Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 479–485
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

MECC determination of oleanolic acid and ursolic acid isomers in *Ligustrum lucidum* Ait

Haixing Liu [a,c], Yuhua Shi [a], Dexian Wang [b], Gengliang Yang [b], Aimin Yu [a,*], Hanqi Zhang [a]

[a] *College of Chemistry, Jilin University, Changchun 130023, PR China*
[b] *College of Chemistry, Hebei University, Baoding 071002, PR China*
[c] *Department of Chemistry, Hebei Vocational Technological Teachers College, Changli 066600, PR China*

Received 16 April 2002; received in revised form 24 January 2003; accepted 13 March 2003

Abstract

In this paper, a novel method to separate and determine oleanolic acid and ursolic acid isomers in *Ligustrum lucidum* Ait was studied by micellar electrokinetic capillary chromatography (MECC). The baseline separation of the two analytes were obtained on the condition that the buffer contained 15 mmol/l disodium hydrogen phosphate, 15 mmol/l disodium tetreborate, 10 mmol/l SDS and 5% (v/v) alcohol. The contents of the oleanolic acid and ursolic acid were determined in *L. lucidum* Ait, *Folium photiniae* and *Flos campsis*; they were 78.3 (R.S.D. = 2.75%) and 20.7 mg/g (R.S.D. = 2.97%), 27.9 (R.S.D. = 3.67%) and 79.8 mg/g (R.S.D. = 3.44%), 65.5 (R.S.D. = 3.73%) and 60.4 mg/g (R.S.D. = 4.06%) ($n = 5$), respectively. The recoveries of the analytes in the extract of *L. lucidum* Ait were 102% (R.S.D. = 2.85%) for oleanolic acid and 104% (R.S.D. = 3.21%) for ursolic acid ($n = 5$). With the emphasis on the effects of SDS and alcohol concentrations on the separation of the isomers were investigated.

Keywords: Micellar electrokinetic capillary chromatography (MECC); Oleanolic acid; Ursolic acid; *Ligustrum lucidum* Ait; *Folium photiniae*; *Flos campsis*

1. Introduction

Over the last years, people gradually recognize that *Ligustrum lucidum* Ait is important on reinforcing organism immunization, anticancer, restraining inflammation and controlling senility [1–3]. Its two effective compounds, oleanolic acid and ursolic acid, have been proved to be effective on restraining inflammation, protecting liver, relieving pain, strength the heart and calming by modern science. More pharmacology effect of ursolic acid is being found gradually such as restraining ulcer, controlling high blood-fat and treatmenting diabetes etc. [4].

The qualitative and quantitative analysis methods of oleanolic acid and ursolic acid are main thin layer chromatography (TLC) [5], high performance liquid chromatography (HPLC) [6,7].

* Corresponding author. Tel.: +86-431-849-9154; fax: +86-431-892-2161.
E-mail address: crdcai@mail.jlu.edu.cn (A. Yu).

0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00235-8

Although TLC and HPLC methods can be employed to analyze herbs and medicines, these traditional methods are time consuming and need a large amount of samples and they are also expensive and difficult to operate. In the past years, capillary electrophoresis (CE) has made a lot of applications in pharmaceutical analysis [8–10]. Micellar electrokinetic capillary chromatography (MECC) is highly efficient analytical technique as it not only possesses advantage similar to CE having high separation efficiency, short analysis time and exceptional resolution, but it is also applicable to the separation of non-charged compounds. Organic solvents are often used as additives to the aqueous buffer in MECC to reduce the retention factors of strongly bound solutes to the micelles and to extend the migration time window and/or enhance selectivities. Different types of organic modifiers such as methanol, acetonitrile, dimethyl sulfoxide (DMSO), acetone and dinethyformamide have been used to improve the resolution in MECC [11–16]. The purpose of this work is to study the effect of organic modifier and SDS concentrations on the separation of the two interest compounds and to establish a new analytical method for the separation and determination of the two compounds simultaneously. The established method was simple, rapid, accurate and convenient.

The determination of active components in traditional Chinese medicine is very important, especially to the pharmaceutical industry. This paper gives a new method of the quality control of the several herbs and relative medicine.

2. Experimental

2.1. Apparatus and materials

The experiment was performed on a Waters Quanta 4000E CE system (Milford, MA, USA) with a built in 0–30 kV high voltage power supply, a fixed wavelength UV detector near the cathodic end and a forced-air cooling system. Uncoated fused capillary (75 μm i.d. total length is 60 cm and effective length is 52 cm) was from Yong Nian Optical Fiber Factory, Hebei Province, China. Data processing was carried out with a Waters Millennium 2010 chromatography system.

Oleanolic acid and ursolic acid were obtained from Drug and Biological Products Examination Center, China. *L. lucidum* Ait, *Folium photiniae* and *Flos campsis* were purchased in medicine shop, China. The other chemical reagents used in the experiment were all of analytical grade.

2.2. Procedures

At the beginning of the working day, the capillary was purged with 0.1 mol/l chromic acid, double distilled water, 0.1 mol/l potassium hydroxide, double distilled water for 5 min and the running buffer for 4 min, respectively. After three runs, the capillary was purged with the above procedure.

The running voltage is 16 W, the UV detector is set at 214 nm, the temperature is 27 °C and the sample is injected by hydrostatic mode for 5 s.

2.3. Treatment of the herb

The dry herbs were ground into fine powder. A certain amount of the powder (*L. lucidum* Ait: 1.53 g, *F. photiniae*: 3.94 g, *F. campsis*: 2.19 g) were extracted with 95% alcohol in a Soxhlet apparatus for 1.5 h. The extracted solution were filtered through a filter-paper when they were hot and the filtered solutions were concentrated, then the volumes of the concentrated solutions were adjusted to 50 ml with 95% alcohol. The solutions were injected directly into the CE system.

2.4. Mobility calculation

The effective electrophoretic mobility of the analytes was calculated from the observed migration time with the equation:

$$\mu_{eff} = \mu_{app} - \mu_{eo} = L_d L_t / V \times (1/t_m - 1/t_{eo}) \qquad (1)$$

where μ_{eff} is the effective electrophoretic mobility of the analytes tested, μ_{app} is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration lime for an unchanged solute (neutral marker), L_t is the total

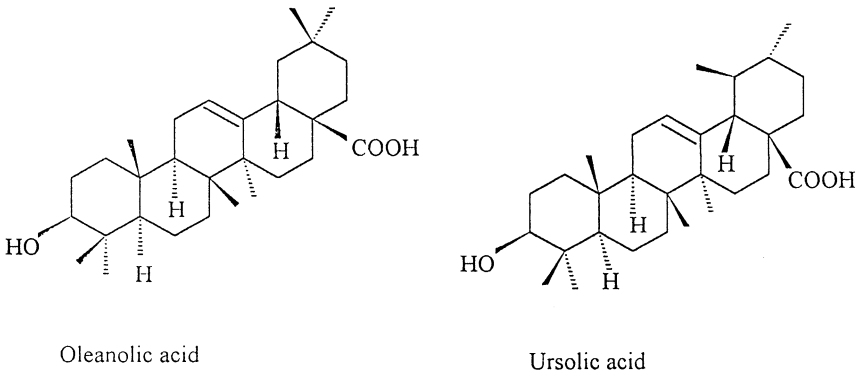

Fig. 1. The structures of oleanolic acid and ursolic acid.

length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

3. Result and discussion

The structures of oleanolic acid and ursolic acid are illustrated in Fig. 1. It can be seen that the structures of the two compounds are similar. In order to obtain successful separation, the buffers of different concentrations of surfactant and organic modifier were tested.

3.1. Effect of SDS concentration

The influence of several different concentrations of disodium hydrogen phosphate and disodium tetreborate systems on the separation of the standard mixture of oleanolic acid and ursolic acid was investigated. Under the condition that the total concentration was 30 mmol/l, by changing the relative contents of phosphate and borate, it was shown that the two compounds could not be separated, the peak was low and wide and tailing. It was also found good that when both phosphate and borate concentrations were 15 mmol/l in the buffer. This was the base of the next search.

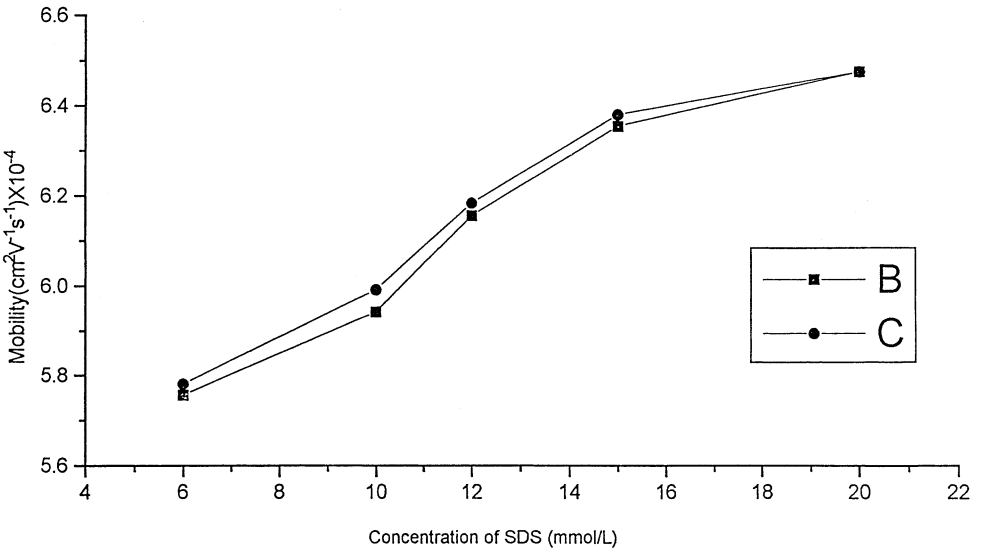

Fig. 2. Effect of the SDS concentration on the effective mobilities of the two compounds B, oeanolic acid; C, ursolic acid.

When disodium hydrogen phosphate and disodium tetreborate concentrations were 15 mmol/l, respectively, the effect of SDS concentration on the separation was tested too. Fig. 2 illustrated the effect of SDS concentration on the effective mobilities of the two compounds. From Fig. 2, it can be seen that when SDS concentration changed from 6 to 20 mmol/l in the buffer, the effective mobilities of oleanolic acid and ursolic acid became larger and larger. But the effective mobilities difference of ursolic acid and oleanolic acid increased first, then decreased. When SDS concentration was 10 mmol/l, the effective mobilities difference of the two compounds was the largest and it was 0.049×10^{-4} cm^2/V per s. When SDS concentration was 20 mmol/l, the effective mobilities difference of the two compounds was almost zero. When SDS concentration was lower than 15 mmol/l, the peaks were wide and were partly separated. When SDS concentration was 20 mmol/l, the two peaks merged to a single peak. Although good separation can be obtained at SDS concentration of 10 mmol/l, baseline separation still can not be achieved. So in the synthetic experiments later, the buffer containing 10 mmol/l SDS was chosen.

3.2. Effect of the organic modifier

Because oleanolic acid and ursolic acid are soluble in alcohol, the buffer system containing different concentration of alcohol was tested to see the influence of organic modifier on the separation of the two compounds. The effect of the concentration of alcohol on the separation of the two compounds was investigated when both disodium hydrogen phosphate and disodium tetreborate concentrations were 15 mmol/l in the buffer.

First, the buffer system was tested without the addition of SDS. In Fig. 3, the effect of the concentration of alcohol on the effective mobilities of the two compounds was shown. It can be seen that the partly separation was obtained around the 5% (v/v) alcohol concentration from the experimental peaks. Though the shape of the electropherogram was poor and completely separation was not obtained, the separation phenomenon was

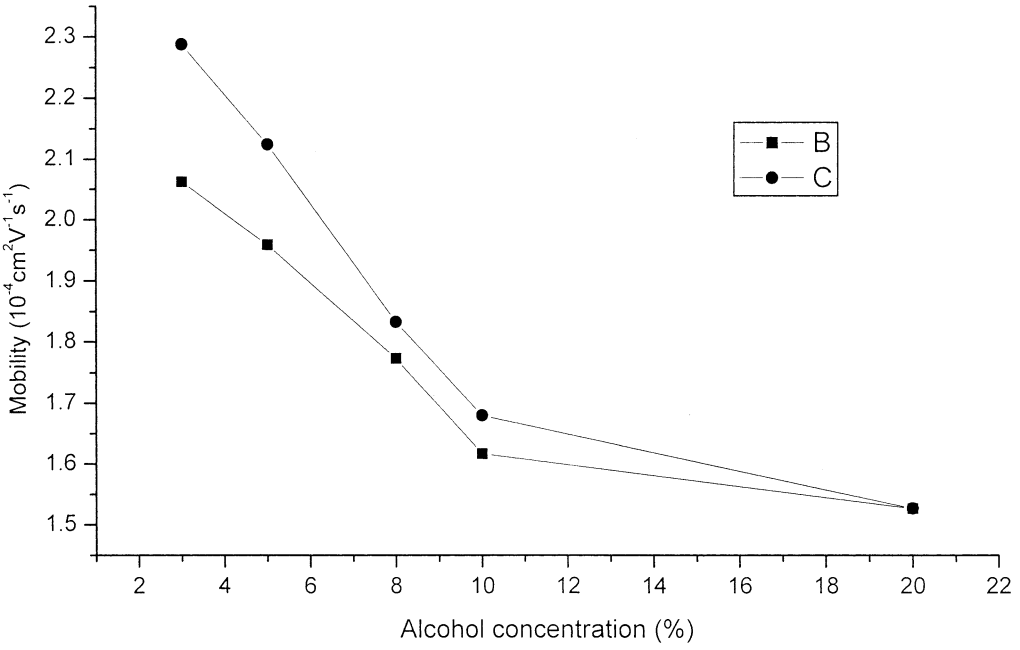

Fig. 3. The effect of alcohol concentration on the effective mobility of the two analytes B, oleanolic acid; C, ursolic acid.

seen when the alcohol was added to the buffer at low concentration.

Then the above buffer system with 10 mmol/l SDS was studied further. In Fig. 4, the effect of alcohol concentration on the resolution of oleanolic acid and ursolic acid was shown. From Fig. 4, it can be seen that the resolution increased as alcohol concentration increased. When the concentration of alcohol changed from 0 to 5% (v/v) in the buffer, the separation of the two compounds was improved increasingly and the peaks of oleanolic acid and ursolic acid became sharper. It was shown in Fig. 5 when alcohol concentration was up to 5% (v/v) in the buffer, baseline separation of oleanolic acid and ursolic acid was achieved. When alcohol concentration was above 5% (v/v), the resolution was still very high, but the peaks of the two compounds became too wide and the difference of the retention time of the two compounds became too big, the shapes of the peaks of the two compounds were too poor. This implies that organic modifier (alcohol) in the buffer play an important role in the separation of the two compounds. The reason may be that organic modifier changes the property both of micelle phase and mobile phase and then changes the distribution of the compounds between the two phases. It can also change the background electrolyte viscosity, the electrophoretic mobilities and the fused silica capillary wall zata potential. However, organic solvent at higher concentration may affect the formation of the micelles and cause

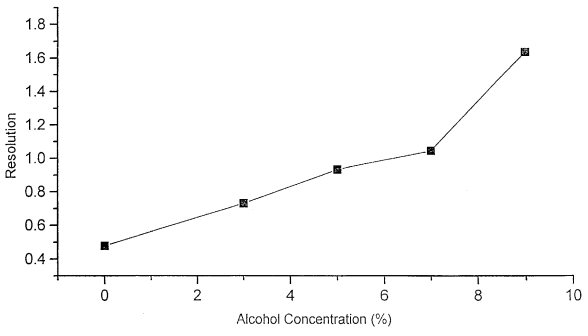

Fig. 4. Effect of alcohol concentration on the resolution of oleanolic acid and ursolic acid.

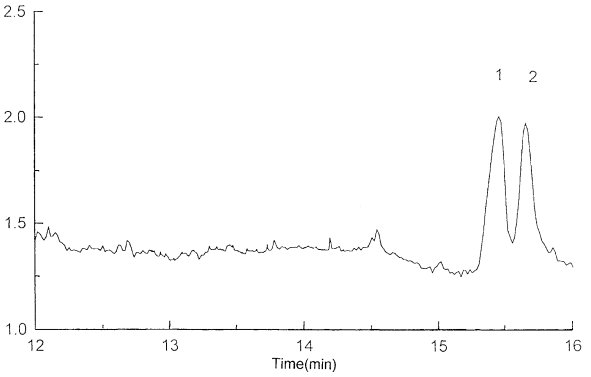

Fig. 5. Electrophorogram of the standard analytes. Experimental condition:buffer solution 15 mmol/l phosphate+15 mmol/l tetreborate+10 mmol/l SDS+5%(v/v) alcohol; applied voltage: 16 kV; column: 60 cm/75 μm; effective length: 52 cm; UV wavelength: 214 nm. Peaks: 1, oleanolic acid; 2, ursolic acid.

an increase in the critical micelle concentration (cmc). This is unfavorable to improve the shapes of the electropherogram and the selectivities of the separation.

3.3. Quantitative analysis

3.3.1. Linearity, reproducibility and limits of detection

The quantitative analysis was carried out under the optimum condition obtained from the above experiments and it was the buffer consisted of 15 mmol/l disodium hydrogen phosphate, 15 mmol/l disodium tetreborate, 10 mmol/l SDS and 5% (v/v) alcohol.

At first, the standards of oleanolic acid and ursolic acid were weighted precisely and dissolved with alcohol into a serious of standard solutions of different concentration. The calibration curve was obtained by using the corrected peak areas of the standards with different concentration. The corrected area for every point in the plot was the average of three runs. Calibration curves were constructed in the range of 0.53–3.56 mg/ml for oleanolic acid, 0.57–4.12 mg/ml for ursolic acid, The linear correlation equations (corrected peak area y mV/s, concentration × g/ml) and coeffi-

cients(r) were: oleanolic acid, $y = -28.5 + 13.4 \times 10^4 \times (r = 0.998)$; (slope = 134 000, intercept = −28.5) ursolic acid, $y = -14.8 + 16.9 \times 10^4 \times (r = 0.997)$; (slope = 169 000, intercept = −14.8).

Repeatability and reproducibility tests based on eight injection of the two standard analytes were performed. The results showed that the reproducibilities of migration times and peak areas for every component were satisfactory. The R.S.D. values of migration times and peak areas were below 2.7 and 4.8%.

The limit of detection (LOD) was obtained as the sample concentration that caused a peak with a height three times the baseline noise level and the limit of quantification (LOQ) was calculated as 10 times the baseline noise level. Thus, the LODs (LOQs) were 2.1 μg/ml (6.9 μg/ml) for oleanolic acid and 0.9 μg/ml (3.2 μg/ml) for ursolic acid.

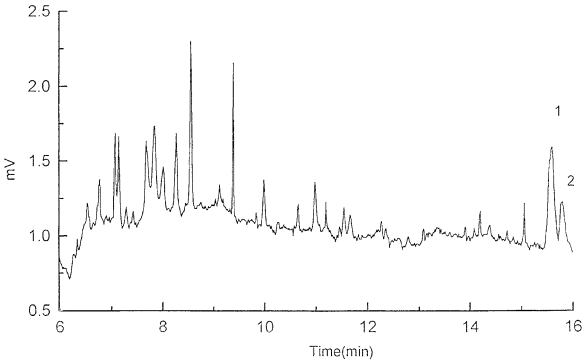

Fig. 6. Electrophorogram of the alcohol extract of *L. lucidum*. Experimental condition:buffer solution 15 mmol/l phosphate+15 mmol/l tetreborate+10 mmol/l SDS+5%(v/v) alcohol; applied voltage: 16 kV; column: 60 cm/75 μm; effective length: 52 cm; UV wavelength: 214 nm. Peaks: 1, oleanolic acid; 2, ursolic acid.

3.3.2. Applications

The electropherogram of the 95% alcohol extract of *L. lucidum* Ait was shown in Fig. 6. It can be seen that the two interest compounds had been well separated under the optimum condition described above. The calculated contents of the two components in *L. lucidum* Ait, *F. photiniae* and *F. campsis* were shown in Table 1.

The contents of the two compounds in *L. lucidum* Ait determined by HPLC were 14.84 mg/g (15.16 mg/g) for oleanolic acid and 6.52 mg/g for ursolic acid in the reference [7] ([6]). From these data, it can be seen that the contents of the two compounds in *L. lucidum* Ait determined by MECC are much larger than that determined by HPLC. This shows MECC has high efficiency and sensitivity.

4. Conclusion

From above discussion, one can see that a new analytical method of MECC can be developed for the separation and the determination of oleanolic acid and ursolic acid isomers in *L. lucidum* Ait, *F. photiniae* and *F. campsis* with good reproducibility and MECC can be used as a very powerful analytical method for the determination and identification of Chinese herbs. MECC is more promising and particularly useful owing to its

Table 1
Contents of the two compounds in *L. lucidum* Ait, *F. photiniae* and *F. campsis*

	Oleanolic acid		Ursolic acid	
	Content (mg/g)	R.S.D. ($n = 5$) (%)	Content (mg/g)	R.S.D. ($n = 5$) (%)
L. lucidum Ait	78.3	2.75	20.7	2.97
F. photiniae	27.9	3.67	79.8	3.44
F. campsis	65.5	3.73	60.4	4.06

The recoveries of the analytes in the extract of *L. lucidiim* Ait were 102% (R.S.D. = 2.85%) for oleanolic acid and 104% (R.S.D. = 3.21%) for ursolic acid ($n = 5$).

simplicity, low cost, low solvent consumption, use of clean solvent, higher analytical speed and higher separation efficiency in the quality control of traditional Chinese pharmaceutical plants.

References

[1] Z. Ying, W. Jie, S. Zhongren, Chinese Pharmaceutical Journal 6 (1990) 47.
[2] F. Zhuohua, F. Xiurong, Chinese Immunity Journal 2 (1986) 88–91.
[3] S. Lifu, C. Yingying, C. Haisheng, D. Jianping, Acta Pharmaceutica Sinica 32 (1997) 442–446.
[4] S. Jing, Z. Qinglin, Chinese Traditional and Herbal Drugs Supplementary Issue 30 (1999) 196–197.
[5] W. Qiuju, W. Tingming, C. Hongying, Chinese Traditional and Herbal Drugs 23 (1992) 467–468.
[6] S. Lifu, C. Qin, W. Guangtong, Y. Shiting, M. Yan, Chinese Journal of Chinese Materia Medica 23 (1998) 77–79.
[7] L. Shuhua, W. Qiang, X. Guangcheng, Chinese Journal of Medicial Analysis 13 (1993) 291–294.
[8] Y. Gengliang, W. Dexian, Journal of Hebei University 17 (1997) 552–554.
[9] Y. Gengliang, S. Xiurong, Z. Hongyi, Journal of Hebei University 18 (1998) 359–363.
[10] H.Y. Zhang, G.L. Yang, Z.D. Hu, Analytical Letters 30 (1997) 2327–2339.
[11] D.X. Wang, G.L. Yang, H. Englhardt, H.Y. Zhang, Electrophoresis 20 (1999) 1895–1899.
[12] Z.P. Zhang, Z.D. Hu, G.L. Yang, Chromatographia 44 (1997) 162–168.
[13] K. Otsuka, M. Higashimori, R. Koike, K. Karuhaka, Y. Okada, S. Terabe, Electrophoresis 15 (1994) 1280.
[14] S. Furlanetto, S. Orlandini, G. Massolini, M.T. Faucci, E. La Porta, S. Pinzanti, The Analyst 126 (2001) 1700–1706.
[15] G. Taibi, M.R. Schiavo, P. Calanni Rindina, R. Muratore, C.M.A. Nicotra, Journal of Chromatography A 921 (2001) 323–329.
[16] S. Liu, X. Tian, X. Chen, Z. Hu, Journal of Chromatography A 928 (2001) 109–115.