China). Xuebijing injection was purchased from Tianjin Chasesun Pharmaceutical Co., Ltd. (Tianjin, China). Methanol was of HPLC grade and obtained from Fisher Scientific Products (Fair Lawn, USA), and triply distilled water was employed. The other chemicals, reagents and solvents used were all of analytical grade.

2.3. Chromatographic condition

The analytical column was an Aglient TC-C_{18} (250 mm × 4.6 mm, 5 μm) column coupled with a C_{18} guard column. The column temperature was controlled at 30.0 ± 0.1 °C. The mobile phase was a mixture of methanol (A) -water containing 0.1% (v/v) formic acid (B) and different concentrations (0.0∼0.6%, v/v) of ILs by gradient elution mode.(0–10 min, 15% A; 10–25 min 15–25% A; 25–40 min, 25–60% A; 40–55 min, 60–100% A). The flow rate of the mobile phase was 0.6 mL/min. The equilibration time for each run was 5 min. The sample injection volume was 20 μL.

For electrochemical detection, a glassy carbon electrode was used as the working electrode, an Ag/AgCl was the reference electrode and a Pt wire was used as the counter electrode.

2.4. Effects of the IL and nano-gold on the HPLC-ECD analytical system

Preparation of nano-gold sol: take 1 mL $HAuCl_4$ aqueous solution (10 mg/mL) into 100 mL aqueous solution, heat to boil and maintain for 5 min, then drip 0.7 mL 1% $C_6H_5Na_3O_7$ (mass fraction) quickly followed by keeping for boiling for 15 min until the solution turns into wine-red. After cooling the solution at the room temperature, settle to 100 mL, then store in dark.

2.4.1. Investigation of the chromatographic behavior

The applied working potential of the electrochemical detector was set at +900 mV. The mobile phase includes different concentrations of additive IL (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, v/v). Mobile phases without additives were also employed for comparative purpose. The variation of the retention time and theoretical plate number of DSS, PA, PAH, HSYA and FA were investigated.

2.4.2. Effects of IL and nano-gold particles on sensitivity of ECD

The working potentials, as the part of the entire methods, were set as +600, 700, 800, 900, 1000, and 1100 mV respectively. In order to acquire the optimal condition for HSYA, DSS, FA, PAH and PA detection in Xuebijing injection, the methods were set as follows:

(1) The chromatographic behavior and the electrochemistry detection for the five phenolic acids with non-ILs and nano-gold added took as 2.3.

(2) The chromatographic behavior with ILs added: The mobile phase for the main flow contained 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and 0.6% ILs as additives, respectively, and the other conditions were the same as (1).
(3) The chromatographic behavior and electrochemical detection with ILs and nano-gold particles added: the mobile phase for the main and side flows contained different concentrations of ILs (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%) and nano-gold sol (57.9, 28.95, 19.3, 11.58, 8.27, and 5.79 mg/L), respectively.

2.5. Application of ILs-HPLC-nanogold-ECD system

2.5.1. Chromatographic conditions

The mobile phase was a mixture of methanol-water containing 0.1% (v/v) formic acid, and the gradient elution condition and flow rate were the same as 2.3. For urine and feces analysis, the gradient elution was as follows: 0–15 min, 26% A; 15–30 min 26–29% A; 30–50 min, 29–35% A; 50–55 min, 35–50% A; 55–65 min, 50–53%). 0.4% (v/v) ILs was added as addictives, and the concentration of nano-gold sol was 8.27 mg/L. The electrochemical detector was set at 900 mV in oxidative mode.

2.5.2. Preparation of calibration and quality control samples

The mixture of stock standard solution containing DSS(0.25 mg/mL), PA (0.12 mg/mL), HSYA (0.05 mg/mL) and FA (0.11 mg/mL) was dissolved in methanol. The working standard solution was prepared by serial dilution of the stock solution with methanol. The calibration samples in biological samples (plasma, urine or feces) were prepared by mixing the standard mixture with rat blank biological sample to form different concentration series (0.2, 1.4, 2.8, 5.7, 11.5, 23 μg/mL of DSS, 0.3, 3.3, 7, 14, 28, 56 μg/mL of PA, 0.5, 1.6, 3, 6, 13, 25, 51 μg/mL of HSYA, and 0.2, 1, 3, 13, 26, 51 μg/mL of FA). All solutions were stored at 4 °C until futher use.

2.5.3. Sampling collection

The Sprague-Dawley (200–230 g) rats were housed (temperature: 20–23 °C; humidity: 50 ± 5%; 12 h light/dark cycle) with unlimited access to food and water except for fasting 12 h before experiment, with water available ad libitum. All the animals were kept according to the Chinese Government Guidelines for the Care and Use of Laboratory Animals. Xuebijing injection was administered intravenously to rats through a cannula inserted into the femoral vein. At fixed intervals, blood was taken from the femoral artery through the cannula. The blood was collected pre-dose and at 2, 5, 10, 15, 20, 25, 30, 40, 50, 60 and 90 min after receiving a single intravenous dose of Xuebijing injection. 200 μL of plasma was separated by centrifuging at 8000 rpm for 10 min, collected and stored at −20 °C until analysis.

Urine and feces samples were collected using metabolic cages. Six male rats were housed in individual metabolism cages designed for separation and collection of feces and urine. Feces and urine samples were collected separately at the interval of 0–4 h, 4–8 h, 8–12 h, 12–16 h, 16–24 h after drug administration. All samples were stored at −80 °C for further analysis.

2.5.4. Sample preparation

Samples of plasma (200 μL) and urine (200 μL) were precipitated with 600 μl acetonitrile. After vortex-mixture for 1 min, the obtained samples were centrifuged at 9000 rpm for 10 min. Each sample was extracted three times with acetonitrie and the upper fractions were combined and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 100 μL of HPLC mobile phase prior to analysis and then 20 μL of sample was injected into the LC system.

Feces were dried, crushed, weighed, and then mixed with water at the rate of 3.2 ml/g. Ultrasonic extraction was performed and the samples were then centrifuged at 9000 rpm for 10 min. Supernatants were transferred to clean tubes and then prepared as urine and plasma samples.

2.5.5. Method validation

The method was validated regarding its selectivity, linearity, limit of quantification (LOQ), accuracy, precision, recovery and stability. To evaluate its selectivity, five independent rat blank biological samples were analyzed by comparing them with the biological-spiked analytes for excluding the interference of endogenous material. The LOQ was considered as the final concentration that produced a signal-to-noise (S/N) ratio of 10. The precision and accuracy of the method were assessed by performing replicate analyses of quality control (QC) samples against calibration standards. The precision was determined from inter-day and intra-day using six determinations of low, medium and high concentrations and expressed as relative standard deviation (RSD%). The extraction recovery was determined by calculating the ratio between the amount of the extracted compounds from drug-free biological samples spiked with known amounts of DSS, PA, HSYA and FA. The stability of the sample was assessed by measuring the analysis data of QC samples under ambient, frozen and froze-thaw storage conditions with freshly prepared QC samples.

2.5.6. Study of animal pharmacokinetic and data analysis

The HPLC procedure was successfully applied to investigate the plasma concentration–time profiles of FA, HSYA and PA in rats. The pharmacokinetic model and the parameters were analyzed by the drug and statistics practical software (DAS software, a recommended pharmacokinetic program in China, version 2.0). The pharmacokinetic parameters were obtained by the software.

The described method was also applied to assay DSS, PA, HSYA and FA in urine and fecal water of six healthy rats. Urine and fecal samples were collected at different times after administration. The excretion ratio in every period for the tested compounds was calculated using the formula:

$$\mathrm{Fr} = \frac{\text{amount detected from urine or fecal water}}{\text{dosage administrated} \times 100\%}$$

3. Results and discussion

3.1. Effects of IL and nano-gold particles on HPLC-ECD

3.1.1. Effects of ILs on the chromatographic behavior of DSS, PA, PAH, HSYA and FA

Table 1 shows the effects of different concentrations of IL as the mobile addictive on the retention time (RT) of the five phenolic acids. With the increase of IL concentration in the mobile phase, the RTs of DSS, PA, PAH, HSYA and FA had a forward trend. Among them, the RT of DSS had the maximum forward value (2.851 min), and the forward values for PA, PAH, FA and HSYA were 1.532, 1.530, 0.552 and 0.818, respectively. It could suggest that the polarity of the mobile phase decreased and the solubility increased with the increase of concentration of the added ILs, so the solubility of these phenolic acids in the mobile phase increased and made the RT forward.

The effects of different concentrations of IL on the theoretical plate number (TPN) are shown in Fig. 1. With the increase of IL concentration, it demonstrated a slow increasing trend, and the maximum TPN appeared at the concentration of 0.3% IL.

Table 1
Effects of different concentrations of ILs on the retention time.

Concentrations of ILs	t_{DSS}	t_{PA}	t_{PAH}	t_{HSYA}	t_{FA}
0.0	19.35	23.18	26.66	34.69	40.03
0.1	19.06	23.09	26.61	34.54	39.91
0.2	18.72	22.86	26.46	34.39	39.72
0.3	18.19	22.57	26.25	34.24	39.60
0.4	17.50	22.34	25.87	34.13	38.63
0.5	16.80	21.85	25.62	33.98	39.51
0.6	16.50	21.65	25.13	33.87	39.48

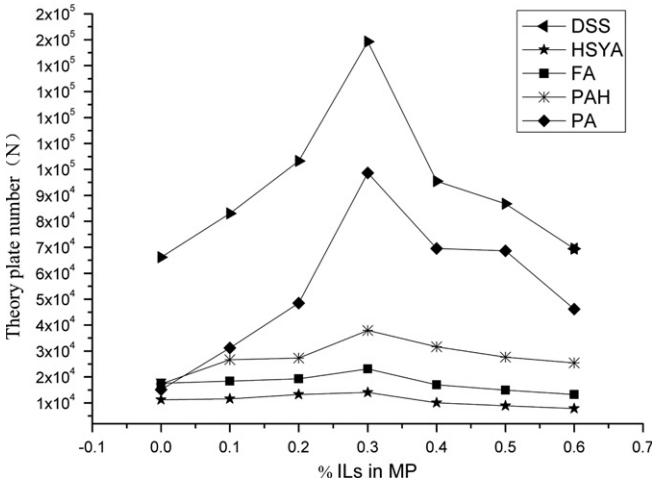

Fig. 1. Effects of different concentrations of ILs on the theoretical plate number.

With further increase of IL concentration, the TPN showed a decreasing trend.

The changes of the RT and TPN might be related to the group of Br ion in IL, which has the obvious promoting function for the five phenolic acids elution. Additionally, the group of 1-butyl-3-methylimidazolium ion, having the similar function of the ion-pair, could also improve the function of stationary phase and increase the ability of adsorption–desorption. However, this ability gradually disappeared with the increase of ILs concentration, which was mainly due to the function of the cross-linking with the silanol groups. This result is consistent with the previous report [2] that the ILs can act as the solid phase in the liquid chromatography to improve the separation. Based on the change trend of RT and TPN for the five phenolic acids as shown in Table 1 and Fig. 1, the addition of 0.3% IL was regarded as the optimal concentration.

3.1.2. *Effects of IL and nano-gold on the area response*

With the addition of different concentrations of IL into the mobile phase followed by detection of the analytes with ECD, the area response was selected as the evaluation index to optimize the working potential.

As shown in Fig. 2, the area responses of the five phenol acid compounds mainly demonstrated an increasing trend with the increase of the working potential, followed by keeping constant except for HSYA and FA. For example, the area response of DSS displayed the optimal value at 800 mV of working potential, and then remained stable with further increase of the applied potential. For PA and PAH, the area responses reached the maximum value at 900 mV. However, the area responses of HSYA and FA kept increasing within the studied working potential range.

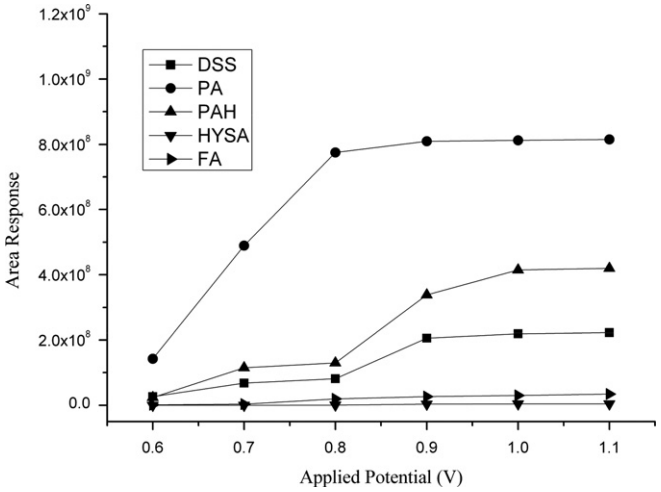

Fig. 2. Area response versus potential for the five phenol acids (0.4% ILs).

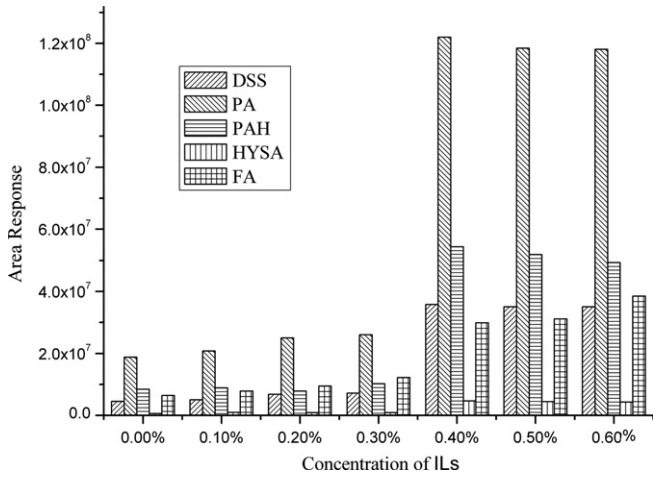

Fig. 3. The area response of the five phenol acid compounds versus different concentrations of ILs (working potential: 900 mV).

Considering the tolerable potential of GC electrode and the average area response, the working potential for the five phenol acid compounds was set as 900 mV in the following study.

Fig. 3 shows the area response of the five investigated phenol acids with the addition of different concentrations of IL into the mobile phase under 900 mV of working potential. For those acids, the area response increased with the IL concentration, then reached the maximum value at 0.4% (v/v) IL except FA. The area responses of DSS, PA, PAH, HSYA and FA were 772%, 628%, 584%, 703% and 600%, respectively, in comparison with the conditions without IL in the mobile phase. Also, the background signals of the five phenol acids decreased in different degrees, especially at the concentration of 0.6% IL added. Taking the RT, TPN and the area response into account, the optimal concentration of IL was set as 0.4%..

This study also investigated the influence of nano-gold particles on the area response of DSS, PA, PAH, HSYA and FA. Fig. 4 shows the corresponding responses with the increase of the concentration of nano-gold, and all of them presented a gradually increasing trend under the concentration of 0.4% IL. With the nanogold particles concentration smaller, the peak areas for five phenol acids increased and the peak area of DSS increased by 5.74 times, PAH, FA and PA were 6.21, 8.48 and 66.48 times, respectively. The area response of HSYA reached the minimum

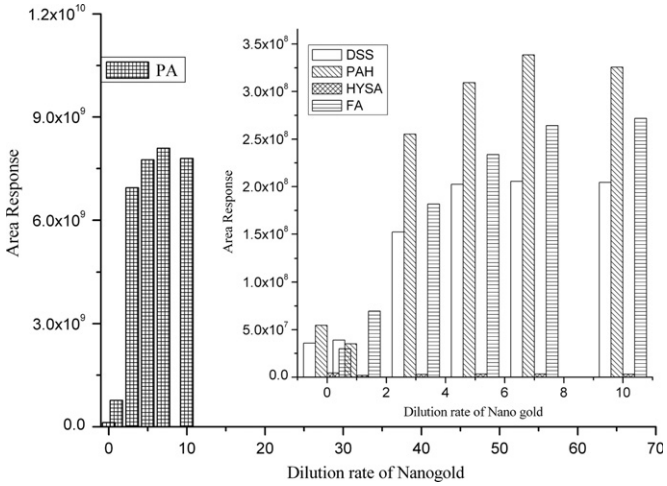

Fig. 4. The area response of the five compounds in different dilution rates of nanogold.

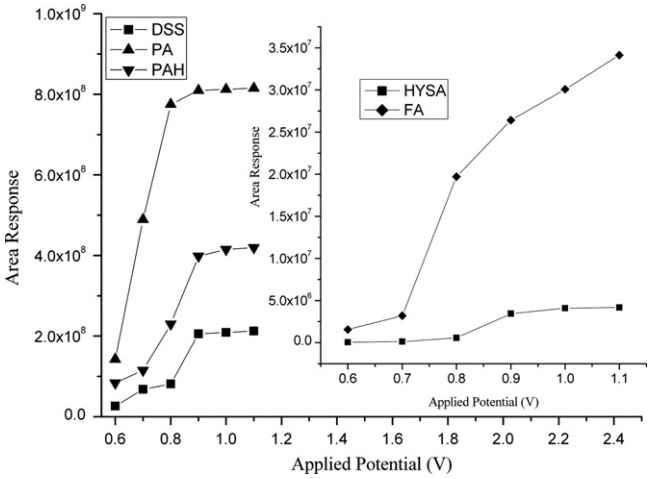

Fig.5. The working potential of the five compounds versus the area (tested in the 4% ILs and 8.27 mg/L nanogold system).

when nanogold sol was diluted to 28.95 mg L^{-1}, which was still 1.8 times higher than the general system. The lower response at a higher nanogold concentration could be attributable to the following reason. The number of nanogold will increase with its concentration, so some nanogold will adsorb at the surface of the electrode, which will block electron transfer. As a result, this effect leads to the decrease of the detection sensitivity. On the contrary, the nanogold enhances electron transfer at a lower concentration, and the sensitivity increases.

Because DSS, PA, PAH and FA have phenolic hydroxyl groups in their structures, it is easy to proceed redox reaction under the electrochemical condition. With the addition of the nanogold particles, the electron transfer rate at the surface of the electrode would be accelerated, and the detection sensitivity is improved. For HSYA, however, the electron transfer rate on the electrode surface is prohibited due to its relatively large molecular structure and steric hindrance and the response decreased.

This study also studied the effect of the nanogold particle system on the working potential. Under the conditions of 0.4% IL and 8.27 mg/L nanogold particles, different working voltages were investigated, as shown in Fig. 5. Under the conditions of 0.4% IL and seven-time dilution (8.27 mg/L) of nanogold particles, the five tested compounds showed an increasing trend in the area response with the increase of the sweep voltage. The area response of PA displayed a platform when the working voltage was higher than 800 mV. For DSS, PAH and HSYA, the platform was not present until the working voltage was 900 mV, while the response of FA continuously increased with the operating voltage. This trend was the same as the case when only IL was added into the mobile phase. The results demonstrated that, to a certain degree, the added IL had a greater effect on the working potentials of the five tested compounds, whereas the effect of nanogold particles was smaller.

From the above discussion, it is obvious that the optimal condition for the five phenolic acids is 0.4% IL and seven times dilution of nanogold.

3.2. Investigation of the pharmacokinetics of Xuebijing injection

The results for selectivity are shown in Fig. 6. The analysis of the biological samples (plasma, urine and feces) showed that there were no endogenous substance peaks or drug metabolite peaks which interfere with the analytes, suggesting that this method is selective and specific for the investigated analytes. By analyzing the relationships between the peak areas of the analytes and their corresponding concentrations in rat biological samples, they displayed a good linear relationship in the investigated concentration range. Liner regression analysis for the four phenolic compounds (DSS, PA, HSYA and FA) were performed in a range of spiking levels from 3.9 to 250 μg/L, 3.7–110.9 μg/L, 8.5–50.0 μg/L and 7.2–594.5 μg/L in rat plasma, respectively. The linear ranges of DSS, HSYA and FA expanded from 3.9 to 250 μg/L, 8.5–125 μg/L, 7–112.5 μg/L in urine and feces, respectively. The sensitivity was evaluated by determining the LOQ, having a precision smaller than 10% and signal/noise ratio higher than 10. DSS 3.9 ng/mL, PA 3.7 ng/mL, HSYA 8.5 ng/mL, FA 7.0 ng/mL (S/N = 10). The extraction recoveries of PA, HSYA, and FA from rat plasma were evaluated. The mean recoveries of the samples were more than 70% which indicated that the extraction recoveries of DSS, PA, HSYA, and FA from the biological samples (plasma, urine and feces) were concentration-independent in the concentration range evaluated. The mean recoveries of DSS were 79.7–92.5% in plasma, 81.1–92.8% in urine and 81.1–92.2 in feces; those of PA were 76.2–96.9% in plasma; those of HSYA were 77.0%–95.0% in plasma, 88.8–95.7% in urine and 88.2–96.3% in feces; those of FA were 79.0–96.3% in plasma, 88.8–97.7% in urine and 86.8–94.2% in feces, and they were all more than 75%, which indicated that the extraction recoveries of PA, HSYA, and FA from the biological samples were concentration-independent in the concentration range evaluated. The precision and accuracy of the method were assessed by performing replicate analyses of spiked samples against the calibration standards. The within-day precision and between-day precision (R.S.D %) were all less than 8%, indicating that the precision and accuracy of the method were acceptable. The stability of the solution were kept at 4 °C and frozen plasma samples (−20 °C). The stability (R.S.D %) of these plasma samples were all less than 10%, thus confirming the overall stability of DSS, PA, HSYA and FA in biological samples under frozen storage, assay processing and freeze-thaw conditions.

The HPLC method showed satisfactory results for the simultaneous determination of PA, HSYA and FA in rat plasma and was successfully used for the pharmacokinetic study of Xuebijing injection followed by an intravenous administration in rats. The main pharmacokinetic parameters of PA, HSYA and FA in rats are listed in Table 2.

From the above results, the relationship between the drug concentrations (e.g., PA, HSYA, and FA) in rat plasma and time displayed a good correlation and fits a two-compartment open model. There were some differences in the distribution and elimination among DSS, PA, HSYA, and FA. Also, it was shown that the diffusion rates of the three drugs in rats were fast. The elimination of

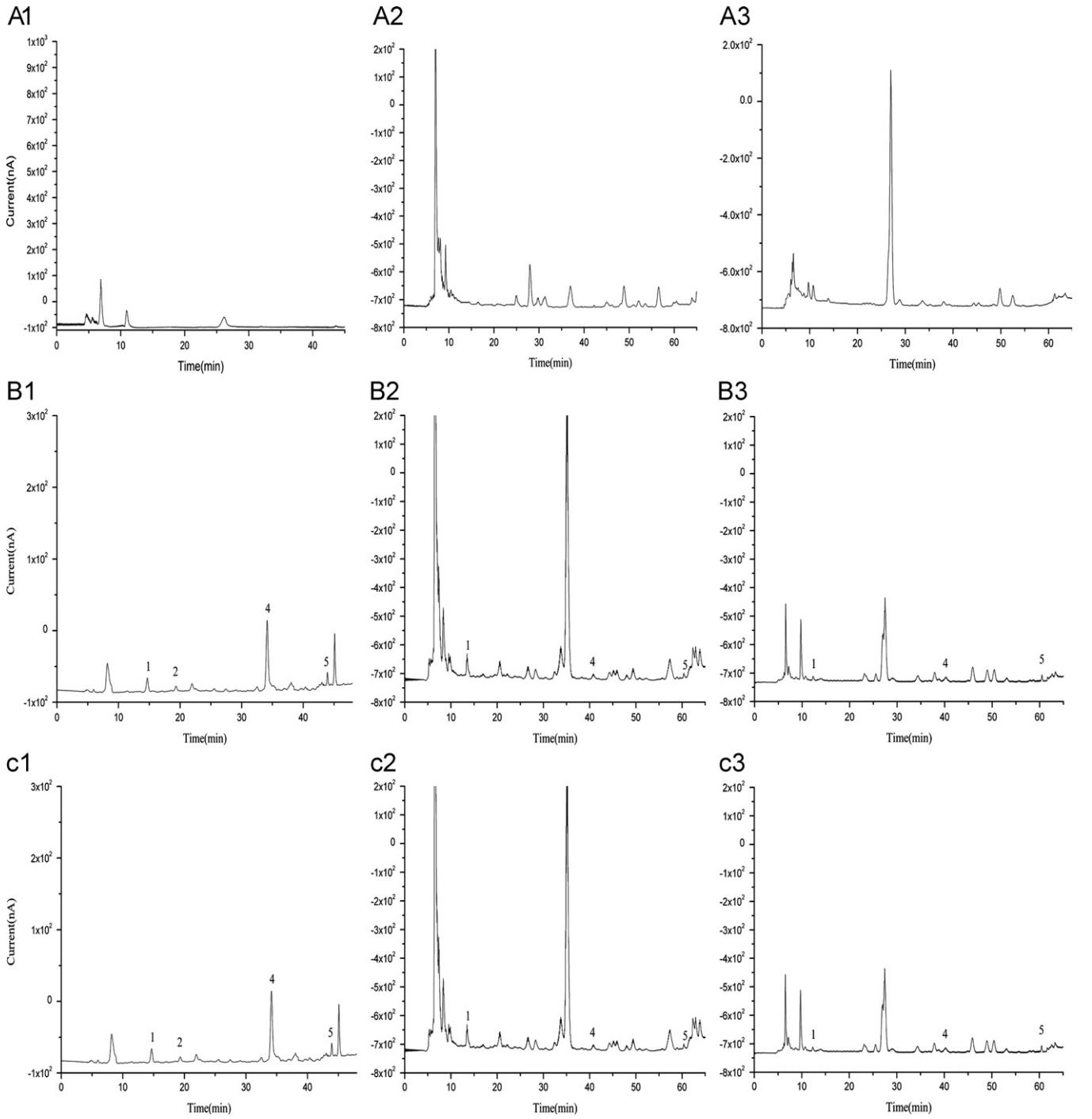

Fig. 6. Representative HPLC chromatography of blank rat biological samples (A1—plasma, A2—urine and A3—feces), biological sample spiked with PA, HSYA and FA (A1—plasma, A2—urine and A3—feces) and biological samples after Xuebijing injection administration. A1—plasma, A2—urine and A3—feces (1—DSS 2—PA 3—PAH 4—HSYA 5—FA).

Table 2
Pharmacokinetic parameters of intravenous Xuebijing injection in rats (n=6).

Parameters	DSS (mean ± S.D.)	HSYA (mean ± S.D.)	PA (mean ± S.D.)	FA (mean ± S.D.)
$t_{1/2\alpha}$ (min)	3.72 ± 2.50	19.96 ± 2.89	6.87 ± 3.02	2.63 ± 1.40
$t_{1/2\beta}$ (min)	43.87 ± 3.05	37.29 ± 2.21	69.32	61.72 ± 8.62
V1(L)	194.05 ± 2.89	39.17 ± 2.01	9.74 ± 3.00	84.12 ± 3.03
CL (L min^{-1})	89.4 ± 3.08	1.05 ± 0.70	0.11 ± 0.06	1.37 ± 1.06
$AUC_{(0-\infty)}$(mg min L^{-1})	1.35 ± 1.04	86.66 ± 14.52	3.89 ± 4.02	12.08 ± 13.22
K_{12}(min^{-1})	0.01 ± 0.03	0.07 ± 0.08	0.23 ± 0.29	0.21 ± 0.52
K_{21}(min^{-1})	0.09 ± 0.04	0.06 ± 0.06	0.11 ± 0.09	0.06 ± 0.50
C_{max}/(mg L^{-1})	0.40 ± 0.002	0.55 ± 0.19	0.02 ± 0.01	0.06 ± 0.03
T_{max} (min)	7.50 ± 2.12	8.33 ± 0.06	5	5

DSS, PA and FA in rabbit blood was also fast, but for HSYA it was relatively slow. The results showed that DSS, PA and FA in Xuebijing injection transported rapidly from the blood into the tissues and organs and eliminated from the blood rapidly. It is reported that the substance with two adjacent phenolic groups usually has a shorter biological half-life [48]. The $t_{1/2\alpha}$ of PA is only 6.8 min, while that of HSYA is about 20 min. The above results indicate that HSYA is transported quickly from the central compartment to the periphery compartment and then is eliminated slowly which is different from the previous report on HSYA [49]. The cause may be attributed to the complex components system of the periphery compartment. The pharmacokinetics of FA fits the two-compartment open model [50, 51], which coincides with this result except for the rates of absorption and elimination.

3.3. Excretion of DSS, HSYA and FA in feces and urine

Tables 3 and 4 showed the excretion ratios of the analytes in urine and fecal water within 24 h. For HSYA and FA, the excretion ratio in urine and feces reached the maximum value during 8–12 h after administration, nevertheless which of DSS in urine reached maximum value during 16–24 h. Fig. 7 shows cumulative amounts of DSS, HSYA and FA excreted in rat urine(A) and feces(B) after Xuebijing injection administration. It can be observed that the cumulative amount of DSS, HSYA and FA excreted in urine and feces keep increasing after administration. It is notable that about 50% HSYA and 65% FA excreted in feces was detected in 25 h after administration of Xuebijing injection, however, 3.41% of HSYA and 3.03% of FA in urine, which indicate that the unchanged HSYA and FA were predominantly excreted in feces after intravenous administration. In addition, only 32% of DSS was found in feces in 24 h after administration, while 47% in urine. It is reported that for DSS and FA, totally one primary metabolite (Phase I) and Phase II secondary metabolites (polar groups catalyzed by transferases and accelerated drug excretion) were found after dosing. Prototype and Phase II metabolites were the majority after drug dosing, which indicates that for these water soluble drugs it was possible that they were excreted from kidney without transformation, and the polar groups could be catalyzed by transferases directly to form the Phase II metabolites [52]. M. Xu [53] found eleven metabolites including PAH, PA and their methylated, glucuronized or glycine conjugates in rat plasma and urine on the basis of their MS fragmentation behaviors, nevertheless, PA and PAH were not detected after administrated Xuebjing injection.

Table 3
Excretion of DSS, HSYA and FA in urine after intravenous administration of Xuebijing injection ($\overline{X} \pm s$, $n=6$).

Time (h)	DSS (%)	HSYA (%)	FA (%)
0–4	2.68 ± 1.22	0.12 ± 1.12	
4–8	6.67 ± 2.42	0.53 ± 1.57	
8–12	9.54 ± 0.93	2.19 ± 2.01	0.70 ± 1.67
12–16	8.55 ± 1.85	0.34 ± 1.23	1.74 ± 2.30
16–24	25.79 ± 2.07	0.23 ± 0.02	0.59 ± 3.02
Total	47.23	3.41 ± 1.78	3.03 ± 4.91

Table 4
Excretion of DSS, HSYA and FA in feces after intravenous administration of Xuebijing injection ($\overline{X} \pm s$, $n=6$).

Time (h)	DSS (%)	HSYA (%)	FA (%)
0–4		2.40 ± 0.02	
4–8	8.44 ± 0.04	8.90 ± 0.07	
8–12	12.72 ± 0.51	16.01 ± 0.09	52.46 ± 0.18
12–16	9.33 ± 0.04	13.65 ± 0.08	10.63 ± 0.17
16–24	1.98 ± 0.12	8.59% ± 0.02	2.39 ± 0.23
Total	32.47 ± 3.17	49.55 ± 2.02	65.48 ± 1.39

4. Conclusions

A high effective and sensitive analytical method was developed and applied for the determination of phenolic acids with IL and nanogold particles as additives. ILs can significantly decrease the retention time and improve the TPN of the five compounds of Xuebijing injection in HPLC-ECD. Moreover, ILs could greatly enhance the area response, which was more than 600% compared to the non-ILs. Upon the effect of the nanogold particles, the peak area increased at least 5.74 times on the basis of the change resulted from IL.

Traditional Chinese medicines and their preparation are commonly complex systems which usually consisted of dozens of chemical components, conventional approaches generally use one or few mark component with higher content to control the quality of TCM, which is insufficient to reveal their synergistic effects,

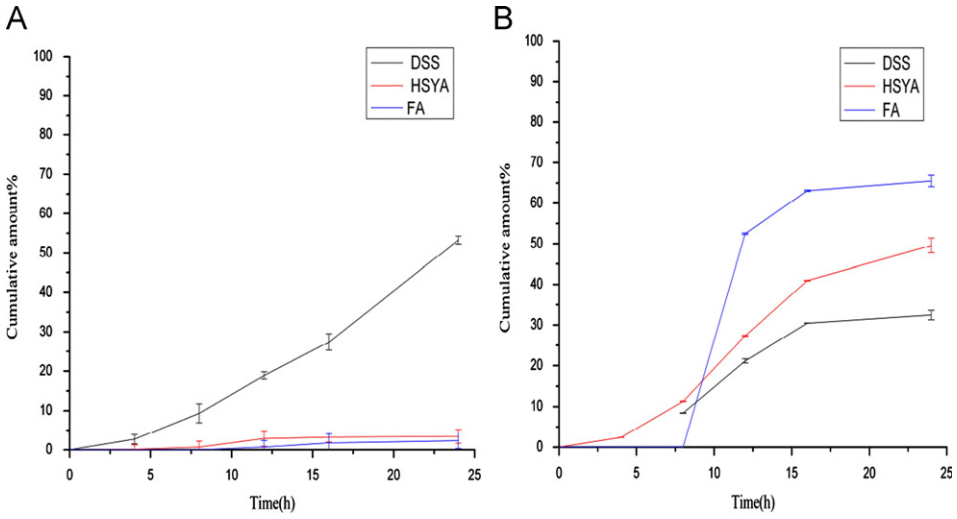

Fig. 7. Cumulative amounts of DSS, HSYA and FA excreted in rat urine (A) and feces (B) after Xuebijing injection administration.

therefore, quality control is one of the problems for the application and development of TCMs. The World Health Organization recognized this in a document entitled "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines" [54–56]. This method may provide a new strategy for the trace detection of some active chemical constituents and their biological samples.

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