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Migration behavior and separation of active components in *Glycyrrhiza uralensis* Fisch and its commercial extract by micellar electrokinetic capillary chromatography

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Abstract

A micellar electrokinetic capillary chromatography (MECC) method for the separation and determination of five components, glycyrrhizic acid (GA), glycyrrhetinic acid (GTA) and 3,4′-dimethoxy-5-hydroxychalone, fermononetin and isoliquiritigenin, in extracts of *Glycyrrhiza uralensis* Fisch root was developed. Migration behavior of these analytes was studied by the systematic examination of the borate and sodium dodecyl sulfate (SDS) concentrations in the run buffer. The optimum separation for these analytes was achieved using 10 mmol l^{-1} of tetraborate and 25 mmol l^{-1} of SDS as the running buffer, with 17 kV of applied voltage. All experiments were performed using a 50.0 cm (42.4 cm effective length)×75 μm I.D. of fused-silica capillary. The apparent pK_a values of GA and GTA and the binding constants for the association between the above five analytes and SDS were calculated in this study. A comparison of the extraction efficiency for GA and GTA from *Glycyrrhiza uralensis* Fisch root was made using ethanol, distilled water and chloroform as extraction reagents, respectively. These results provided very useful information to select the proper solvent to extract the desired components in *Glycyrrhiza uralensis* Fisch. The MECC method established in this paper was employed to analysis the above five active components.

Keywords: *Glycyrrhiza uralensis*; Pharmaceutical analysis; Glycyrrhizic acid; Glycyrrhetinic acid; Dimethoxyhydroxychalone; Fermononetin; Isoliquiritigenin

1. Introduction

Glycyrrhiza uralensis Fisch root has been used as a Chinese traditional medicine for over 1000 years. The medicine is frequently used to treat diseases such as phthisis, contagious hepatitis, bronchitic asthma and ague. It has been demonstrated that the major bioactive components in *Glycyrrhiza uralensis* Fisch root are glycyrrhizic acid (GA), glycyrrhetinic acid (GTA) and 3,4′-dimethoxy-5-hydroxychalone (DH), fermononetin (FN) and isoliquiritigenin (IQ) (their structures are shown in Fig. 1) [1]. The five most important bioactive components can be considered as indices for estimation of quality. Therefore, a simple and rapid method for determination of these five components in *Glycyrrhiza uralensis* Fisch root and its commercial extract is needed.

Several methods including polarography [2,3] and

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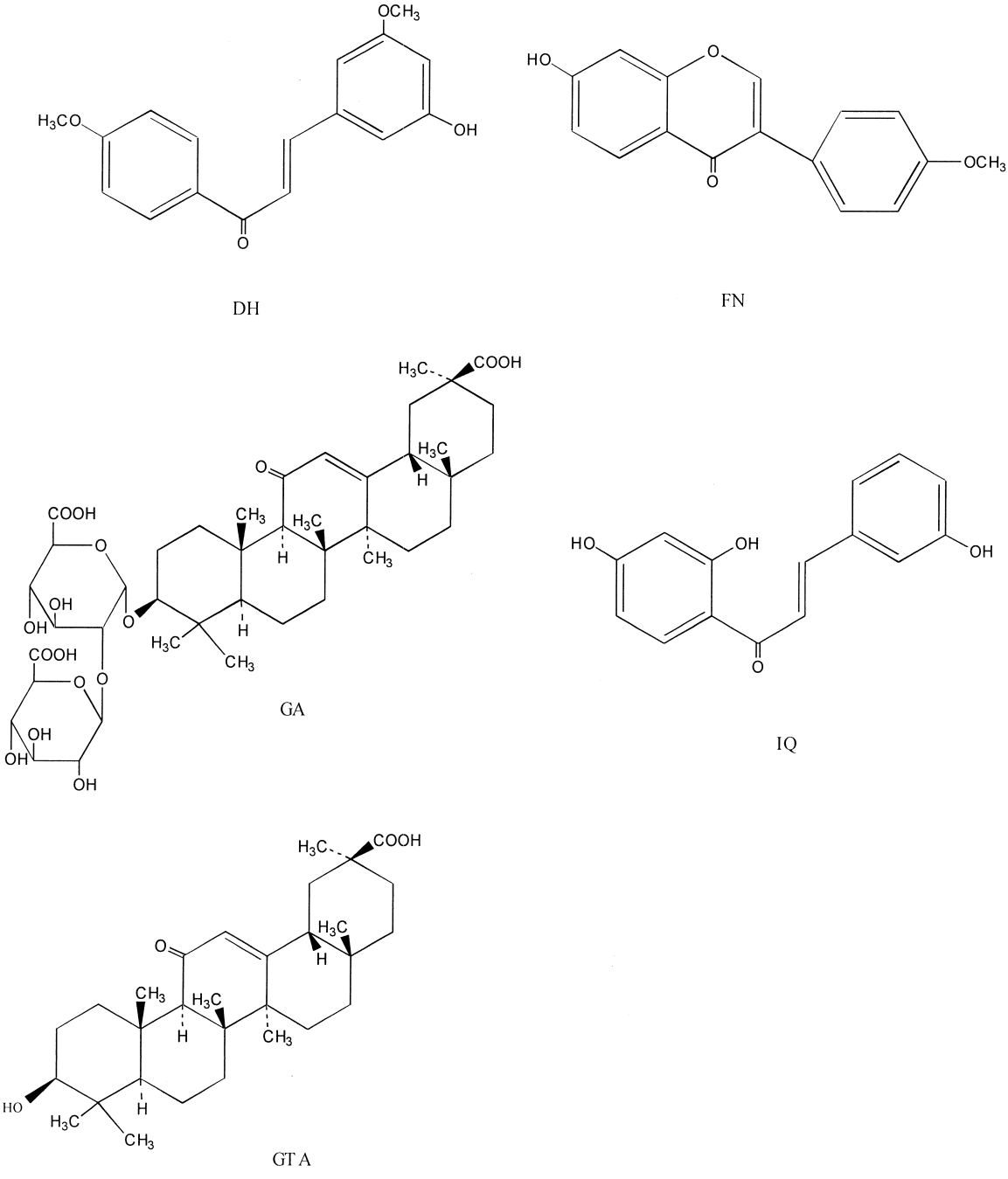

Fig. 1. Structures of analytes.

high-performance liquid chromatography (HPLC) [4–6] have been reported to determine the contents of GA and GTA in *Glycyrrhiza uralensis* Fisch and herbal preparations. Capillary electrophoresis (CE) has been used in the separation of inorganic [7] and organic [8] analytes. Recently, much attention has

focused on the application of this technique to studying the pharmaceuticals [9,10]. Because it often provides higher resolving power, short analysis time and lower operating costs than HPLC and thin layer chromatography, CE has been accepted by more and more people as an attractive method for the separation and identification of Chinese traditional medicine [11–19]. In this paper, a new and simple method of micellar electrokinetic capillary chromatography (MECC) for simultaneous separation of these chalcones and organic acids in *Glycyrrhiza uralensis* Fisch and its commercial extract (including finery extract and raw extract) is presented.

2. Experimental

2.1. Instrumentation

All experiments were carried out on a Waters Quanta 4000 system (Waters Chromatography Division of Milford, MA, USA). The temperature was kept at 20°C and the wavelength of the UV detector was set at 254 nm. Hydrodynamic injection (10.0 cm) was set 2 s. A 75 μm I.D. fused-silica capillary from Waters Accasep, total length 50.0 cm, distance between injection and detection, 42.4 cm. A 5 min wash cycle with 0.5 mol l^{-1} NaOH followed by 3 min with distilled water, and 5 min with the separation buffer was necessary to condition the capillary. Between runs, the capillary was washed with 0.5 mol l^{-1} NaOH for 3 min, followed by distilled water for 3 min and buffer for 5 min.

2.2. Reagents

Authentic GA, GTA, DH, FN and IQ were obtained from the Organic Chemistry Laboratory at the Department of Chemistry of Lanzhou University. Structures and names of these five compounds were shown in Fig. 1.

Samples of *Glycyrrhiza uralensis* Fisch and the commercial extract of *Glycyrrhiza uralensis* Fisch root were obtained from Lanzhou Lanzheng pharmaceutical store and Lanzhou pharmaceutical market. The finery extract of *Glycyrrhiza uralensis* Fisch root was obtained from the International Import and Export Corporation of Gansu province. Ethanol was used as electroosmotic flow marker, sudan (III) was used as marker of sodium dodecyl sulfate (SDS) surfactant. All chemicals were of analytical reagent grade unless otherwise specified.

2.3. Preparation of electrolyte

The run buffer was prepared from 0.1 mol l^{-1} $Na_3B_4O_7$ solution and adjusted to the desired pH with H_3PO_4. At MECC mode, SDS was dissolved in the plain $Na_2B_4O_7$ buffer.

2.4. Sample preparation

Three methods were used to prepare the extract from *Glycyrrhiza uralensis* Fisch. For method I, 5.0 g of root of this pharmaceutical plant was refluxed with 100 ml ethanol for 1 h, and this extraction solution was injected directly in the CE system after filtering. For method II, 5.0 g of this sample was refluxed with 100 ml of chloroform for 1 h, the chloroform was evaporated off after filtering the chloroform extraction solution, and the residue of the extract was dissolved in with 100 ml ethanol. For method III, a 5.0 g sample was extracted with distilled water for 1 h. This procedure is similar to the common method for preparing Chinese traditional medicines in hospitals. The aqueous solution of the extract was injected directly to the CE system after filtering.

A 0.3 g sample of commercial raw extract of *Glycyrrhiza uralensis* Fisch was refluxed with 100 ml of ethanol for 1 h, then the extract solution was filtered and the residue of the sample was refluxed with 50 ml of ethanol for 30 min. The above procedures were repeated for three times. After the mixture of extraction solutions had been partly evaporated, the residue solution was diluted to 100 ml with ethanol. The final solution was injected directly in the CE system.

A 0.3 g amount of the commercial finery extract of *Glycyrrhiza uralensis* Fisch root was treated with the last method described. The residue after evaporating the ethanol was diluted to 250 ml with ethanol for the determination of GA and to 25 ml for the determination of GTA.

2.5. Calculations

Because these compounds migrated to the opposite direction of elecctroosmotic flow, the effective mobility was defined by the following equation [20]:

$$\mu_{\text{eff}} = \left(\frac{1}{t_R} - \frac{1}{t_0}\right) \cdot \frac{lL}{V} \quad (1)$$

μ_{eff} is the effective mobility of analyte, t_R is the migration time of analyte and t_0 is the migration of ethanol.

3. Results and discussion

3.1. Capillary zone electrophoresis study of the migration behavior of GA and GTA

In capillary electrophoretic studies of ionisable compounds, pH plays a fundamental role as it determines the extent of ionisation of a solute, and this can be used for the determination of pK_a by measuring the ionic mobility of a solute as a function of pH [20]. The influence of the buffer solution pH value on effective mobilities for GA and GTA was shown in Fig. 2. The apparent pK_a values for GA and GTA calculated by the method in [21] are 7.09 and 6.53, respectively.

In addition, the change of the pH value affects the dissociation of sinanol of capillary inner face, resulting in the change of the effective charge on the inner face of capillary and electroosmotic flow. It was found from our experiment that a higher pH value could shorten the analysis time. In the method described here, a solution of sodium tetraborate was selected as the working buffer (pH 9.20). However, DH and FN could not be separated in the pH range examined in this study (6.98–9.20). Hence, the MECC mode should be tried so as to separate all five compounds listed in Fig. 1.

3.2. Influence of SDS concentration

The concentration of SDS surfactant is very important in the separation of these five compounds. The influence of SDS concentration on the effective mobility of the analytes is shown in Fig. 3. As demonstrated in Fig. 3, the higher SDS concentration results in a sufficient separation, although the migra-

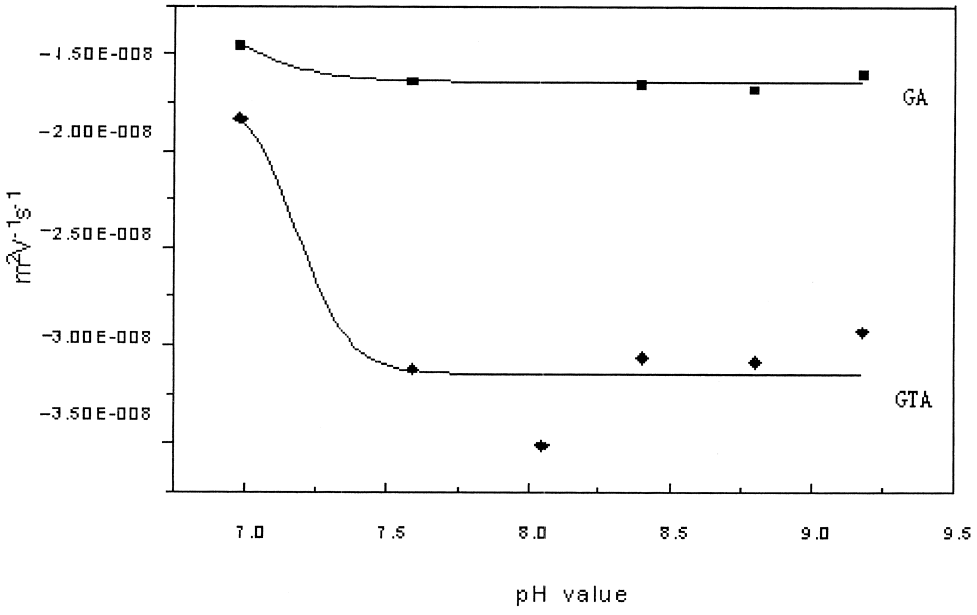

Fig. 2. Effective mobilities of two chargeble analytes vs. pH. Conditions: 10 mmol l^{-1} of teraborate buffer without SDS, 17 kV applied voltage.

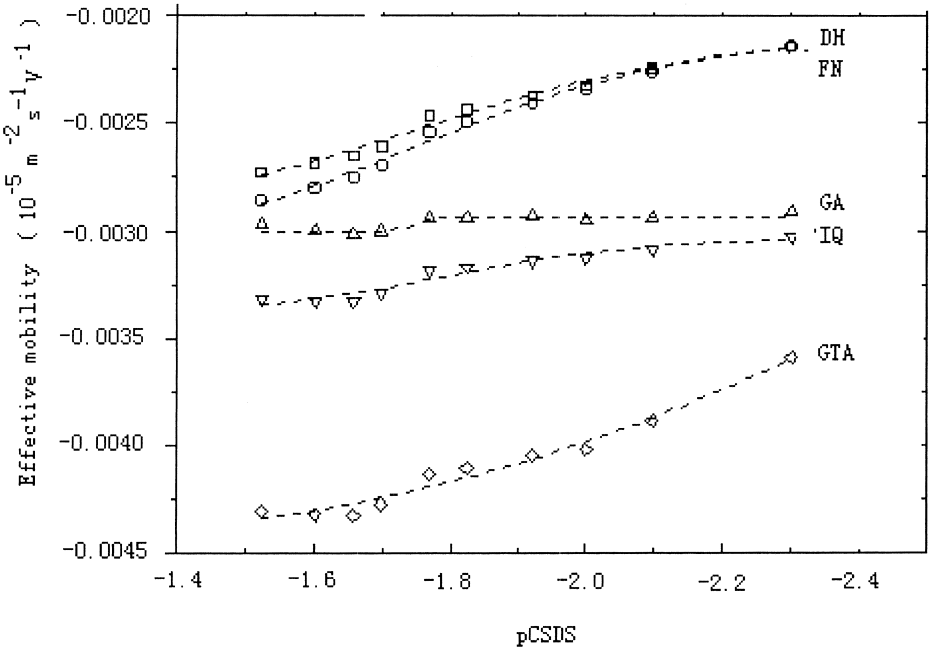

Fig. 3. Dependence of effective mobility of analyts (GA and GTA) on pC_{SDS}. Conditions: 10 mmol l^{-1} of tetraborate buffer, pH 9.20, 17 kV applied voltage.

tion time is long compared with the low SDS concentration. The optimum separation for the selected five compounds is shown in Fig. 4. From our experiment it was found that the resolution for DH and FN, which were difficult to be separated, was 1.31 when SDS concentration was 25 mmol l^{-1}, whereas it was only 0.98 when SDS concentration was 15 mmol l^{-1}. On the other hand, the SDS concentration also influences the current across the capillary. The current across the capillary increases with increasing SDS concentration. The higher SDS concentration is disadvantageous to the separation. Considering the above two sides, a SDS concentration of 25 mmol l^{-1} was selected as the optimum condition.

The binding constants were measured for the association of the selected five compounds to SDS. As early as 1951, Alberty and King [22] presented an equation relating the apparent binding constant to the electrophoretic mobilities of free and uncomplexed analytes for zone electrophoresis. Here we provide an equation to estimate the binding constant for the five compounds to SDS under the similar idea to Albert's:

$$\mu_{\text{eff}} = \mu_1 \frac{1}{1 + 10^{\frac{pC - pK}{B}}} + \mu_2 \cdot \frac{10^{\frac{pC - pK}{B}}}{1 + 10^{\frac{pC - pK}{B}}} \qquad (2)$$

where $pC = -\log C_{SDS}$, pK is an apparent equilibrium constant, B is a constant, μ_1 and μ_2 are the effective mobilities of analytes without and with SDS, respectively. The parameters in Eq. (2) for different analytes are listed in Table 1. The dependence of effective mobility of analytes on pC is shown in Fig. 3. The dashed lines are the results obtained by Eq. (2) with parameters in Table 1.

3.3. Influence of $Na_2B_4O_7$ solution concentration

To investigate the dependence of migration behavior of analytes on borate concentration, the electrophoretic measurements were performed with running electrolytes of 20 mmol l^{-1} SDS, pH 9.20, at various tetraborate concentrations. The migration time of micelles, t_m, increased substantially with increasing the tetraborate concentration from 3 to 17 mmol l^{-1}, while the migration time of the electroosmotic flow marker, ethanol, was almost un-

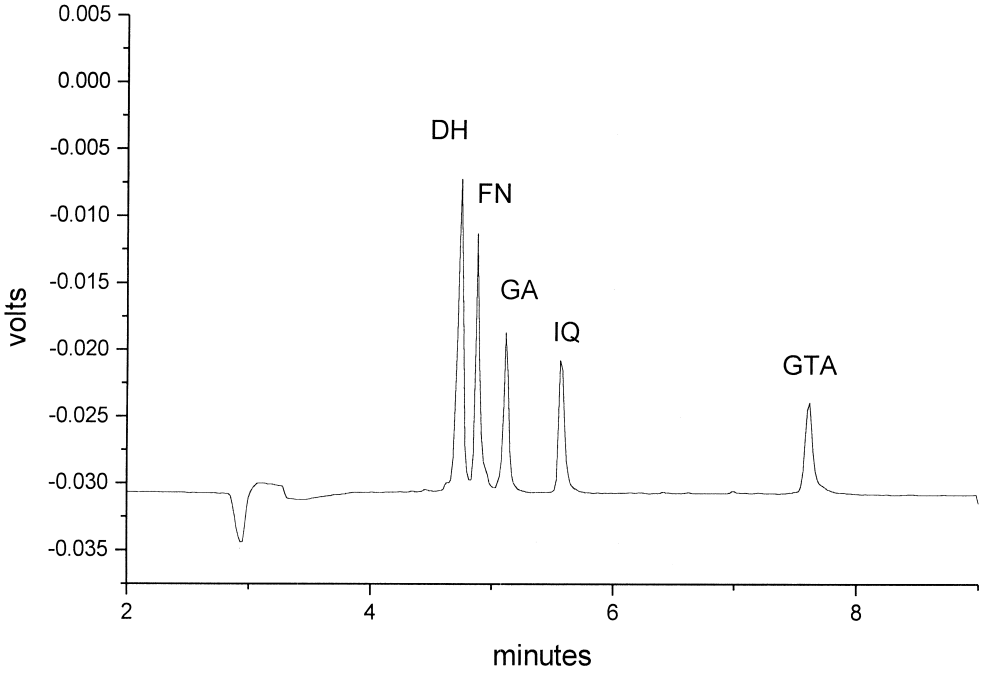

Fig. 4. Electropherogram for standard analytes. Conditions: 25 mmol l^{-1} SDS, 10 mmol l^{-1} teraborate, 17 kV applied voltage.

changed. The migration window was slightly broadened with the increase of tetraborate concentration. It can be found from Fig. 5 that the migration times for the selected analytes increased with increasing the tetraborate concentration from 3 to 17 mmol l^{-1}.

3.4. Applied voltage

Higher applied voltages resulted in decreased migration times, and in the mean time the peak broadened due to the excess Joule heat and diffusion. The optimized applied voltage in the method presented was selected as 17 kV.

3.5. Relative migration time reproducibility and calibration curve

A frequent drawback of the MECC mode is the poor reproducibility of absolute migration times [23], which makes the identification of drugs unreliable, especially in biological matrices. Usually the repeatability of the migration times can be improved by replacing absolute values with relative migration times [24]. In this work, the relative migration times for the selected compounds were calculated against the migration time of ethanol. Table 2 lists the relative migration time, reproducibilities, correlation coefficients for linear calculation graphs and concentration ranges for the five analytes in optimum

Table 1
Parameters of Eq. (2) obtained by simulation

Analytes	μ_1 ($\times 10^{-8}$ m^2 V^{-1} s^{-1})	μ_2 ($\times 10^{-8}$ m^2 V^{-1} s^{-1})	p*K*	B
DH	−2.08	−3.00	1.74	0.53
FN	−2.08	−3.15	1.74	0.48
GA	−2.93	−3.00	1.76	0.20
IQ	−3.04	−3.39	1.79	0.34
GTA	−1.73	−4.56	2.58	0.99

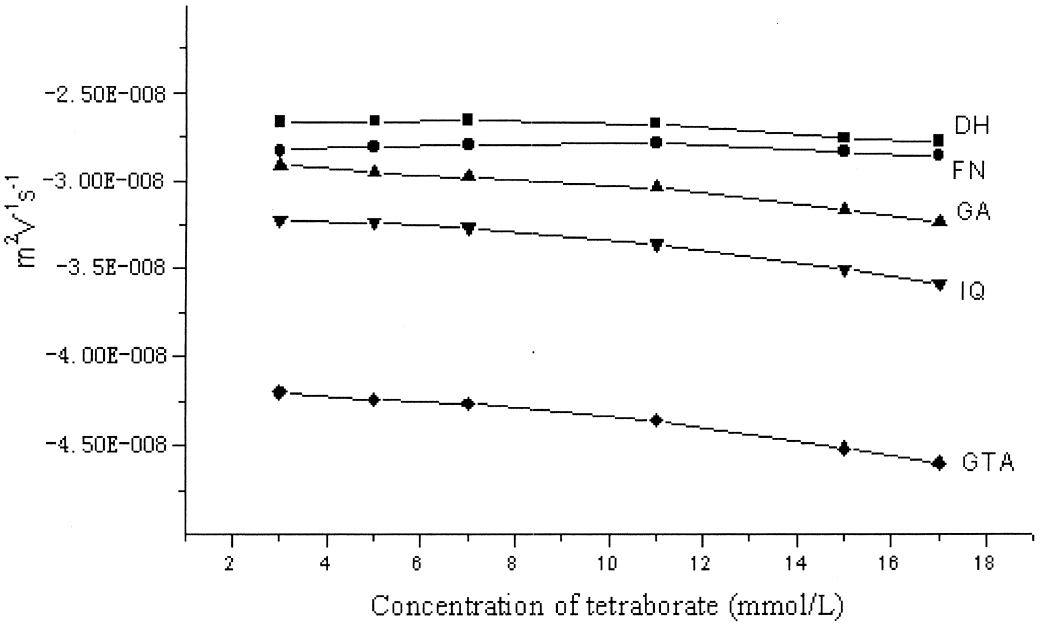

Fig. 5. Influence of tetraborate concentration on effective mobilities of analytes. Conditions: 25 mmol $l^{-1}$of SDS, pH 9.20, 17 kV applied voltage.

condition. The corrected area of the electropherogram was employed for the quantitation of the analytes. The correlation coefficient of calibration was greater than 0.9982.

3.6. Applications

In order to find out the most suitable extraction solvent for GA and GTA from *Glycyrrhiza uralensis* Fisch, we compared the extraction results obtained by the different extraction solvents ethanol, chloroform and distilled water. The typical electropherograms for a sample extracted with the various solvents are shown in Fig. 6a–c. It was found that more than ten peaks appeared in the electropherograms of Fig. 6a–c. The specific compounds were identified by comparing the relative migration time of the standards with those in the actual samples or by spiking standards in the actual samples. From our experiment, it was found that the order of extraction efficiency for GA was water>ethanol>chloroform, and for GTA it was ethanol>chloroform>water. The contents of GA and GTA in *Glycyrrhiza uralensis* Fisch were 1.69 and 0.80%, respectively.

There is difference between the migration time of GTA in Fig. 6c and in the other graphs, due to the

Table 2
Relative migration time, reproducibilities, correction coefficient of linear calculation graphs and concentration range for five analytes in optimum condition

Analytes	Relative migration time	RSD for relative migration time (%)	Correlation coefficient	Concentration range ($\mu g\ ml^{-1}$)
DH	1.914	0.64	0.9993	2.0–50.0
FN	2.054	1.97	0.9978	5.5–110.0
GA	2.352	2.03	0.9985	11.0–120.0
IQ	2.740	1.61	0.9981	5.0–80.0
GTA	4.964	2.29	0.9982	10.4–125.0

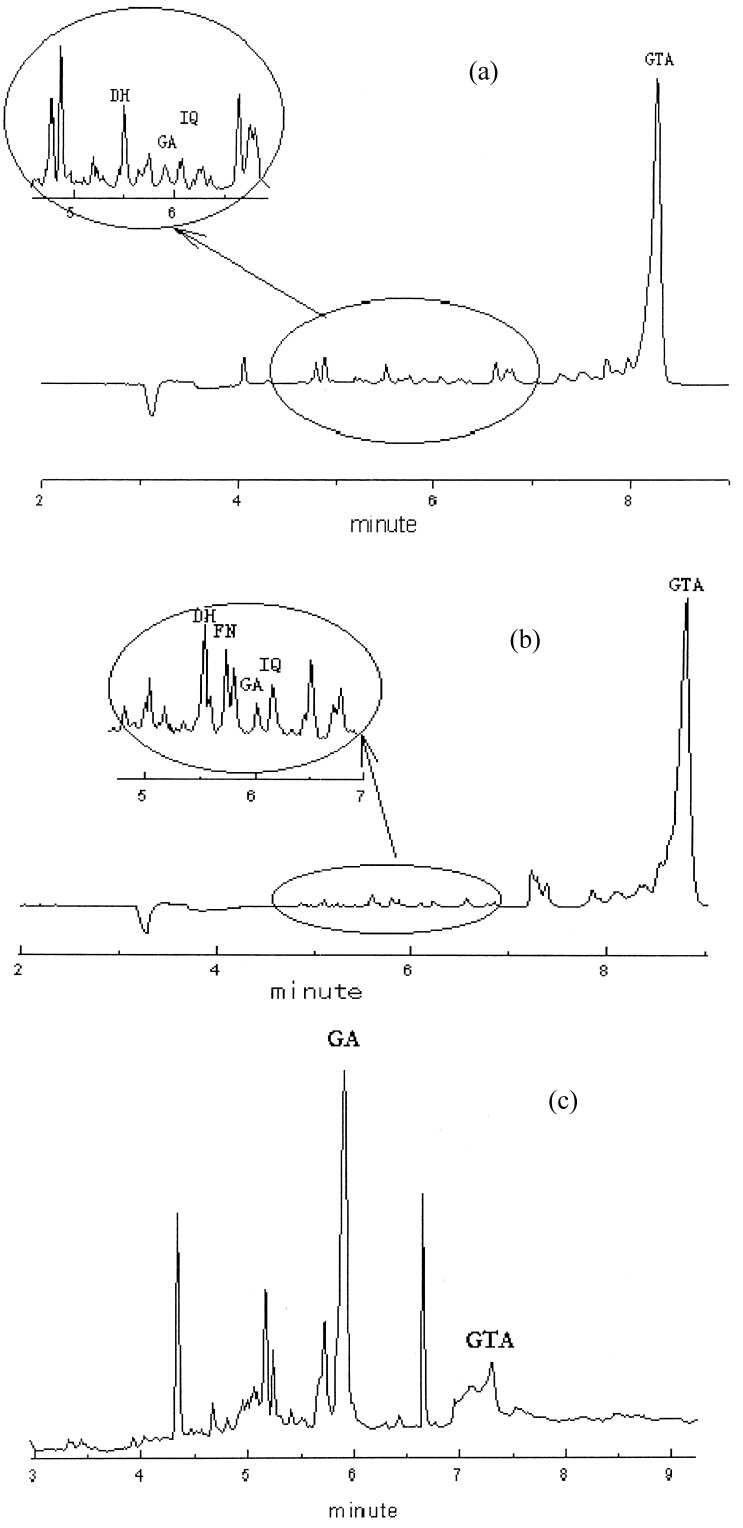

Fig. 6. Electropherogram of sample extracted by various solvents. Conditions: 25 mmol l^{-1} of SDS buffer, 10 mmol l^{-1} of tetraborate buffer, pH 9.20, 17 kV applied voltage. (a) By ethanol, (b) by chloroform, (c) by distilled water.

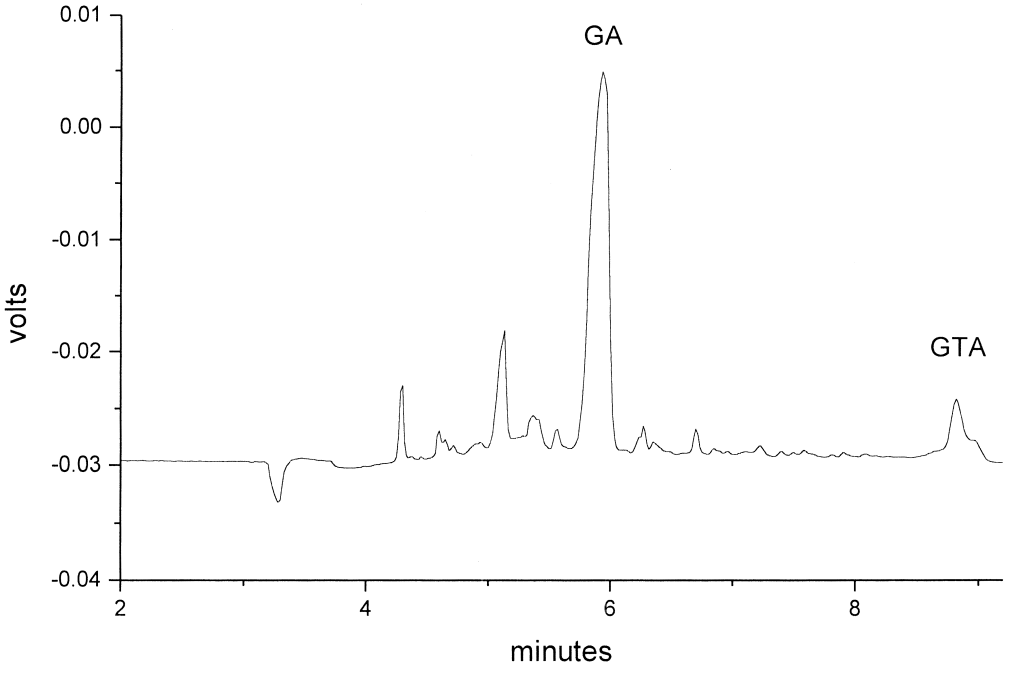

Fig. 7. Electropherogram of the commercial extract finery extract of *Glycyrrhiza uralensis* Fisch root. Conditions: 25 mmol l^{-1} SDS, 10 mmol l^{-1} tetraborate, pH 9.20, 17 kV applied voltage.

difference in the extraction conditions. Some water-soluble components in the complicated sample will be extracted into the sample solution after it has been pretreated with water. These components will be absorbed on the inner surface of capillary and change the characteristics of the capillary inner surface. In addition, the coexistance of multi-substances will affect the migration time of some active components as well. Therefore, the migration time of some components will be changed.

Similarly, two commercial products, the finery and raw extracts of *Glycyrrhiza uralensis* Fisch root, were analyzed. The electropherogram for the finery extract of *Glycyrrhiza uralensis* Fisch root is shown in Fig. 7. The amounts of GA and GTA in the commercial finery extract of *Glycyrrhiza uralensis* Fisch root were 47.4 and 2.4%, respectively. The relative standard deviation was 2.1% for GA and 5.4% for GTA. It should be pointed out that the contents of GA and GTA in the commercial raw extract were very low compared with those in the commercial finery extract.

4. Conclusion

In this paper, we propose a simple and rapid MECC method to separate and identify some chargeable and unchargeable components in *Glycyrrhiza uralensis* Fisch and its commercial extract. The successful separation of five substances extracted from this pharmaceutical plant shows that MECC is a powerful technique for the analysis of complex components in Chinese traditional pharmaceuticals and industrial products.

References

[1] New Medical College of Jiangsu, in: Dictionary of Traditional Chinese Medicine, Vol. Part II, People's Publisher of Shanghai, 1996, p. 567.
[2] W. Guo, M. Zhao, J. Song, Y. Liu, Chem. J. Chin. Univ. 17 (1996) 1541.
[3] W. Guo, J. Song, H. Du, Fenxi Huaxue 24 (1996) 835.
[4] R. Wang, S. Yang, X. Hu, L. Fang, H. Zhang, Y. Zhang, Chin. Pharm. J. 31 (1996) 549.

[5] L. Zeng, Z. Lou, R. Zhang, Acta Pharm. Sinica 26 (1991) 788.
[6] W. Zhu, R. Yu, Yaowu Fenxi Zazhi 14 (1994) 47.
[7] H. Zhang, L. Jia, Z. Hu, J. Chromatogr. A 704 (1995) 242.
[8] L. Jia, H. Zhang, Z. Hu, Chromatographia 41 (1995) 605.
[9] Z. Zhang, Z. Hu, G. Yang, Chromatographia 44 (1997) 162.
[10] H. Zhang, T. Ding, Z. Hu, Fenxi Huaxue 25 (1997) 318.
[11] H. Zhang, Z. Hu, G. Yang, Z. Shi, H. Sun, Chromatographia 49 (1999) 219.
[12] H. Zhang, Z. Hu, G. Yang, Z. Shi, H. Sun, Anal. Lett. 30 (1997) 2327.
[13] G. Li, X. Chen, Q. Pu, M. Liu, Z. Hu, Analyst 123 (1998) 1501.
[14] S.-J. Sheu, Huaxue 54 (1996) 55.
[15] H.-K. Wu, W.-C. Chuang, S.-J. Sheu, J. Chromatogr. A 803 (1998) 179.
[16] C.Y. Wang, H.-Y. Huang, K.-L. Kuo, Y.-Z. Hsieh, J. Chromatogr. A 802 (1998) 225.
[17] K. Ohba, M. Minoura, M.M. Safarpour, G.L. Picard, H. Safarpour, Nippon Nayaku Gakkaishi, 22 (1997) 227, Chemical Abstract 128 (1998) 47444z.
[18] Y.-R. Ku, Y.-T. Lin, J.-H. Lin, K.-C. Wen, C.-H. Liao, J. Chromatogr. A 805 (1998) 301.
[19] C.-M. Liu, Y.-M. Tzeng, J. Chromatogr. A 809 (1998) 258.
[20] S.C. Smith, J.K. Strasters, M.G. Khaledi, J. Chromatogr. 559 (1991) 57.
[21] J.-P. Mercier, Ph. Morin, M. Drenx, A. Tambute, Chromatographia 48 (1998) 529.
[22] R.A. Alberty, E.L. King, J. Am. Chem. Soc. 75 (1951) 517.
[23] J.L. Beckers, F.M. Everaerts, M.T. Ackermans, J. Chromatogr. 537 (1991) 407.
[24] M. Krogh, S. Brekker, F. Tonnesen, K.F. Rasmussen, J. Chromatogr. A 674 (1994) 235.