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Analysis of terpene compounds in *Cimicifuga foetida* L. by reversed-phase high-performance liquid chromatography with evaporative light scattering detection

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Abstract

An RP-HPLC method with evaporative light scattering detection (ELSD) was developed for the analysis of terpene compounds in traditional Chinese medicine. Actein, 27-deoxyactein and cimicifugoside in a typical Chinese medicine of *Cimicifuga foetida* L. were quantitatively analyzed. Comparing ELSD with UV detection under the same eluent conditions, the former showed better sensitivity and a more stable baseline. The ELSD responses versus sample size of three terpenes and those in double logarithmic were investigated. The good calibration curves in double logarithmic coordinator for actein, 27-deoxyactein and cimicifugoside were obtained. Three solutions for the extraction of the terpene compounds were also compared, the results indicated that methanol–water (80:20) is the best among them. The method was applied to quantify actein, 27-deoxyactein and cimicifugoside in *Cimicifuga foetida* L. from Hunan, China. It was shown that ELSD is an effective detection method for the analysis of the non-volatile terpenes in traditional Chinese medicine. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Cimicifuga foetida* L.; Pharmaceutical analysis; Terpenes

1. Introduction

Cimicifuga foetida L., a traditional Chinese medicine, is widely used for antimicrobial, tranquilizer and hypotensive treatments [1]. As a raw material of natural product, the herb has been exported to North America and Europe recently. Actein, 27-deoxyactein and cimicifugoside are the main bioactive compounds [2–4] responsible for the pharmacological effects of *Cimicifuga foetida* L., so that the quantity of the three ingredients is often taken as the norm for quality control. As yet, the method for determining the bioactive compounds in *Cimicifuga foetida* L. by reversed-phase high-performance liquid chromatography (RP-HPLC) has not been reported, especially with the use of evaporative light scattering detection (ELSD). Since actein, 27-deoxyactein and cimicifugoside are terpene compounds, and do not contain conjugated radicals, their wavelengths for maximal UV absorption are under 200 nm which results in low sensitivity for detecting terpenes with UV detection. In addition, during the analysis of a herb by HPLC, bioactive compounds with low UV absorption may be seriously interfered with by other compounds with high UV absorption. Both the specificity of the method and detection sensitivity can be improved by adopting ELSD for the compounds with low UV absorption.

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ELSD is a detection method based on the large differences between the vapor pressure of mobile phase and that of most analytes in liquid chromatography [5–7]. The detector responds to the mass flow of non-volatile analytes. Even though the more universal refractive index detectors can be used for detection of non-volatile compounds, it is restricted in practice because of its poor detection limit and high sensitivity to chromatographic conditions, such as flow-rate and temperature. Moreover, it is very difficult to satisfy the separation of complex samples from natural sources, particularly herb, which usually requires the use of gradient elution. In contrast to refractive index detection, ELSD produces a very stable baseline even while a steep composition gradient elution used. In fact, ELSD has been used widely for the detection of non-volatile compounds such as carbohydrates [8–10], steroids [11,12], lipids [13–16], amino acids [17], medicines [18–20], etc.

This paper describes an application of HPLC–ELSD to the analysis of terpene compounds including actein, 27-deoxyactein and cimicifugoside in the extractions of *Cimicifuga foetida* L. from Hunan, China.

2. Experimental

2.1. Equipment

The HPLC system was composed of a LC-10ATvp pump, a SPD-10Avp UV detector (Shimadzu, Japan), an evaporative light scattering detector (Varex MKIII ELSD, Alltech, USA), a Rheodyne injector valve with 20 μl loop and a WDL-95 chromatographic workstation (National Chromatographic R&A Center, Dalian, China). An ODS2 column (200×4.0 mm I.D., 5 μm, Hypersil, UK) was used to perform the HPLC experiments. Three ODS solid-phase extraction (SPE) columns (100 mg/2 ml, National Chromatographic R&A Center, Dalian, China) were used for concentrating the fraction collected from the ODS2 column.

2.2. Reagents

The standards of actein, 27-deoxyactein, cimicifugoside used in this work were prepared by middle-pressure preparative chromatography in our laboratory, and their purities were above 97% tested by RP-HPLC, the molecular masses of standards were identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS. The traditional Chinese medicine of *Cimicifuga foetida* L., was obtained from the Oriental Import & Export Company of Dalian, China. Methanol and ethanol were of HPLC grade. Water was purified by a Millipore device (Millipore, MA, USA).

2.3. Sample preparation

The standards of actein, 27-deoxyactein, cimicifugoside with molecular structures shown as in Fig. 1 were weighed accurately and dissolved in methanol–water (80:20), then prepared for a series of standard solutions.

The *Cimicifuga foetida* L. was crushed with a grinder. A 160-mg amount of powder was weighed accurately, then extracted by 25 ml methanol–water

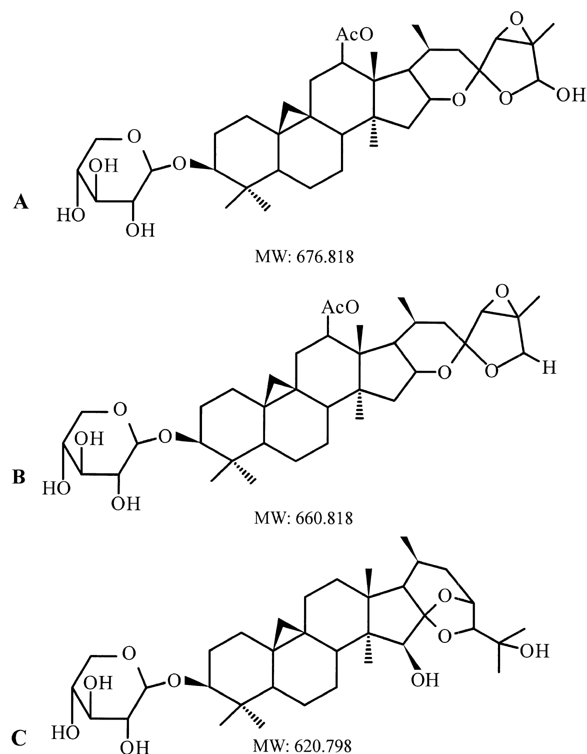

Fig. 1. Molecular structures of (A) actein, (B) 27-deoxyactein and (C) cimicifugoside.

(80:20) for 15 min under ultrasonic vibrations. After centrifugation at 12000 rpm for 12 min, the supernatant of the extracted solution was obtained for analysis.

2.4. Chromatography conditions

The mobile phases used in the HPLC experiments were (A) methanol–water (67.5:32.5) and (B) methanol–water (80:20). The flow-rate of the mobile phase was 0.8 ml/min. Stepwise gradient elution profile was adopted with 0~8 min for mobile phase A and followed by 8~20 min for mobile phase B. Quantification was obtained with external standard method. The drift tube temperature of the ELSD system was at 119°C, the gas flow-rate was 2.30 l/min. The UV detection wavelength was set at 200 nm.

3. Results and discussion

3.1. Identification of actein, 27-deoxyactein, cimicifugoside in Cimicifuga foetida L.

Standard samples of actein, 27-deoxyactein and cimicifugoside are separated by RP-HPLC with diode array detection (DAD), and the obtained chromatogram and UV spectra are shown in Fig. 2. Extraction of *Cimicifuga foetida* L. by methanol–water (80:20) was achieved under the same experimental conditions for standard samples, and the obtained chromatogram and UV spectra are shown in Fig. 3. It can be seen that the spectra of peaks 1, 2 and 3 in Fig. 3 were consistent with those of actein, 27-deoxyactein, cimicifugoside as shown in Fig. 2. Although the DAD spectra in Figs. 2 and 3 are not very informative for structure identification, the impurity with strong UV absorption can be excluded. According to the retention time of the standard sample of actein, 27-deoxyactein and cimicifugoside, three fractions were collected. After multiple collections, the fractions were concentrated by SPE cartridges and identified by MALDI-TOF-MS with a technique of desorption/ionization on silicon [21,22], the molecular masses of compounds in peaks 1, 2 and 3 in Fig. 3 corresponded with the standard samples of actein, 27-deoxyactein and cimicifugoside, respectively. All of above results indicated that the peaks 1, 2 and 3 are compounds of the actein, 27-deoxyactein, cimicifugoside, which are the active components in *Cimicifuga foetida* L. for quality control.

3.2. Comparing ELSD with UV detection of terpenes in Cimicifuga foetida L.

Because the terpene compounds of actein, 27-deoxyactein, cimicifugoside in *Cimicifuga foetida* L. do not have UV absorption radicals as the molecular structures show in Fig. 1, they show poor UV adsorption and their λ_{max} values were below 200 nm. Therefore, their UV detection sensitivity is quite low. In some cases, it simultaneously brings the disturbance of background and baseline drift, especially for analysis of them with gradient elution. In Figs. 2 and 3, it can be observed that 27-deoxyactein and cimicifugoside were hardly detected by UV detection.

ELSD is a detection method based on the large difference between the vapor pressure of mobile phase and that of most the analytes in liquid chromatography. The detector responds to the mass flow of non-volatile analytes. RP-HPLC-ELSD was applied to the purity test of the fractions collected, and the obtained chromatograms are shown in Fig. 4. It can be seen that all of the fractions showed a single peak on RP-HPLC, which indicated the good purity of them. Furthermore, the extracted solution of *Cimicifuga foetida* L. was separated by RP-HPLC-ELSD under the same experimental conditions as in Fig. 3, and the obtained chromatogram is shown in Fig. 5. It can be seen that the peak area of actein in chromatogram with UV detection is much lower than that with ELSD. The detection sensitivity with ELSD and UV detection for the terpene mixture was compared by setting a peak height of solute as twice the baseline noise, and it was observed that the limits of ELSD for actein, 27-deoxyactein and cimicifugoside are 40, 33 and 33 ng, respectively. Accordingly, those of UV detection are 606, 880 and 427 ng. The result showed that ELSD has significant advantages for detecting non-volatile compounds with low UV absorption. Furthermore, comparing the chromatograms by UV detection (Figs. 2 and 3) with those by ELSD (Fig. 5), ELSD detection produces a

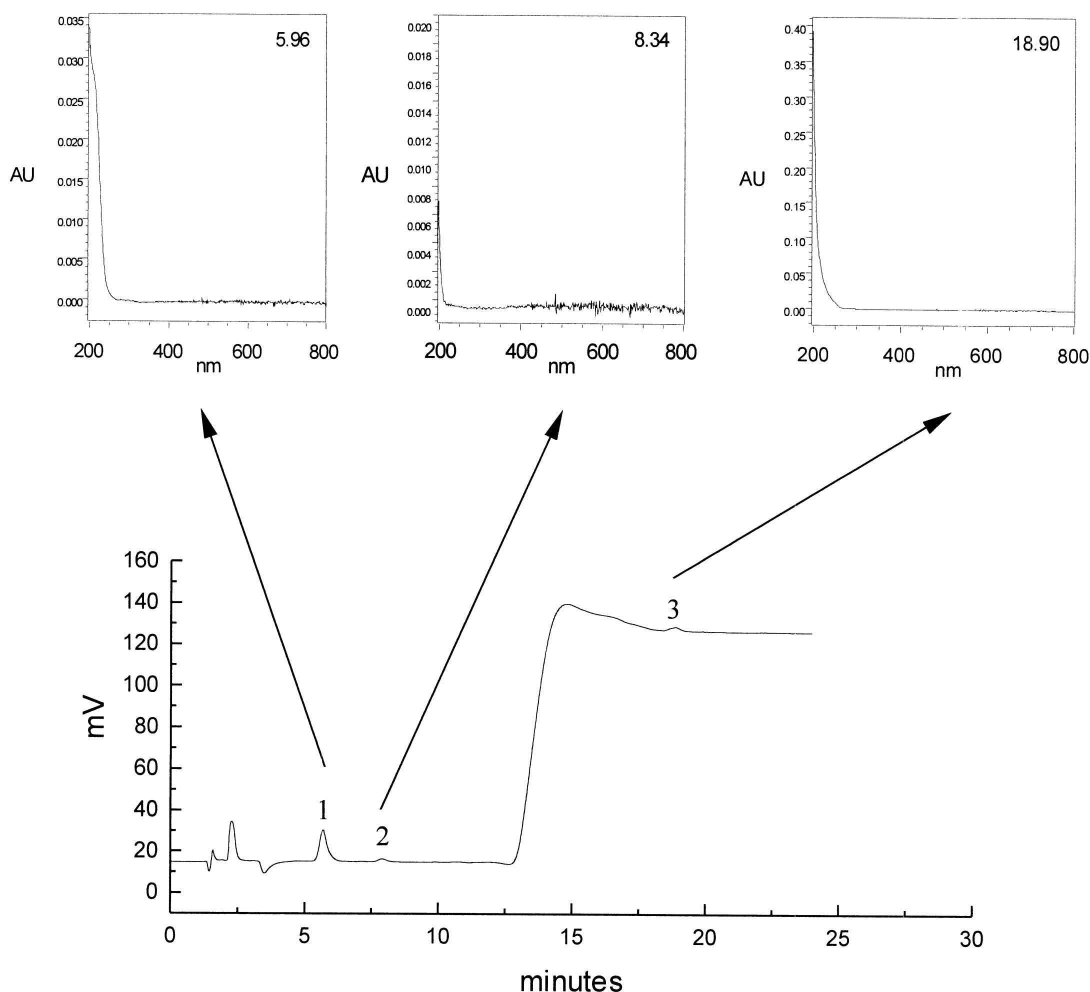

Fig. 2. Chromatogram and UV spectra for standard samples of actein, 27-deoxyactein, cimicifugoside on RP-HPLC–DAD. For conditions see Experimental section. Peaks: 1=actein; 2=27-deoxyactein; 3=cimicifugoside.

better baseline without drift owing to the volatility of solvents (methanol and water) and non-volatility of terpene. Thus, ELSD was selected for the quantitative analysis of terpene compounds in *Cimicifuga foetida* L.

3.3. Relationship of ELSD response and sample size

As shown in Fig. 6, a plot of peak area versus sample size by ELSD is not linear, but the plot of peak area versus sample size in double logarithmic is linear as shown in Fig. 7. The latter plot can be used as the calibration curves for quantitative analysis of terpene compounds. In fact, the non-linear behavior has been observed in other studies. It conforms to the mechanism of ELSD explained by Stolyhwo and co-workers [6,7]. The strength of scattered light is a function of the particle diameter, the light wavelength and the angle of scattered light. It is related to the concentration of the solute, but not proportional. The relationship between the response (R) and the concentration (C) follows an exponential equation of $R=C^x$. When a peak is eluted, the number of droplets formed in the nebulizer per unit volume of solution is constant. On the contrary, the concentration of the

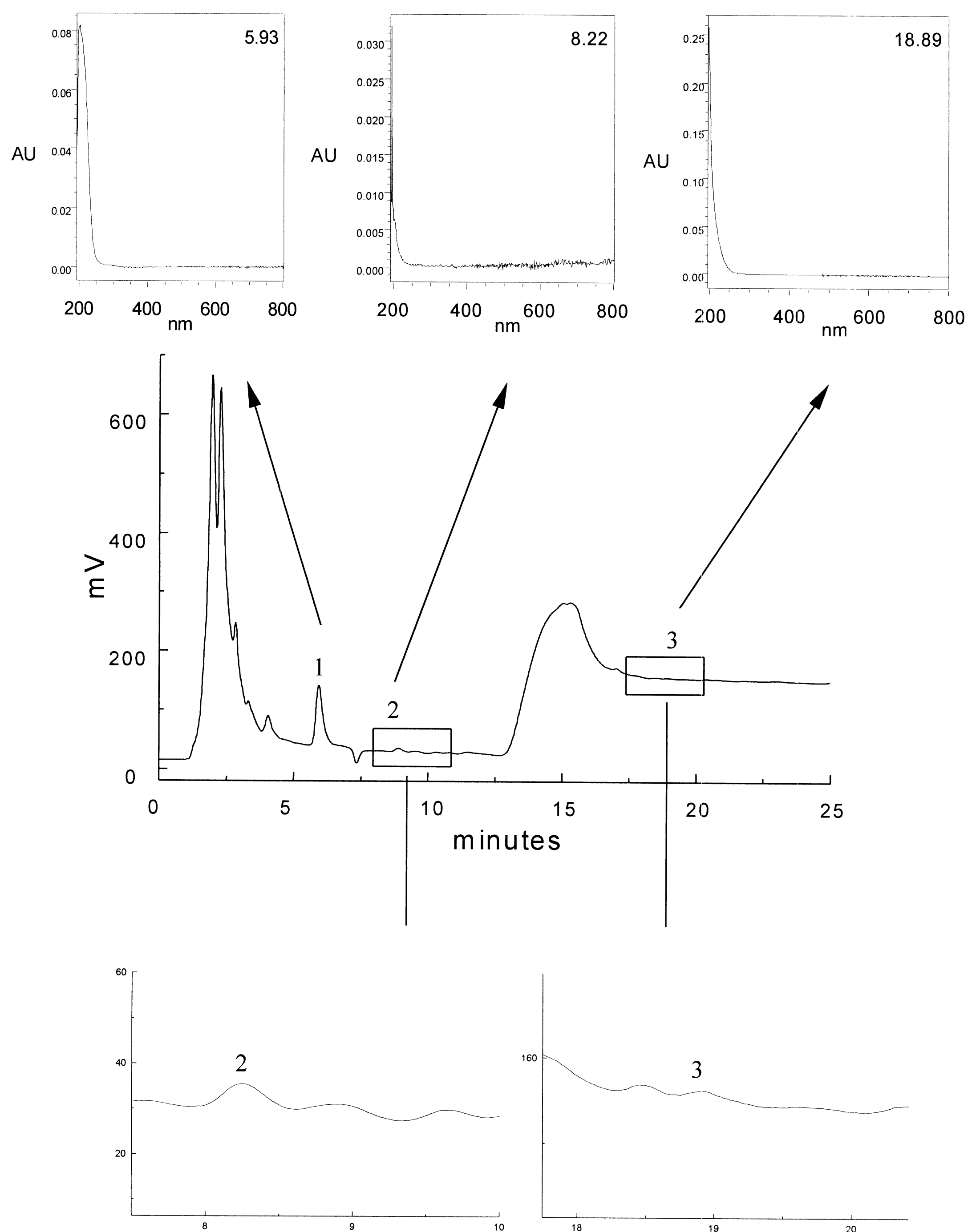

Fig. 3. Chromatogram and UV spectra for actein, 27-deoxyactein, cimicifugoside in *Cimicifuga foetida* L. on RP-HPLC–DAD. For conditions see Experimental section. Peaks: 1=actein; 2=27-deoxyactein; 3=cimicifugoside.

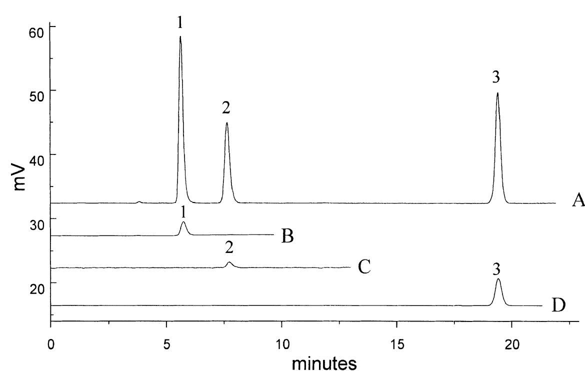

Fig. 4. Purity test for LC fractions of *Cimicifuga foetida* L. extraction by RP-HPLC–ELSD. For conditions see Experimental section. Chromatograms: (A) standard sample, (B) fraction of peak 1, (C) peak 2 and (D) peak 3 of *Cimicifuga foetida* L. extraction in Fig. 3. Peaks: 1=actein; 2=27-deoxyactein; 3=cimicifugoside.

solute in droplets varies. After solvent vaporization, the solute particle is formed, and its diameter is equal to the droplet size generated by the ELSD nebulizer multiplied by the cube root of the solute concentration [6,7]. Thus, the response is a function of the solute particle diameter. Since the particle diameter of solute is of the same order of magnitude as the wavelength of the scattered light, the relationship between the strength of light scattered and the particle diameter is exponential. However, the plot of the peak area versus sample size in double logarithmic coordinates is linear and can be used as the calibration curves for quantitation of analytes.

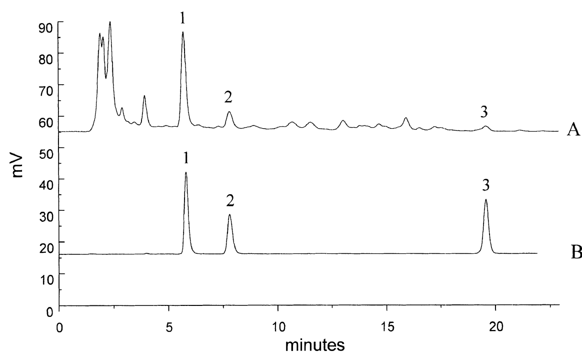

Fig. 5. Chromatograms for (A) *Cimicifuga foetida* L. extracted with methanol–water (80:20) and (B) standard compounds on RP-HPLC–ELSD. For conditions see Experimental section. Peaks: 1=actein; 2=27-deoxyactein; 3=cimicifugoside.

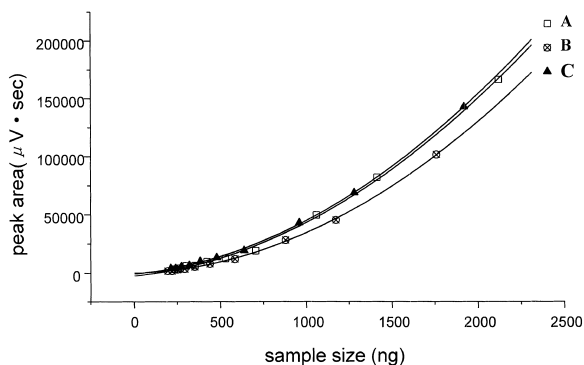

Fig. 6. Plots of peak area versus size of standard sample by ELSD. Curves: (A) actein; (B) 27-deoxyactein; (C) cimicifugoside.

3.4. Quantitative analysis of terpene compounds in *Cimicifuga foetida L.*

Quality control is very important in the identification and characterization of medicine materials and crude drugs. The above results demonstrated the possibility for separating and detecting the terpene compounds actein, 27-deoxyactein and cimicifugoside in *Cimicifuga foetida* L. The amounts of them in *Cimicifuga foetida* L. extracted with methanol–water (80:20) as shown in Fig. 5 were determined with the calibration curves shown in Fig. 7.

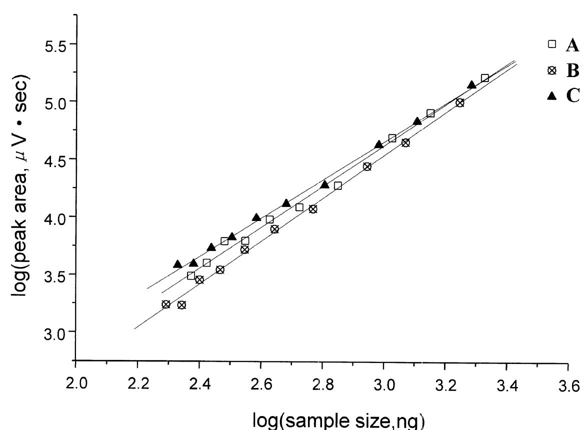

Fig. 7. Plots of peak area versus sample size in double logarithmic by ELSD. Curves: (A) actein, $y=(1.781\pm0.049)x-(0.7166\pm0.1370)$, $R=0.997$, $N=10$, SD=0.0484, $P<0.0001$; (B) 27-deoxyactein, $y=(1.871\pm0.037)x-(1.072\pm0.099)$, $R=0.998$, $N=10$, SD=0.0362, $P<0.0001$; (C) cimicifugoside, $y=(1.668\pm0.031)x-(0.3433\pm0.0843)$, $R=0.999$, $N=10$, SD=0.0302, $P<0.0001$.

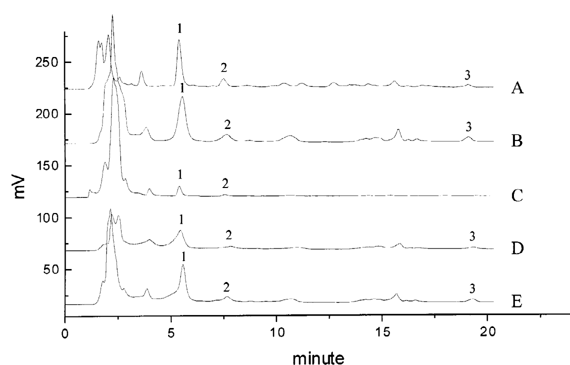

Fig. 8. Chromatograms for *Cimicifuga foetida* L. extracted with different solutions. For conditions see Experimental section. Extraction solutions: (A) methanol–water (80:20), (B) ethanol–water (70:30), (C) water, (D) ethanol and (E) methanol. Peaks: 1=actein; 2=27-deoxyactein; 3=cimicifugoside.

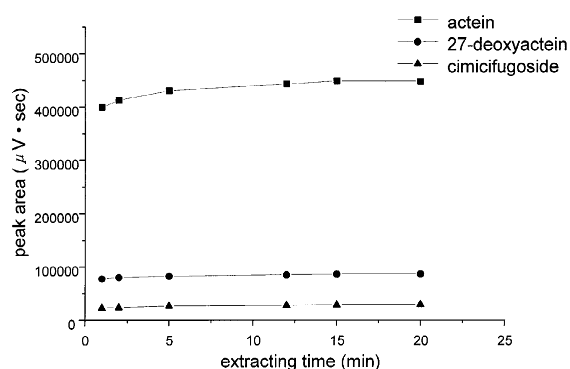

Fig. 9. Effect of the extraction time on the extracting amounts of three terpene compounds in *Cimicifuga foetida* L. extracted with methanol–water (80:20).

In other cases, we have investigated the effect of extraction solution on the extracted amounts of actein, 27-deoxyactein and cimicifugoside from *Cimicifuga foetida* L. Fig. 8 showed the chromatograms for the extractions of *Cimicifuga foetida* L. by solutions of methanol–water (80:20), ethanol–water (70:30), water, ethanol and methanol, respectively. From chromatograms D and E in Fig. 8, it can be observed that the peaks of terpene compounds were overlapped by some unknown impurities, and which makes it difficult to quantify the terpene compounds accurately. The disadvantage was overcome by adopting the extraction solution of methanol–water and ethanol–water. The amounts of actein, 27-deoxyactein and cimicifugoside in *Cimicifuga foetida* L. extracted by the solutions of water, methanol–water and ethanol–water are listed in Table 1.

It is obvious that methanol–water and ethanol–water are better than pure water for extracting actein, 27-deoxyactein and cimicifugoside because more amounts of terpenes can be extracted. Cimicifugoside even was not be detected by using an extraction solvent of water, which indicated that water is not good solvent for extracting the three terpene components because of its poor extraction rate, although water is mostly used as extracting solvent in traditional Chinese medicine. Comparing the extraction solution of ethanol–water with methanol–water, the separation of terpene components in latter case is better, because the peaks 1 and 2 in chromatogram (B) of Fig. 8 are broader. Extraction reproducibility by the solution of methanol–water (80:20) was determined, and the observed RSD values are 2.4, 1.1 and 2.6% ($n=3$) for actein, 27-deoxyactein and cimicifugoside, respectively.

The effect of extraction time on the extraction rate was determined, and the obtained results are shown in Fig. 9. It can be seen that extraction time had

Table 1
Amounts of actein, 27-deoxyactein and cimicifugoside in the *Cimicifuga foetida* L. extracted with different solutions

Extracting solution	Concentration (%, w/w)		
	Actein ($n=3$)	27-Deoxyactein ($n=3$)	Cimicifugoside ($n=3$)
Methanol–water (80:20)	3.44±0.019	1.42±0.008	0.93±0.011
Ethanol–water (70:30)	3.33±0.022	1.36±0.015	0.81±0.011
Water	1.09±0.007	0.46±0.01	–

some influence on the peak area of three terpenes with methanol–water (80:20) as the extraction solution. The peak area of actein gradually increased, but 27-deoxyactein and cimicifugoside increased very slightly with an increasing of extraction time. The experimental result in Fig. 9 indicated that the good extraction rate of the three terpenes in *Cimicifuga foetida* L. can be reached with an extraction time longer than 15 min.

4. Conclusion

The performance of ELSD makes it useful for the analysis of terpene compounds with weak UV absorption above 200 nm and non-volatility, and the detection method is attractive for the separation of complex samples, such as those in traditional Chinese medicine, which necessitate the use of gradient elution. Compared with UV detection, ELSD shows much lower detection limits. The terpenes in *Cimicifuga foetida* L. can be quantified by ELSD at a linear range.

Acknowledgements

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