

Determination of curcumins in turmeric by micellar electrokinetic capillary chromatography

Xiuli Lin^a, Ling Xue^b, Huiyun Zhang^b, Chenfu Zhu^{c*}

Contribution from: ^aSchool of Pharmacy, Shandong University, Wen Hua Xi Road 44, Jinan 250012, P.R.China

^bSchool of Chinese Pharmacy, Shandong University of Traditional Chinese Medicine, Jing Shi Road 53, Jinan 250014, P.R.China, ^c School of Chemistry and Chemical Engineering, Shandong University, Shan Da Nan Road 27, Jinan 250100, P.R.China

Received: September 6, 2005

Accepted (in revised form): December 17, 2005

Abstract

A rapid method for the determination of curcumins in Chinese herbal medicine turmeric by micellar electrokinetic capillary chromatography (MEKC) has been developed. Curcumin, dimethoxy curcumin and bis-dimethoxy curcumin were separated in less than 10 minutes using a 60 cm × 50 μm I.D uncoated fused-silica capillary column with a buffer consisting of 25 mM hydroxypropyl-β-CD (HP-β-CD), 10% methanol, 40 mM sodium borate and 40 mM SDS (pH 9.50). The recovery efficiencies were 95.7 - 106.3%. The calibration curves exhibited good linearity in the range of 90 - 1220 μg/mL (R = 0.9996) for curcumin, 80 - 1120 μg/mL (R = 0.9998) for dimethoxy curcumin and 80 - 1200 μg/mL (R = 0.9998) for bis-dimethoxy curcumin. Contents of curcumins in a methanol extract of turmeric sample could easily be determined by this method. The effect of pH, buffer, surfactant and organic modifier concentration on the migration behavior of the solutes were discussed.

Keywords: Turmeric, Curcumins, MEKC

Résumé

Nous avons développé une méthode rapide pour la détermination des curcumines dans l'herbe médicinale chinoise curcuma, en utilisant la chromatographie capillaire électrocinétique micellaire (MEKC). La curcumine, la déméthoxy curcumine et la bis-déméthoxy curcumine ont été séparées en moins de 10 minutes en se servant d'une colonne capillaire de silice fusionnée, non enduite, de 60 cm × 50 μm I.D, avec une solution

tampon composée de 25 mM en hydroxypropyle-β-CD (HP-β-CD), de 10% méthanol et 40 mM en borate de sodium et de 40 mM en SDS (pH 9.50). Nous avons obtenu des niveaux de recouvrement de 95.7 à 106.3%. Les droites de calibration ont affiché de bonnes linéarités dans la gamme de 90 à 1220 μg/mL (R = 0.9996) pour la curcumine, de 80 à 1120 μg/mL (R = 0.9998) pour la déméthoxy curcumine et de 80 à 1200 μg/mL (R = 0.9998) pour la bis-déméthoxy curcumine. Grâce à cette méthode, nous avons pu déterminer aisément les contenus en curcumines dans un extrait méthanol d'un échantillon de curcuma. Nous avons aussi discuté de l'effet du pH, du tampon, du surfactant et de la concentration du modificateur organique sur le comportement de migration des solutés.

Introduction

Turmeric (*Curcuma longa*) is commonly and widely used in traditional medicine in China, Japan and South-eastern Asia. Curcumins, isolated from turmeric (*Curcuma longa*) have been known to have several pharmacological properties. It is reported to be cytotoxic and tumor-reducing (1) and are natural antioxidants (2-4) Because of their antioxidant activity, curcumins have been found to exhibit anti-mutagenic (5) and anti-carcinogenic (6) properties. Moreover, curcumins have been known to inhibit phorbol ester-induced tumor promotion in mouse skin (7). It is an inhibitor of arachidonic acid metabolism (8) and is a good anti-inflammatory agent (9). Recent studies indicate its possible use as an antiviral agent against the human immunodeficiency virus (10). TLC (11,12) and high-performance liquid chromatography (HPLC) (13) methods have reported the determination of these curcumins. However, the proce-

*Author to whom correspondence should be addressed: chenfu@zhu.sdu.edu.cn

dures of TLC and HPLC usually require a large amount of organic solvents. Thus, capillary electrophoresis (CE), which is characterized by its high efficiency, low cost and easy turnover mode, has been used for analysis of many compounds (14-21).

Matthias Lechtenberg *et al.* (22) and Kailong Yuan (23) have developed capillary zone electrophoretic (CZE) method for the separation and quantification of the three curcuminoids. Wanatabe *et al.* (24) succeeded in developing micellar electrokinetic chromatography (MEKC) separation of these curcumins using a high molecular mass surfactant (a butyl acrylate/butyl methacrylate/methacrylic acid (BBMA) copolymer), but this method suffered from the disadvantage that this amphiphilic polymer is not commercially available.

The aim of the present work was to develop a viable MEKC method for the rapid separation and determination of curcumins in Chinese herbal medicine. In this work, the buffer media parameters such as the concentrations of HP- β -CD, the concentrations of background electrolyte, surfactant and pH were considered and their effects on the separation were also studied.

Experimental

Apparatus

The analysis was carried out in a home-made capillary electrophoresis system equipped with a UV detector and a power supply (The Institute of Chemistry, Jinan, China). Measurements were carried out in a fused-silica capillary of 50 μ m I.D. and 375 μ m O.D. with a total length of 60 cm and a detection length of 50 cm (Yongnian, Hebei Province China). A pH meter (Delta 320, Mettler Toledo Instrument Company, Shanghai, China) was used for pH measurement. The electrolyte was filtered through a 0.45 μ m filter before use. The operating wavelength was 258 nm and the voltage of the power supply was 20 kV.

Chemicals and reagents

The standard curcumins (their structural formulae are shown in Figure 1), β -CD, HP- β -CD and sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO, USA). Methanol, which was used as an additive to buffer electrolyte in MEKC, was of analytical grade. Sodium borate, boric acid, and sodium hydrox-

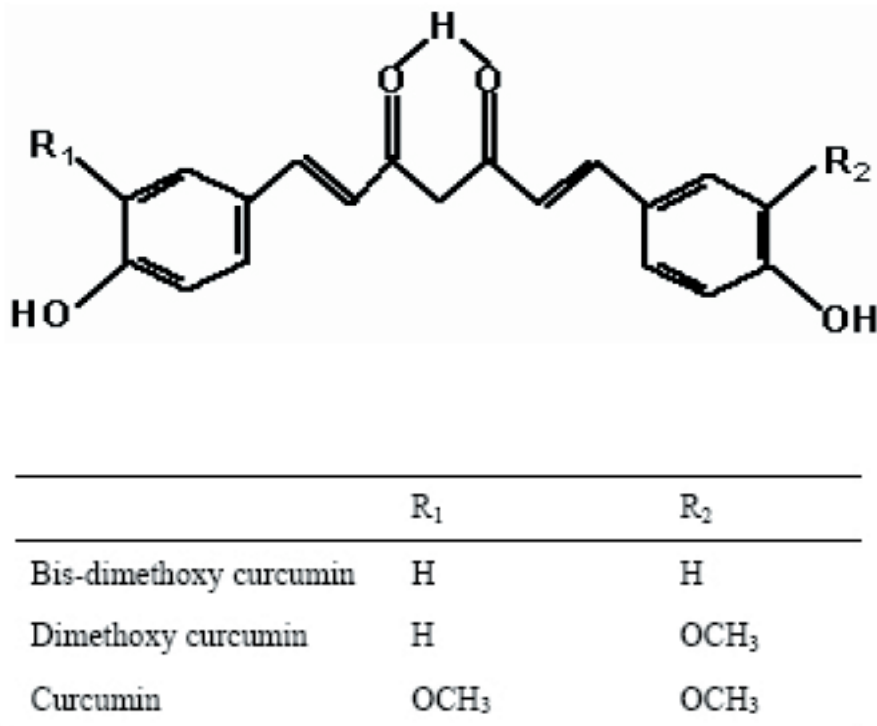


Figure 1. Structural formulae of curcumins.

ide (NaOH) are also of analytical grade. They were purchased from Jinan chemical factory. The pH of the buffer electrolyte was adjusted with 0.1 M boric acid or 0.1 M NaOH. Double-distilled water was used to prepare all solutions.

Preparation of standard solution

Stock solutions of three curcumins were prepared at 1 mg/mL in methanol. A working solution was prepared by diluting the stock solutions with water. Standards were prepared fresh and in low actinic glass to protect against photo-decomposition.

Sample preparation

Crude turmeric was obtained from two different places: Jinan Shandong and Chengde Hebei. Turmeric was ground finely, then 1.000 g was accurately weighed out, dissolved in 100 mL of water and vortexed. The sample was centrifuged at 2000 rpm and the water was decanted. 3 mL of methanol was added and the sample was vortexed again. The sample was centrifuged for 30 min at 2000 rpm. This extraction procedure was repeated three times before the extracts were combined together. The final solution was diluted with methanol to the 10-mL mark and was filtered through a 0.45 μm filter. The filtrate was injected into the capillary electrophoresis system directly at an injection voltage of 20 kV for 5 second.

Electrophoretic procedure

A new capillary was conditioned by flushing successively with 1.0 mol/L NaOH solution for 60 min, 0.1 mol/L NaOH for 30 min and double distilled water for 30 min before use. Between each injection, the capillary was rinsed with 0.1 mol/L NaOH for 2 min, double distilled water for 2 min and with the corresponding running buffer for 2 min.

Results and Discussion

Effect of HP- β -CD

The MEKC mode was used to separate the three curcumins. In preliminary experiments, the stability of the three curcumins in the buffer containing 10% methanol, 40 mM sodium borate (pH 9.50) and 40 mM SDS was tested. The results showed that the three curcumins were stable for 7h. When HP- β -CD was added to the buffer, the three curcumins were stable for 10 h.

When the buffer containing 10% methanol, 40 mM sodium borate (pH 9.50) and 40 mM SDS was used, all

the three compounds co-eluted. When different concentrations of β -CD (1, 5, 10, 15 mM) were used, all the three compounds still co-eluted. Therefore, the effect of different concentrations of HP- β -CD (10, 15, 20, 25 mM) on the resolution and migration time were studied. When the concentration of HP- β -CD was 10 mM, all the three compounds could not be baseline separated. When the concentration of HP- β -CD was 15 mM, bis-dimethoxy curcumin could not be baseline separated from dimethoxy curcumin. All three compounds were baseline separated when the concentration of HP- β -CD was 20 and 25 mM. The migration time of the three compounds all decreased with increased concentration of HP- β -CD. With respect to the baseline separation and the migration time, an optimum value of 25 mM HP- β -CD was chosen.

Effect of pH

The pH of choice in MEKC is as important as it is in CZE to ensure that weakly ionized compounds are separable. The states of ionization of compounds in the sample zone and their apparent mobility change with the buffer pH. An electrolyte system containing 25mM HP- β -CD, 40 mM $\text{Na}_2\text{B}_4\text{O}_7$, 40 mM SDS and 10% methanol at five different pH values from 8.0 – 10.0 was used in order to study the effect of pH on the separation. Figure 2 shows the dependence of the migration times of the compounds on pH. As can be seen from Figure 2 that the migration time of all compounds increased as the buffer pH increased in the range of 8.0 – 10.0. At pH 8.0 – 8.5, all three compounds almost co-eluted. At pH 9.0, bis-dimethoxy curcumin almost co-migrated with dimethoxy curcumin. At pH 9.5, all three compounds were well separated. At pH 10.0, although all three compounds were well separated, the migration time of all three compounds increased but with no increase in resolution and the peak of curcumin was broader. Therefore, the best pH of the buffer was 9.5.

Effect of sodium borate concentration

Sodium borate improved the buffer capability in the range of 8 – 10, but also showed good complex-forming characteristics (25). Therefore, sodium borate was chosen as the background electrolyte of the buffer for the separation of these compounds. In the presence of 25 mM HP- β -CD, 40 mM SDS, 10% methanol and pH 9.5, a range of 20 mM to 50 mM sodium borate was tested. The results obtained are shown in Figure 3 where the migration time was plotted against sodium borate concentrations. In Figure 3, the migration times of all

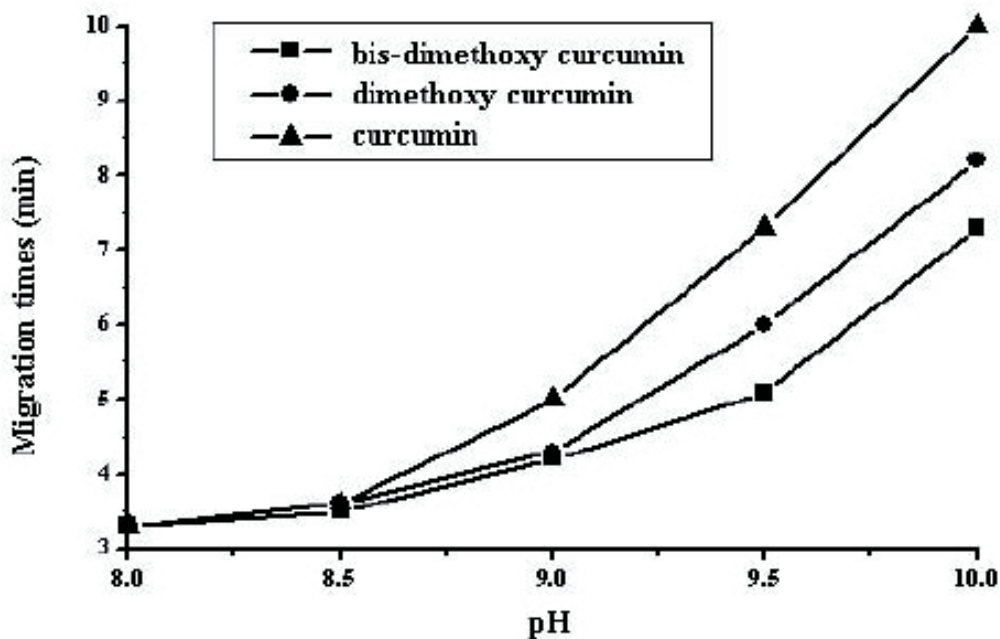


Figure 2. Effect of buffer pH on migration time. Electrophoretic conditions: 25 mM HP- β -CD, 40 mM sodium borate, 40 mM SDS, 10% methanol at with various pH values. Fused-silica capillary, 50 μ m I.D., detection length 50 cm, total length 60 cm; injection : 20 kV for 5s; temperature: 22°C; UV detection: 258 nm.

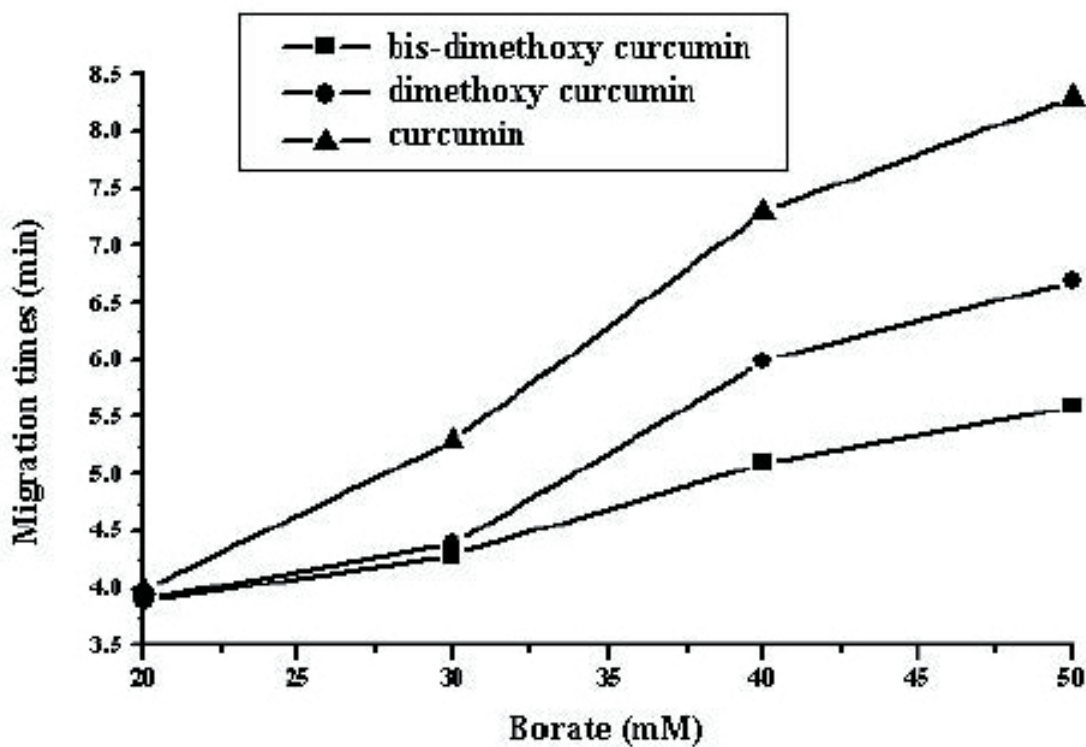


Figure 3. Migration times versus borate concentration for the three curcumins. Electrophoretic conditions: 25 mM HP- β -CD, 40 mM SDS, 10% methanol, pH 9.5 at various concentrations of borate; other conditions were set identical to those in Figure 2.

three compounds increased with increased concentrations of sodium borate. When the concentration of borate was 20 mM, the three compounds co-migrated. When the concentration of borate was 30 mM, bis-dimethoxy curcumin almost co-migrated with dimethoxy curcumin. When the concentration of borate was 40–50 mM, all the three compounds were well separated. When the concentration of borate was 50 mM, the current was high and resulted in Joule heating. In order to achieve good baseline separation and migration time, an optimum value of 40 mM borate was chosen.

Effect of SDS concentration

Electrolyte systems containing 25 mM HP- β -CD, 40 mM sodium borate and 10% methanol at five different SDS concentrations in the range of 10–50 mM at pH 9.5 were used to study the effect of SDS concentration on the separation. The results obtained are showed in Figure 4, where the migration times are plotted against SDS concentrations. One can see from Figure 4 that the migration times of the three compounds increased with increasing SDS concentration. The elution order of the compounds was: bis-dimethoxyl curcumin eluted, then

dimethoxy curcumin, and followed by curcumin. This shows that the methylated compounds are more lipophilic and therefore more soluble in micelles. When the concentration of SDS was lower than 20 mM, the three compounds were co-eluted. When the concentration of SDS was 30 mM, bis-dimethoxy curcumin almost co-migrated with dimethoxy curcumin. At a concentration of 40–50 mM, all three compounds were well separated. At a concentration of 50 mM SDS, the migration increased rapidly with no increase in resolution but unsymmetrical peaks appeared. To achieve good baseline separation, symmetrical peaks and short migration time, an optimum value of 40 mM SDS was chosen.

Effect of methanol concentration

In MEKC, the addition of organic solvent to the buffer can lengthen the migration time and widen the migration window (26,27). The organic solvent also modifies the entity of micelles. Electrolyte systems containing 25 mM HP- β -CD, 40 mM sodium borate, 40 mM SDS and pH 9.5 were used to study the effect of four methanol concentrations on the separation. The results obtained are given in Figure 5. When the concentration of metha-

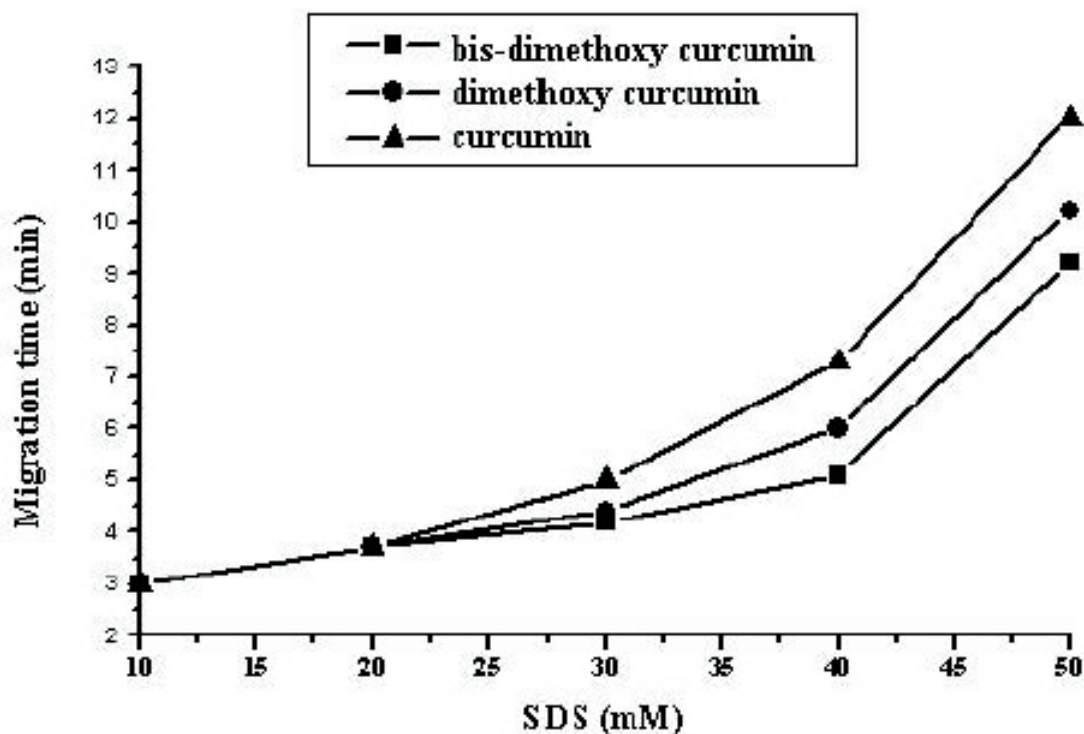


Figure 4. Effect of SDS concentration on migration time. Electrophoretic conditions: 25 mM HP- β -CD, 40 mM sodium borate, 10% methanol, pH 9.5 at various concentrations of SDS. Other conditions were as set identical to those Figure 2.

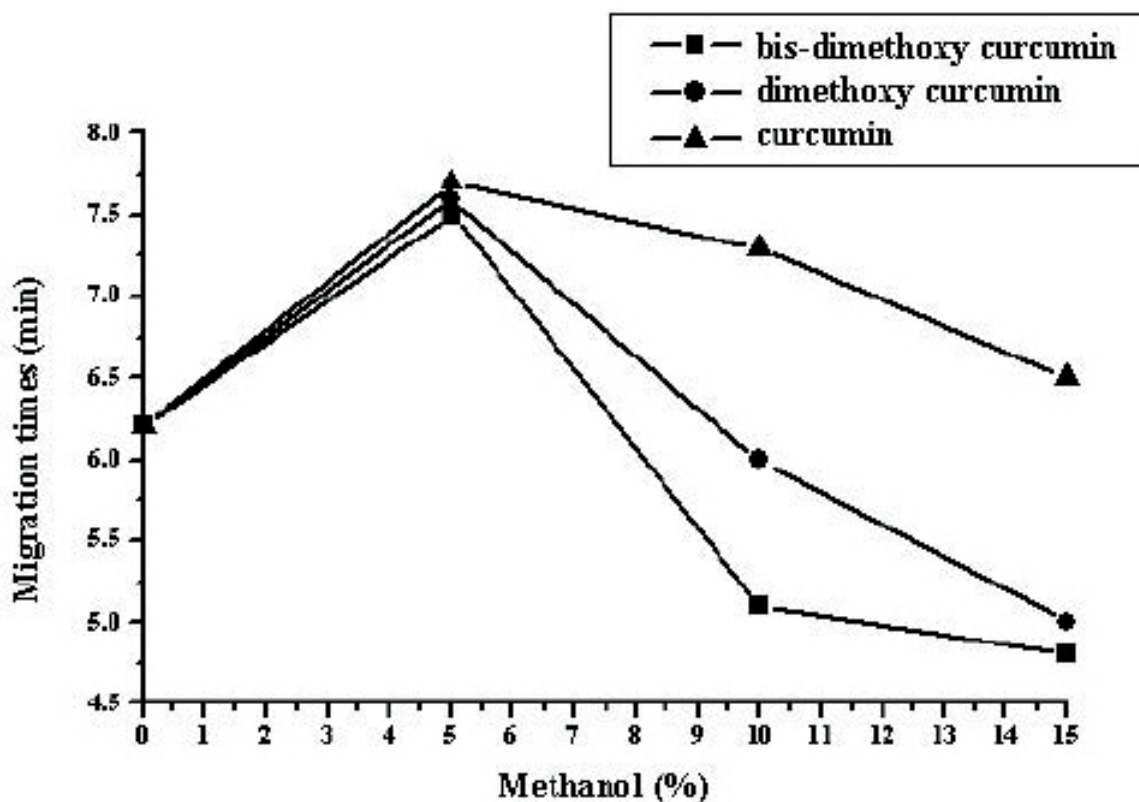


Figure 5. Effect of methanol concentration on migration time. Electrophoretic conditions: 25 mM HP- β -CD, 40 mM sodium borate, 40 mM SDS, pH 9.5 at with various concentrations of methanol. Other conditions were set identical to those in Figure 2.

nol was in the range of 0 – 5%, the three compounds almost co-migrated and the migration times of the three compounds increased. This behavior can be explained by the decreased electro-osmotic flow (EOF). When the concentration of methanol changed from 10% to 15%, the migration times of the three compounds decreased. This might be attributed to the increased partitions of the diphenolics into the aqueous phase, which caused the three compounds to migrate faster. At 10% methanol, the three compounds were well separated. At 15% methanol, bis-dimethoxy curcumin and dimethoxy curcumin were not baseline separated, so an optimum value of 10% methanol was chosen.

Calibration graphs for curcumins

Calibration graphs (peak-area ratio, y , vs. concentration, x , mg/mL) were constructed in the range 0.09 – 1.20 mg/mL for bis-dimethoxy curcumin, 0.08 – 1.12 mg/mL for dimethoxy curcumin, and 0.08 – 1.22 mg/mL for curcumin. The regression equations of these curves and their correlation coefficients were calculated as fol-

lows: bis-dimethoxy curcumin, $y = 423.15 - 0.28x$ ($r = 0.9998$, $n = 6$); dimethoxy curcumin, $y = 689.23 - 0.17x$ ($r = 0.9998$, $n = 6$); curcumin, $y = 536.55 - 0.31x$ ($r = 0.9996$, $n = 6$).

System suitability and recovery of method

The method was validated for reproducibility of the migration time and the peak area of the analytes. The relative standard deviations (R.S.D.) of the migration time and the peak area of each of the peaks for six replicate injections were (1.50 – 2.08%) and (3.15 – 4.56%), respectively. The LOD was determined at a signal-to-noise ratio of 3 and was found to be 0.012 mg/mL. Accuracy of the method was determined by adding suitable amounts of the analytes of known concentration to plant extracts. Recovery experiments were performed four times by adding 0.25 mg/mL bis-dimethoxy curcumin, demethoxy curcumin and curcumin into the extract of turmeric obtained from Jinan and Chengde. The recoveries were 103.2, 97.8, 95.7% and 101.8, 98.4, 106.3%, respectively.

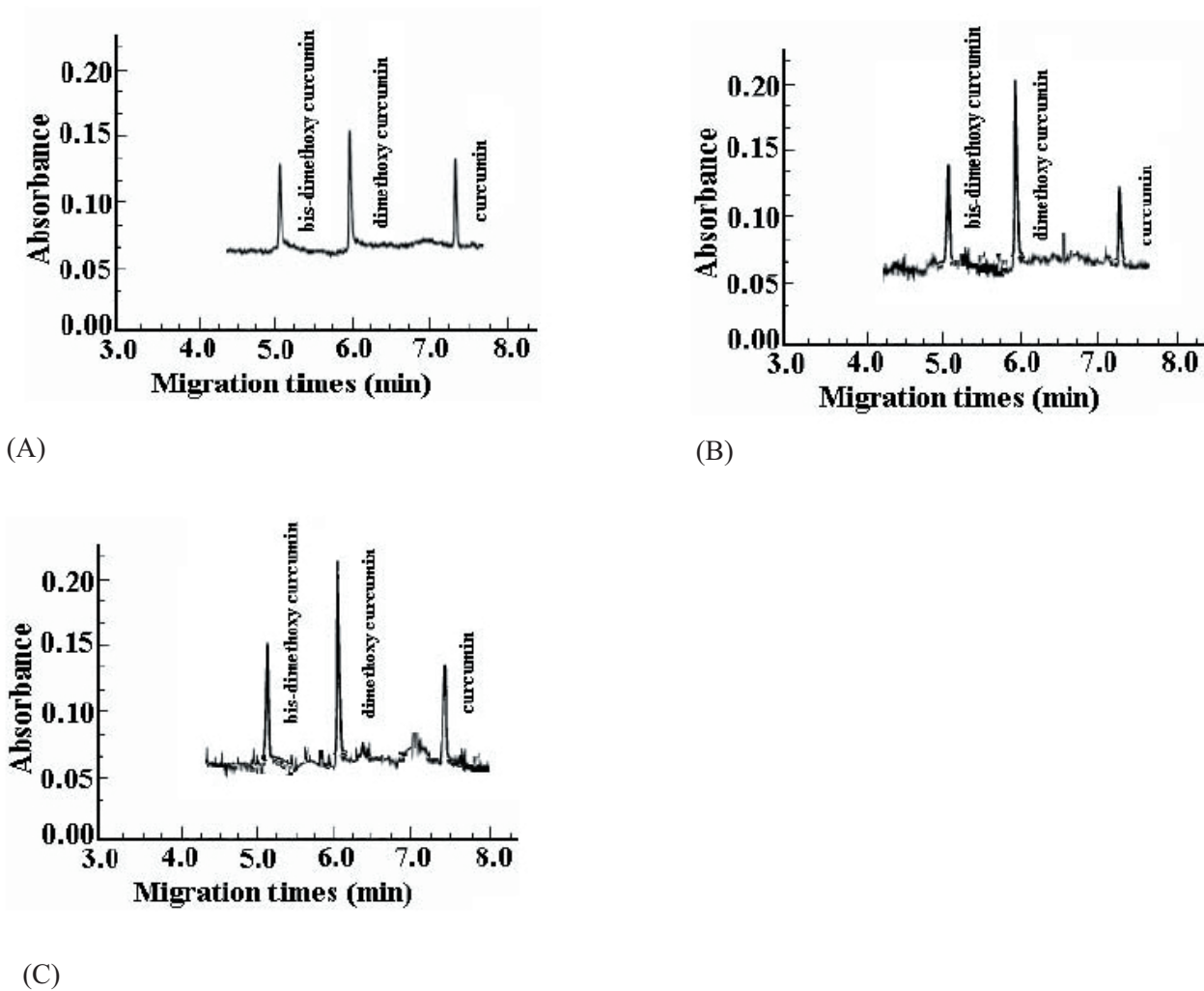


Figure 6. The electropherograms of the standards mixture solution and the real samples. (A) The standards mixture, (B) The sample from Jinan, (C) The sample from Chengde. Electrophoretic conditions: 25 mM HP- β -CD, 40 mM sodium borate, 40 mM SDS, 10% methanol, buffer pH 9.5; fused-silica capillary, 50 μ m I.D., detection length 50 cm, total length 60 cm; injection : 20 kV for 5s; temperature: 22°C; UV detection: 258 nm.

Table 1. Quantitative results of curcumins content in turmeric.

Sample	Component	Content of Curcumins (%)	RSD (%)
Turmeric from Jinan	bisdimethoxy curcumin	1.10	4.31
	dimethoxy curcumin	1.42	3.57
	curcumin	0.98	2.52
Turmeric from Chengde	bis-dimethoxy curcumin	1.25	3.91
	dimethoxy curcumin	1.53	3.26
	curcumin	1.30	1.97

Determination of curcumins in turmeric

The tested solutions of turmeric were analyzed under the selected conditions. The electrophoretic graphs were obtained as shown in Figure 6A, B and C. The content of each curcumin in turmeric was obtained as shown in Table 1. The results were the same as the results used by the HPLC method carried out in our lab.

Conclusion

MEKC is a viable method for the separation of curcumins. In the present work, a rapid method for the separation and determination of curcumins in crude turmeric by MEKC is described. The proposed method is simple, economic, rapid and robust.

Acknowledgements

This work was supported by the Doctoral Program Foundation of Shandong Natural Science (No. 03BS046).

References

1. K.K. Soudamini, R. Kuttan, *Indian J. Pharmacol.*, **20**, 95 (1988).
2. I. Nishigaki, R. Kuttan, H. Oku, F. Ashoon, H. Abe, K. Yagi, *J. Clin. Biochem. Nutr.*, **13**, 23-29 (1992).
3. K.K. Soudamini, M.C. Unnikrishnan, K.B. Soni, R. Kuttan, *Indian J. Physiol. Pharmacol.*, **36**, 239 (1992).
4. K. Elizabeth, M.N.A. Rao, *Int. J. Pharm.*, **58**, 237 (1990).
5. M. Nagabhushan, A.J. Amonkar, S.V. Bhide, *Food Chem. Toxicol.*, **25**, 545 (1987).
6. K.K. Soudamini, R. Kuttan, *J. Ethnopharmacol.*, **27**, 227 (1989).
7. M.T. Huang, R.C. Smart, C.Q. Wong, A.H. Conney, *Cancer Res.*, **48**, 5941 (1988).
8. A.H. Conney, T. Lysz, T. Ferraro, T.F. Abidi, P.S. Manchand, J.D. Laskin, M.T. Huang, *Adv. Enzyme Regul.*, **31**, 385 (1991).
9. A. Mukhopadyay, N. Basu, N. Ghatak, P.K. Gujral, *Agents Actions*, **12**, 508 (1982).
10. C.J. Li, L.J. Zhang, B.J. Dezube, C.S. Crumpacker, A.B. Pardee, *Proc. Natl. Acad. Sci.*, **90**, 1839 (1993).
11. M.J. Ansari, S. Ahmad, K. Kohli, J. Ali, R.K. Khar, *J. Pharma. Biomed. Anal.*, **39**, 132 (2005).
12. L. Péret-Almeida, A.P.F. Cherubino, R.J. Alves, L. Dufossé, M.B.A. Glória, *Food Res. Int.*, **38**, 1039 (2005).
13. H.H. Tonnesen, J. Karlsen, *J. Chromatogr.*, **259**, 367 (1983).
14. N.M. Vizioli, M.L. Russel, C.N. Carducci, *Anal. Chim. Acta*, **514**, 167 (2004).
15. X.L. Lin, C.F. Zhu, A.Y. Hao, *Anal. Chim. Acta*, **517**, 95 (2004).
16. A.G. Jensen, S.H. Hansen, *J. Pharm. Biomed. Anal.*, **27**, 167 (2002).
17. X.L. Lin, C.F. Zhu, A.Y. Hao, *J. Chromatogr. A*, **1059**, 181 (2004).
18. E. Stellwagen, R. Ledger, *Anal. Biochem.*, **321**, 167 (2003).
19. D.H. Na, C.K. Cho, Y.S. Youn, Y. Choi, K.R. Lee, S.D. Yoo, K.C. Lee, *Toxicol.*, **43**, 329 (2004).
20. S.W. Sun, H.M. Tseng, *J. Pharm. Biomed. Anal.*, **36**, 43 (2004).
21. Y.Q. Yu, P.L. Ding, D.F. Chen, *Anal. Chim. Acta*, **523**, 15 (2004).
22. L. Matthias, Q. Bettina, N. Adolf, *Phytochem. Analysis*, **15**, 152 (2004).
23. K.L. Yuan, Q.F. Weng, H.Y. Zhang, J.H. Xiong, G.W. Xu, *J. Pharma. Biomed. Anal.*, **38**, 133 (2005).
24. T. Wanatabe, T.K. Mazumder, A. Yamamoto, S. Nagai, S. Terabe, *Nippon Shokuhin Kagaku Kaishi*, **47**, 780 (2000).
25. S.W. Sun, S.S. Lee, L.Y. Chen, C.K. Chen, *J. Chromatogr. A*, **767**, 277 (1997).
26. J. Gorse, A.T. Balchunas, D.F. Swaile and M.J. Sepaniak, *J. High Res. Chromatogr.*, **11**, 554 (1988).
27. A.E. Bretnall, G.S. Clarke, *J. Chromatogr. A*, **716**, 49 (1995).