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Short communication

Simultaneous determination of andrographolide and dehydroandrographolide in *Andrographis paniculata* and Chinese medicinal preparations by microemulsion electrokinetic chromatography

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Abstract

The present paper describes the development of a microemulsion electrokinetic chromatographic (MEEKC) method for simultaneous determination of andrographolide and dehydroandrographolide in traditional Chinese medicines and Chinese medicinal preparations. The MEEKC method involved the use of sodium dodecyl sulfate (SDS) as surfactant, heptane as organic solvent and butan-1-ol as co-solvent. The effect of temperature and pH of running buffers on separation were examined. The optimized conditions (heptane 0.81% (w/w), SDS 3.31% (w/w), butan-1-ol 6.61% (w/w) and 10 mM sodium tetraborate buffer, pH 9.2) allowed a useful and good reproducible separation of the studied analytes to be achieved.
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Keywords: Microemulsion electrokinetic chromatography; *Andrographis paniculata*; Andrographolide; Dehydroandrographolide

1. Introduction

Andrographis paniculata, also known commonly as "King of Bitters", is a member of the plant family Acanthaceae and an ancient medicinal herb with an extensive ethnobotanical history in Asia. The most common reported uses were for digestive problems, snakebite, and infections ranging from malaria to dysentery [1,2]. The primary medicinal component of *A. paniculata* is andrographolide. It has a very bitter taste, is a colorless crystalline in appearance, and is called a "diterpene lactone"—a chemical name that describes its ringlike structure (see Fig. 1). Besides andrographolide cited above, other related diterpenoid components include dehydroandrographolide, neoandrographolide, andrographiside, etc. [3]. These bitter constituents are believed to have immune stimulating, anti-inflammatory, fertility-decreasing, liver-protective, and bile secretion-stimulating actions [4–6]. However, each one of these constituents exhibits varying degrees of anti-inflammatory effects and antibacterial activities, and furthermore, both growing region and seasonality play a role as to the concentration of these diterpene lactones. Recent studies reveal that andrographolide in the herb demonstrates better hypoglycemic, choleretic and hepatoprotective activity against CCl_4, as well as galactosamine and paracetamol intoxication, while dehydroandrographolide is a better in vitro inhibitor against the human immunodeficiency virus (HIV) [7]. These different effects imply that qualitative and quantitative control of the herbs to ensure its maximal therapeutic value is very necessary. Several methods have been reported for the determination of these lactones, including TLC [8–10], LC [11], HPLC [12–14], high-speed counter-current chromatography [15] and so on. But many of these procedures are time consuming, imprecise and require multiple

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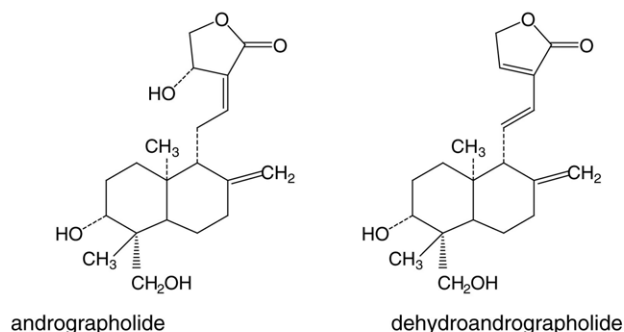

Fig. 1. Molecular structures of andrographolide and dehydroandrographolide.

steps of extraction and purification. Therefore, it is important to establish an improved method for quantitative analysis of these lactones.

Capillary electrophoresis (CE) is another analytical technique, which shows to be speedy and reliable. The method is suitable for simultaneous analysis of mixed compounds and offers advantage of excellent separation in terms of efficiency and resolution of analysis. The use of CE for the analysis of pharmaceuticals has become increasingly popular in recent years. For determination of andrographolide and dehydroandrographolide, micellar electrokinetic chromatography (MEKC) [7,16] is the most applied approach. Microemulsion electrokinetic chromatography (MEEKC) [17–19] is a relatively new technique of CE, which accomplishes electrokinetic separations using buffers containing surfactant coated oil droplets. MEEKC has been shown to be useful for the separation of very lipophilic substances [17,18,20] and very hydrophilic substances [18,21,22] as well as pharmaceuticals [23,24] and natural products [25,26]. The oil-in-water micromulsion systems are similar to micelles in that they can solubilize hydrophobic compounds, but with a much larger capacity. The solvating property of the micromulsion enables resolution of a wide range of solutes of differing hydrophobicity [27]. In addition, organic solvents are widely used in MEKC to improve the separation but in some cases there are problems of solvent evaporation. MEEKC presents some advantages with respect to the use of organic solvent additives in MEKC where microemulsions are relatively non-volatile and may be prepared with a higher proportion of organic additives without loss of solvent through evaporation. Despite the great potential of this technique, few studies into the use of micromulsion system in pharmaceutical analysis have been conducted. To the best of our knowledge, there are no applications on the separation of andrographolide and dehydroandrographolide in *A. paniculata* or in its medicinal preparations by MEEKC. In this paper, we first developed a MEEKC method for determination of bioactive species in the extracts of *A. paniculata* and its medicinal preparations. The results indicate the MEEKC method has better separation efficiency, exceptional resolution, good repeatability and recovery compared with the reported MEKC method.

2. Experimental

2.1. Reagents and materials

Andrographolide and dehydroandrographolide (the structures were shown in Fig. 1), samples of *A. paniculata* (I) and *A. paniculata* (II) were purchased from the National Institute for Control of Pharmaceutics and Biological Products, Beijing, China. The Chuanxinlian tablets (Yuxi Pharmaceutical Limited Company (Yunnan, PR China)) and Xiaoyanlidan tablets (Shantou Pharmaceutical factory (Guangdong, PR China)) were purchased from a local herbal store.

All chemicals were of analytical-reagent grade: sodium dodecyl sulfate, sodium tetraborate-10-hydrate, hydrochloric acid, sodium hydroxide, heptane, butan-1-ol, and methanol were all purchased from Beijing Chemical Reagents Plant. Milli-Q deionized water was used throughout the study (Millipore, Bedford, MA, USA).

2.2. Apparatus

All separations were performed on MDQ CE instrument equipped with a photodiode-array detector Beckman (Fullerton, CA, USA). Beckman Coulter MDQ 32 Karat software was used for instrumental control and data analysis. A 60.2 cm × 50 μm i.d. uncoated fused silica capillary (Yong-Nian Optical Fiber Factory, Hebei, China) was utilized with an effective length of 50 cm. Separation were carried out using an electrical voltage of 20 kV, and the temperature of the capillary was maintained at various values between 25 and 45 °C, While 214 nm was selected as the detection wavelength. Samples were injected by applying a pressure of 0.5 psi, for 3 s.

2.3. Sample and extract preparation

Stock solutions (1 mg/mL) of andrographolide and dehydroandrographolide were prepared in methanol, and the solutions at various concentrations were prepared by appropriate dilution from the stock solutions when needed.

For diterpene lactones extraction, 1.00 g of powdered plant and 2.00 g of powder of Chuanxinlian tablets and Xiaoyanlidan tablets were accurately weighed and extracted with 10 mL methanol separately for 30 min by ultrasonication. After that, it was maintained at room temperature for 30 min. The extracts were filtered through a filter paper. The extraction procedure was repeated three times, and the extracts were combined and concentrated to dryness. The residue was diluted to 50 mL with methanol, which was then passed through a 0.45 μm membrane filter before analysis.

2.4. Preparation of running buffer

The microemulsions were prepared by weighing 0.81 g heptane, 6.61 g butan-1-ol, 3.31 g SDS and 89.27 g of 10 mM sodium tetraborate buffer to a 100 mL volumetric flask.

This was then sonicated for 30 min until a clear and stable micromulsion was obtained. HCl or NaOH adjusted the value of pH of the buffer to desired value. All the running buffers were prepared daily and, prior to use, filtered through 0.45 μm filter to remove particulate matter and degassed in an ultrasonic bath.

3. Results and discussion

3.1. Method optimization

The optimization of the separation of the chosen diterpene lactones was performed with the aim of developing a MEEKC method of general applicability; particular attention was focused on the specific separation of andrographolide and dehydroandrographolide, the marker phytochemicals in the *A. paniculata* and commercial tablets extracts. We investigated the effects of SDS concentration, the pH buffer value, and temperature on the migration times of the studied analytes. The requirements for use of an analytical method in a routine pharmaceutical analysis include a relatively rapid separation and acceptable separation repeatability. In this paper, we chose the standard conditions (0.81% (w/w) of heptane, 3.31% (w/w) of SDS, 6.61% (w/w) of butan-1-ol and 10 mM sodium tetraborate buffer, pH 9–10) that were described by most papers to obtain the microemulsions, which can form highly stable microemulsions with shelf lives exceeding several months. The main disadvantage of MEEKC is the relatively longer times needed for the separation of lypophilic compounds.

Lower concentrations of the surfactant can reduce analysis time, but it will increase surface tension to generate unstable microemulsions. Terabe et al. [28] reported instability of the microemulsions using only 1.6% (w/w) SDS. Ishihama et al. [29] also reported poor repeatability using 1.4% SDS. Miola et al. [30] observed that microemulsions produced with 2% (w/w) or less SDS content disintegrated after only a few hours. In the experiment, we found increasing SDS concentration did not affect selectivity obviously but increase analysis time, so we kept the concentration of the surfactants constant at 3.31% in all experiments.

The effect of buffer pH in the range from 8 to 11 on the separation of the two active components was investigated. The migration times of the two active components decreased with increasing pH value, because the two components are not ionizable and influenced only by EOF (electroosmotic flow). Although there was no problem to separate the two active components in the standard mixture over the whole pH range, the results of experiment showed that the baseline was noise and current increasing highly when pH value exceeded 10. Therefore, pH 9.2 was selected as optimized condition because of a good compromise between resolution and analysis time.

The temperature affects solute solubility, which is related to the partitioning coefficient. The electrophoretic mobility

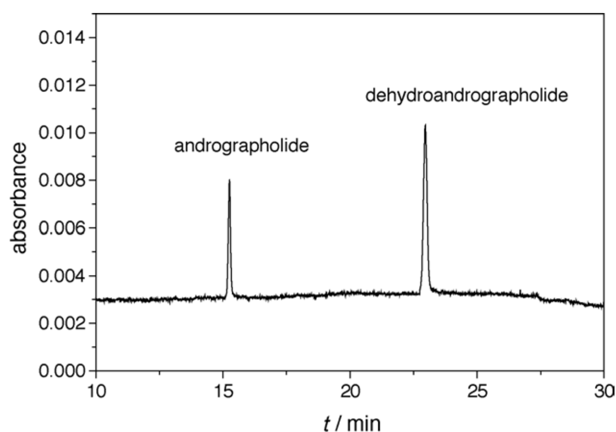

Fig. 2. Optimized separation of andrographolide and dehydroandrographolide.

of an ion is also affected by temperature by 2%/°C. In this study, the temperature was changed between 25 and 45 °C and the observed migration time decreased evidently but the selectivity remained similar with increasing temperature. The decrease in migration time for andrographolide and dehydroandrographolide is primarily due to an increase in EOF resulting from the decrease in viscosity. However, the selectivity of neutral solutes is unaffected by temperature.

We used a high voltage (20 kV) and high temperature (42 °C) to reduce analysis time and limit the generation of excessive operating current (about 80–90 μA). Fig. 2 shows the separation of andrographolide and dehydroandrographolide under the optimal conditions.

Microemulsion conditions: 0.81% (w/w) heptane, 3.31% (w/w) SDS, 6.61% (w/w) butan-1-ol and 89.27% (w/w) of 10 mM sodium tetraborate buffer at pH 9.2. Injections: 0.5 psi/3 s. Applied voltage: 20 kV. Direct UV detection at 214 nm.

3.2. Method validation

The calibration curve for andrographolide and dehydroandrographolide was constructed in the concentration range of 15.4–450 and 13.9–535 μg/mL, respectively. The linear regression equation and coefficient are as follows:

$$\text{andrographolide}: \quad y = (70.77 \pm 1.60)x + (190.1 \pm 474.1) \quad (R = 0.9992)$$

dehydroandrographolide :

$$y = (59.41 \pm 1.24)x + (4628.8 \pm 367.4) \quad (R = 0.9994)$$

where y is the integrated peak area (integration units) and x the concentration (μg/mL). Thus, the amounts of andrographolide and dehydroandrographolide in *A. paniculata* and the commercial Chinese herbal preparations can be quantified according to the above regression lines of equations. It can be seen that the linearity was satisfactory with a correlation coefficient (R) greater than 0.999.

Table 1
Percentage content of andrographolide and dehydroandrographolide in real samples (dry plants or tablets) ($n = 3$)

Analyte	*Andrographis paniculata* (I)		*A. paniculata* (II)		Chuanxinlian		Xiaoyanlidan	
	Content (%)	R.S.D. (%)	Content (%)	R.S.D. (%)	Content (%)	R.S.D. (%)	Content (%)	R.S.D. (%)
Andrographolide	1.27	2.28	0.57	1.93	0.061	3.89	0.34	3.31
Dehydroandrographolide	1.12	2.56	1.97	2.17	3.57	2.95	5.19	3.67

The limits of detection (LODs) for andrographolide and dehydroandrographolide, on the basis of a signal-to-noise of 3 (S/N) were determined to be 0.30 and 0.26 μg/mL, respectively. The limit of quantification (LOQ) was calculated as 10 times the baseline noise level. The LOQs were 1.0 μg/mL (R.S.D. = 1.89%) for andrographolide and 0.93 μg/mL (R.S.D. = 2.12%) for dehydroandrographolide, respectively.

The precision of CE depends on the repeatability of migration times and peak areas. In order to obtain good repeatability, separate vials were used for each injection and adequate rinses were applied between two injections. The standard samples were injected six times within the same day, the R.S.D. of migration time was 0.82% for andrographolide and 1.16% for dehydroandrographolide, and the R.S.D. of area was 2.89 and 3.13%, respectively. The inter-day reproducibilities were determined by examining six runs of the same sample in three continuous days, and the R.S.D. values of migration time and peak area for andrographolide and dehydroandrographolide were less than 4%. The results showed the method had a satisfactory reproducibility.

The recoveries were determined with the standard addition method for andrographolide and dehydroandrographolide in the real sample. 6.35 mg of andrographolide and 5.60 mg of dehydroandrographolide were accurately weighed and mixed with 1.00 g of a fine powder of *A. paniculata* (I), the mixture was extracted and analyzed using the proposed method. The recoveries of the andrographolide and dehydroandrographolide were 105 and 97.6% ($n = 5$) with the R.S.D. of 3.25 and 4.18%, showing that the sample preparation method could provide acceptable extraction efficiency and recovery of this analysis method was good.

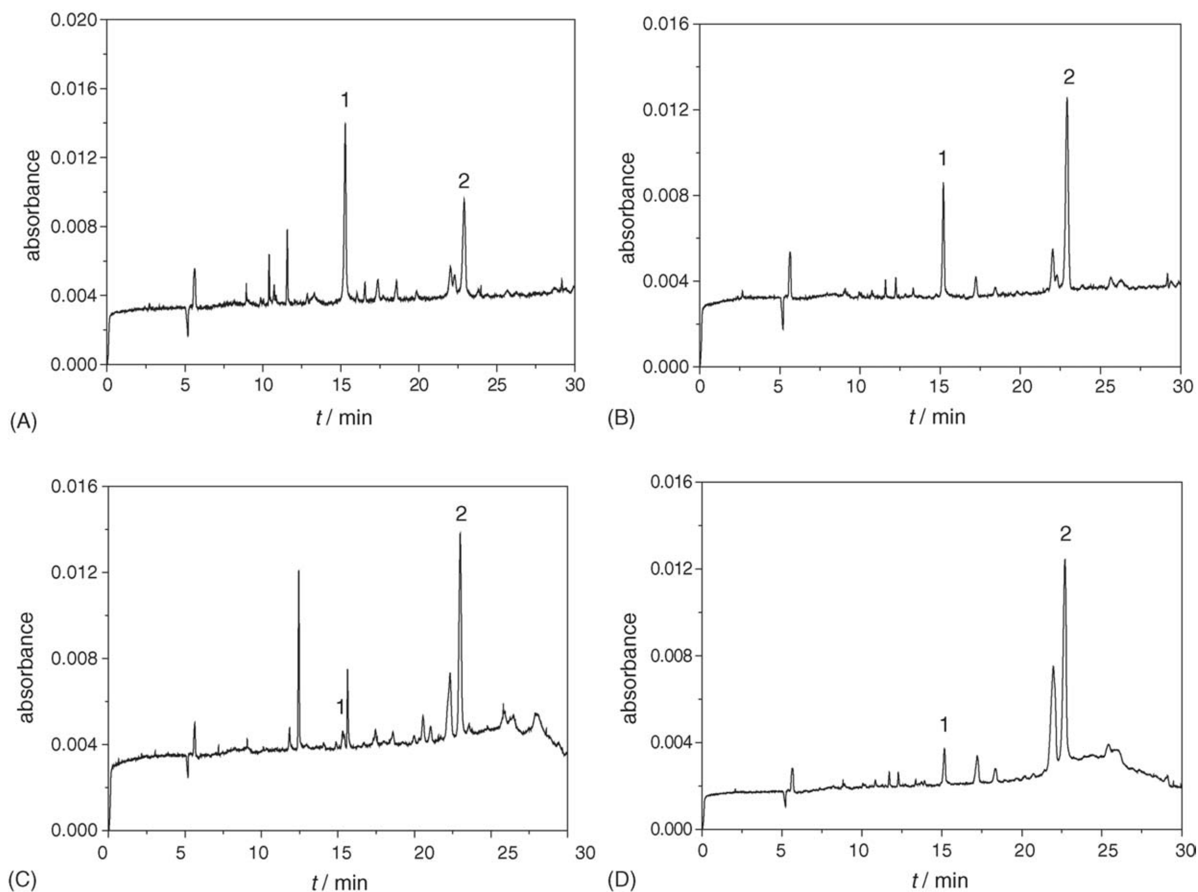

Fig. 3. Electropherograms of real samples. Peaks: 1, andrographolide; 2, dehydroandrographolide. Analytical conditions were same as in Fig. 2A. *A. paniculata* (I), (B) *A. paniculata* (II), (C) Chuanxinlian tablet, (D) Xiaoyanlidan tablet.

3.3. Determination of andrographolide and dehydroandrographolide in Chinese herb and Chinese medicinal preparations

The extracts of *A. paniculata* and the two *Andrographis paniculata*—containing Chinese medicinal preparations were determined under the optimum conditions mentioned earlier, and the calculated contents of andrographolide and dehydroandrographolide were shown in Table 1. The electropherograms of real samples were shown in Fig. 3. It was observed that andrographolide and dehydroandrographolide and other unknown compounds were baseline resolved within 25 min. Peaks were identified by the addition of standard andrographolide and dehydroandrographolide.

4. Conclusion

The present investigations confirm the earlier observations that microemulsion electrokinetic capillary chromatography (MEEKC) provide good selectivity and high separation efficiency. The contents of andrographolide and dehydroandrographolide in *A. paniculata* and its medicinal preparations were successfully determined with satisfactory recoveries, sensitivities and reproducibility. The proposed method is simple, economic, and rapid and is especially suitable for quality control in pharmaceutical plants.

References

[1] A. Leelarasamee, S. Trakulsomboon, N. Sittisomwong, J. Med. Assoc. Thailand 73 (1990) 299–304.
[2] D. Bensky, A. Gamble, T. Kaptchuk, Chinese Herbal Medicine Materia Medica, Revised Edition, Eastland Press, Seattle, 1993, p. 95.
[3] P. Siripong, B. Kongkathip, K. Preechanukool, P. Picha, K. Tunsuwan, W.C. Taylor, J. Sci. Soc. Thailand 18 (1992) 187–194.
[4] K. Bone, Nutr. Healing (1998) (review).
[5] P.K. Singha, S. Roy, S. Dey, Fitoterapia 74 (2003) 692–694.
[6] S. Rajagopal, R.A. Kumar, D.S. Deevi, C. Satyanarayana, R. Rajagopalan, J. Exp. Ther. Oncol. 3 (2003) 147–158.
[7] H.Y. Cheung, C.S. Cheung, C.K. Kong, J. Chromatogr. A 930 (2001) 171–176.
[8] The Pharmacopoeia Commission of the People's Republic of China, Pharmacopoeia of PRC, Chemical Industry Press, Beijing, 2000, p. 220.
[9] S. Saxena, D.C. Jain, M.M. Gupta, Phytochem. Anal. 11 (2000) 34–36.
[10] A. Srivastava, H. Misra, R.K. Verma, Phytochem. Anal. 15 (2004) 280–285.
[11] D.C. Jain, M.M. Gupta, S. Saxena, S. Kumar, J. Pharm. Biomed. Anal. 22 (2000) 705–709.
[12] W.K. Li, J.F. Fitzloff, J. Liq. Chromatogr. R.T. 25 (2002) 1335–1343.
[13] W.K. Li, J.F. Fitzloff, J. Liq. Chromatogr. R.T. 27 (2004) 2407–2420.
[14] N. Pholphana, N. Rangkadilok, S. Thongnest, Phytochem. Anal. 15 (2004) 365–371.
[15] Q. Du, G. Jerz, P. Winterhalter, J. Chromatogr. A. 984 (2003) 147–151.
[16] J. Zhao, G. Yang, H. Liu, D. Wang, X. Song, Y. Chen, Phytochem. Anal. 13 (2002) 222–227.
[17] K.D. Altria, J. Chromatogr. A 892 (2000) 171–186.
[18] K.D. Altria, J. Chromatogr. A 844 (1999) 371–386.
[19] R. Pomponio, R. Gotti, N.A. Santagati, V. Cavrini, J. Chromatogr. A 990 (2003) 215–223.
[20] S. Pedersen-Bjergaard, S. Moestue, K.E. Rasmussen, J. Chromatogr. A 876 (2000) 201–211.
[21] G.-H. Zhou, G.-A. Luo, X.-D. Zhang, J. Chromatogr. A 853 (1999) 277–284.
[22] I. Miksik, J. Gabriel, Z. Deyl, J. Chromatogr. A 772 (1997) 297–303.
[23] M.S.A. Prado, C.A. Silva, M.F.M. Tavares, K.D. Altria, J. Chromatogr. A 1051 (2004) 291–296.
[24] T. Bo, X. Yang, F. Liu, K.A. Li, L. Xiu, H. Liu, Anal. Chim. Acta 474 (2002) 37–48.
[25] C.G. Jensen, S.H. Hansen, S.P. Bjergaard, Electrophoresis 22 (2001) 1330–1336.
[26] L. Debusschere, C. Demesmay, J.L. Rocca, G. Lachatre, H. Lofti, J. Chromatogr. A 779 (1997) 227–233.
[27] S.W. Sun, P.C. Yeh, J. Pharm. Biomed. Anal. 36 (2005) 995–1001.
[28] S. Terabe, N. Matsubara, Y. Ishihama, Y. Okada, J. Chromatogr. 608 (1992) 23–29.
[29] Y. Ishihama, Y. Oda, K. Uchikawa, N. Asakawa, Anal. Chem. 67 (1995) 1588–1595.
[30] M.F. Miola, M.J. Snowden, K.D. Altria, J. Pharm. Biomed. Anal. 18 (1998) 785–797.