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Capillary electrophoresis analysis of mangiferin extracted from *Mangifera indica* L. bark and *Mangifera persiciformis* C.Y. Wu et T.L. Ming leaves

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Abstract

The flavonoid compound mangiferin is found in the leaves, stem bark, fruit peels and root of *Mangifera indica* L. and in many other herbal species with many potential pharmacological properties. We have established an analytical method of mangiferin extracted from *M. indica* L. bark and *Mangifera persiciformis* C.Y. Wu et T.L. Ming leaves utilizing CZE. An electrolytic buffer containing 0.05 M borate buffer, pH 7.4 with methanol (1:0.3, v/v) was deemed suitable for mangiferin analysis. An ideal mangiferin electropherogram with a migration time at approximately 10.50 min was obtained. Repeatability tests showed that the R.S.D.s for both intra- and inter-day migration time and peak area for all manigferin sources tested were less than 4%. The linearity range of this method was 5–1000 μg/ml. The detection limit of this method was 1.5 μg/ml. Quantitative analysis of mangiferin was also performed with this method. The accuracy of quantitation at 10, 500 and 1000 μg/ml of control mangiferin were 99.00, 99.38 and 99.14%, respectively ($n = 10$). The repeatability of quantitation (R.S.D.) was below 3%. Our results demonstrated that CZE is a simple and reliable method in mangiferin analysis and more studies are needed to detect other mangiferin resources, such as clinical biological samples, in pharmacology and pharmacokinetic studies.

Keywords: Capillary electrophoresis; Flavonoid; Mangiferin; *Mangifera indica*; *Mangifera persiciformis*

1. Introduction

The flavonoid compound mangiferin (Fig. 1) (2-beta-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthen-9-one), molecular formula: $C_{19}H_{18}O_{11}$, occurs in the leaves, stem bark, fruit peels, and roots of *Mangifera indica* L. and other higher plants [1–4]. Studies on the pharmacological properties of mangiferin reveal that this flavonoid compound possesses antitumor [5–8], antiviral [7,9], antioxidant [10–15], antidiabetic [16], immunomodulatory [3,17–20], and vascular modulatory activity [21]. High-performance liquid chromatography (HPLC) alone or HPLC combined with mass spectrometry [22–26] is widely employed for determination of mangiferin quality and quantity in pharmaceutical, pharmacokinetic or pharmacological studies. Although HPLC is a reliable method and LC–MS has very powerful resolution for the detection of mangiferin and other flavonoid compounds, they remain a costly procedure. Alternatively, capillary electrophoresis (CE) has powerful resolving ability and is a simpler, more efficient, and less costly procedure in comparison to HPLC. The most successful techniques for flavonoid compound resolution using CE are micellar electrokinetic capillary chromatography (MEKC) and capillary zone electrophoresis (CZE) [27] while the latter is simpler. Some researchers reported recently the successful use of CE for separation and determination of flavonoid compounds extracted from herbal plants and wine [27–37]. We previously reported a preliminary, but not yet fully optimized or validated, method for the analysis of mangiferin extracted from *M. indica* L. leaves utilizing CZE [38]. Here, we report an optimized and updated analysis of mangiferin extracted from *M. indica* L. bark (commercial) and *Magifera persiciformis* C.Y. Wu et T.L. Ming leaves (lab-extracted) using CZE.

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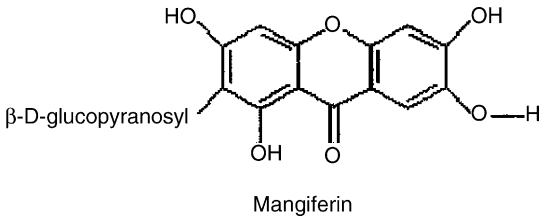

Mangiferin

Fig. 1. Structure of mangiferin.

2. Experimental methods

2.1. Reagents

HPLC grade methanol was purchased from Siyou Chemical Company (Tianjin, China). All other chemicals and reagents were of analytical reagent grade purchased from chemical companies in China.

2.2. Mangiferins

Commercially available Mangiferin from *M. indica* L. stem bark was purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA), Cat. No. M3547, Lot No. 79H0548, with purity >99% by thin layer chromatography. Mangiferin from *M. persiciformis* C.Y. Wu et T.L. Ming leaves was extracted in our lab using a previously reported protocol [39]. In brief, the fresh leaves were collected from local *M. persiciformis* C.Y. Wu et T.L. Ming trees in Nanning city in Guangxi province of southern China. The plant was authenticated at the Guangxi College of Traditional Chinese Medicine. The leaves were cleansed by rinsing with tap water, and then air-dried at room temperature. They were then milled into a fine powder and four kilograms of this powder were extracted three times with 95% ethanol in a 10-l percolator for 24 h each at room temperature. Filtrate collection was followed by ethanol harvesting with vacuum evaporation. The condensed marc was resuspended in distilled water. The dissolved marc was further processed with vacuum evaporation using petroleum ether (60–90 °C) and ethyl acetate consecutively. Hundred grams of marc from the petroleum extraction (extract A), 46 g of marc from the ethyl acetate extraction (extract B) and 120 g of water phase marc (extract C) were obtained at this stage. Extract C was re-extracted with hot ethanol (55%) four times, then combined with the extractions from extraction C. The combined ethanol solution was then heated until near boiling and then filtered through 3 M paper filter (Millipore). The filtrates were collected and dried at 50 °C in an oven. Lastly, 7.5 g of golden mangiferin (m.p. 271–273 °C) were obtained and further characterized using TLC, HPLC, IR, ^{1}H NMR, LC–MS, and spectrophotometry scanning (data not shown). The mangiferin obtained at this stage contains mangiferin (approximately 97.39%, see below), which was characterized by means of the methods described above. This product was used in our on-going pharmacological study. To obtain a control pure mangiferin, the above mangiferin product was further purified by a silica gel column chromatography in our lab and was further characterized by ^{1}H NMR and LC–MS.

2.3. Instrumentation

A Beckman P/ACE 5000 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with an UV detector and a laser-induced fluorescence detector and System Gold software was used in the mangiferin CE analysis. A 67 cm (50 cm to the detector) length, 75 μm I.D., 375 μm OD uncoated fused silica capillary (Beckman) was used in electrophoresis. The capillary was activated at ambient temperature before use by rinsing sequentially with 1 mol/L NaOH for 2 h, water for 30 min, 1 mol/L HCl for 2 h, and then water for 30 min. The treated capillary was equilibrated with electrolyte buffer for 1 h before injection and pre-equilibrated by applying the voltage on the capillary for 10 min. The capillary was also rinsed with running buffer for 2 min between each run.

2.4. Preparation of control and sample mangiferin solutions

Control mangiferin was weighed and dissolved in 70% methanol solution and then sonicated five times for 5 s each. Serial dilutions of concentrations ranging 10–1000 μg/ml of the control mangiferin were prepared. Sample mangiferin was prepared at a concentration of 375 μg/ml and dissolved as described above. All solutions were filtered by a 0.22 μm filter (Millipore, Bedford, MA, USA) and stored at room temperature until needed.

2.5. Electrophoresis

Free solution capillary zone electrophoresis was employed in this study. After the optimization analysis, the electrophoretical buffer of a filtered mixture containing 0.05 M of borate, pH 7.4 and methanol (1:0.3, v/v) was adopted as CZE running buffer for mangiferin analysis. The capillary temperature was set to 25 °C and samples were injected by applying injection pressure for 6 s. The separation voltage was set to 20 kV. The detection wavelength was set at 254 nm according to previous report [24] and our spectral scanning results of mangiferins (see below).

2.6. Linearity and detection sensitivity

In order to test the linearity of the detector response in this method, different concentrations of control mangiferin were used under the CZE conditions reported above. The sensitivity (limit of detection) was estimated by means of the baseline noise method when the signal-to-noise ratio was 2.

2.7. Calibration and quantitation

Control mangiferin was used for construction of the calibration curve. The curve was prepared by diluting a control stock of mangiferin solution with 70% methanol resulting in six different concentrations ranging 10–1000 μg/ml. The slope, coefficient determination, and concentration were obtained accordingly. The purity of our sample mangiferin was determined in this report.

2.8. Accuracy and repeatability test

Three different concentrations at 10, 500, 1000 μg/ml of control mangiferin were used in the test ($n = 10$). For the intra-day repeatability test, we injected the same sample 20 times during the period of one day. For the inter-day repeatability test, we prepared the same concentration of mangiferin solution each day and injected the sample twice a day to obtain an average and then performed the same assay for a total of 10 days. R.S.D.s for migration times and peak areas were obtained by statistical analysis.

3. Results and discussion

3.1. Spectral scanning of mangiferin

In order to characterize and determine the purity of the mangiferin produced in our laboratory, and to set a suitable CZE detection wavelength, we performed spectral scanning for all mangiferins including a control mangiferin sample using a Beckman DU-640 spectrophotometer (Beckman Instruments, Fullerton, CA, USA) prior to CE analysis. The spectrum (in ethanol) for mangiferins were as follows: control mangiferin: 241, 258, 316, 366; commercial mangiferin: 238, 258, 316, 368 (the spectrum of commercial mangiferin in manufacturer's data sheet was: 241, 258, 317 and 370 in methanol). Lab-extracted mangiferin: 240, 259, 316, 366. Base on these parameters and our previous CZE data together with the literature reports of HPLC analysis of mangiferin, we set the CZE detection wavelength at 254 nm.

3.2. Effect of borate concentration on separation

In order to understand the influence of borate concentration on mangiferin CZE results, we tested several different borate buffer concentrations including 0.02, 0.025, 0.05 and 0.1 M. All other electrophoresis conditions were set as previously described. We found that all of the concentrations produced adequate resolution, but the borate buffer concentration positively correlated with migration time. The increase in migration time may be secondary to a decreased EOF since this effect is directly related to the decrease of the zeta potential at the capillary wall–solution interface. The total migration times of the analytes in this study increased from 9.71 min (0.02 M) to 10.80 min (0.1 M) (Table 1). Considering peak shape and migration time together, we chose 0.05 M borate buffer for further optimization analysis.

Table 1
Effect of borate buffer concentration on separation ($n = 10$)

Concentrations (M)	Migration times (min ± S.D.)
0.02	9.72 ± 0.05
0.025	10.52 ± 0.06
0.05	10.61 ± 0.04
0.1	10.83 ± 0.05

3.3. Effect of pH on separation

We also tested the impact of 0.05 M borate buffer pH on mangiferin CZE analysis. Seven p*H*a values (p*H*a 6.4, 7.4, 8.4, 9.4, 10.4, 11.4 and 12.4) were used in this study. All seven differing p*H*a buffers contained the same concentrations of methanol that were described above. With p*H*a 6.4, the resolving effect was poor, while at p*H*a 9.4, 10.4 and 12.4, neither baseline nor resolution improved (noisy baseline). Furthermore, the electropherogram became unstable at high p*H*a, varying from time to time. The results indicate that the p*K*a of mangiferin is around neutral p*H*a. When an acidic buffer p*H*a (i.e. p*H*a 6.4) was applied, the hydrogen in this polyphenol did not ionize completely, resulting in poor resolution. On the other hand, at very basic p*H*a, the phenolic hydroxyl groups in the compound became over ionized resulting in high current generation and Joule heating, the latter of which may destroy the structure of the solute, resulting in an unstable electropherogram. Thus, we selected borate buffer, p*H*a 7.4, for further analysis.

3.4. Effect of organic solvent on separation

We also tested the effect of methanol content in the electrolytic buffer on mangiferin CZE analysis. Various borate buffers to methanol ratios (1:0.1, 1:0.2, 1:0.3, 1:0.4 and 1:0.5, v/v) were tested in this study. The purpose of using organic solvent in CE was to enhance the solubility of highly hydrophobic analytes in the aqueous phase and to enlarge the migration time window hence improving the separation efficiency. We found that when the methanol concentration increased, the migration time increased significantly as well, from 4.90 min (pure borate buffer) to 15.34 min (1:0.5) (Table 2). Theoretically, pure borate buffer in this test could gain resolution and baseline. Considering the hydrophobic property of mangiferin and the relatively short migration time in the electropherogram, we determined that the results with pure borate buffer were unstable thus increasing the risk of analytical failure. All ratios except 1:0.3 presented with either peak shape or migration time issues, so we chose the 1:0.3 buffer constituent for use in the optimization test.

3.5. Effect of voltage on analysis

We also tested the effect of voltage on analysis in this study. Various voltages beginning with 10–30 kV in 5 kV intervals were applied. The results show that the voltage had a negative correlation with migration time. Thus it further reflects that increasing

Table 2
Effect of organic solvent (borate buffer:methanol) on separation ($n = 10$)

Composition (v/v)	Migration times (min ± S.D.)
Pure borate buffer	4.83 ± 0.03
1:0.1	6.49 ± 0.09
1:0.2	9.19 ± 0.05
1:0.3	10.53 ± 0.05
1:0.4	13.05 ± 0.07
1:0.5	15.35 ± 0.04

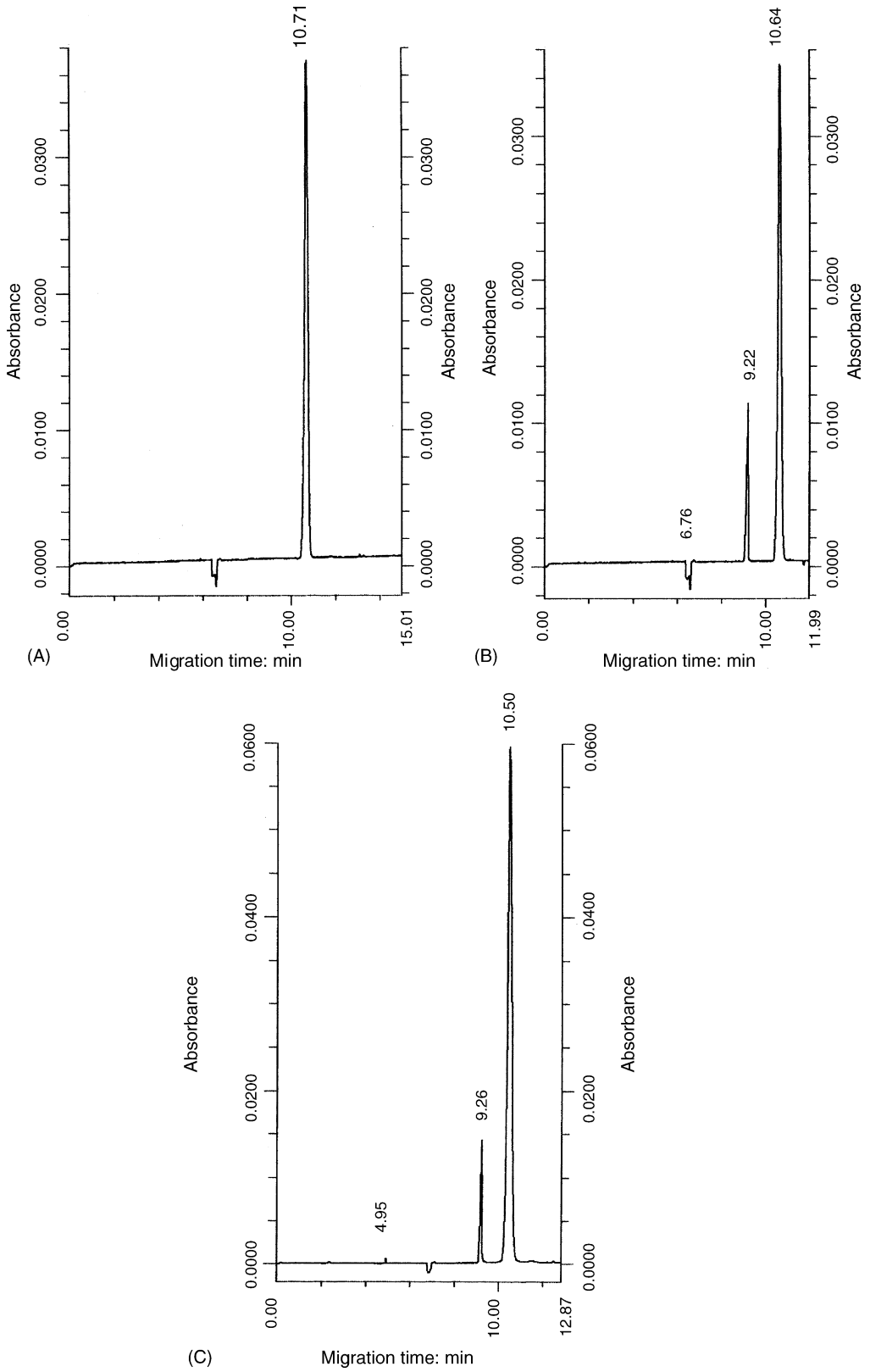

Fig. 2. CZE electropherograms of mangiferins. Electrophoresis conditions. Instrument: Beckman P/ACE 5000 capillary electrophoresis system equipped with an UV detector and a laser-induced fluorescence detector with System Gold software; electrolytic buffer: 0.05 M, pH 7.4 borate buffer:methanol (1:0.3, v/v); voltage: 20 kV; capillary temperature: 25 °C; uncoated fused silica capillary: 67 cm (50 cm to the detector) length, 75 μm ID, 375 μm OD; detection wavelength: 254 nm. Mangiferins (375 μg/ml) were used in the test. (A) Control mangiferin; (B) commercial mangiferin; (C) lab-extracted mangiferin.

high field strength (high voltage) enhances the EOF and electrophoretic velocity giving the short analysis time. This effect can be used to reduce diffusion, which causes band broadening under certain circumstances. We found that in light of peak shape and migration time, the best voltages were 25 and 30 kV, however, higher voltages that induced high current and Joule heating could harm the instrument since the system's maximal voltage setting were approached. Accordingly, we selected 20 kV as our analysis parameter.

3.6. CZE analysis of mangiferin extracted from Mangifera indica L. bark and Mangifera persiciformis C.Y. Wu et T.L. Ming leaves

We performed analysis of mangiferin from *M. indica* L. bark (commercial mangiferin) and *M. persiciformis* C.Y. Wu et T.L. Ming leaves (lab-extracted mangiferin) and compared it with control mangiferin using CZE. Under the electrophoresis conditions described above and with 0.05 M borate buffer (p*H*a 7.4) containing methanol (1:0.3, v/v), we found that the electropherograms of commercial mangiferin and our lab-extracted mangiferin were not significantly different (Fig. 2). All mangiferins were used at a concentration of 375 μg/ml in the resolving comparison test. The resolving was efficient and the peaks were very sharp and clear. The baseline of the electropherogram was very smooth. The electropherograms of both mangiferin sources contained two peaks, one front minor peak and another major peak follows. According to the control mangiferin ^{1}H NMR, LC–MS and CZE results, the rear major peak represents mangiferin while the minor front peak is an unidentified compound.

3.7. Linearity, detection limit and repeatability of the analysis

Under the CZE conditions described above, the linearity of this method was between 5 and 1000 μg/ml, $y = 20.71996x + 0.461884$ ($n = 5$), the correlation coefficient was 0.99862, R.S.D. = 0.25%. The detection limit of this method was 1.5 μg/ml. The repeatability test results for migration time and peak area for both intra- and inter-day were within acceptable limits. R.S.D.s were less than 5% as shown in Table 3.

Table 3
Intra- and inter-day R.S.D.s of migration times and peak areas in mangiferin CZE

Analytes	Intra-day R.S.D. ($n = 20$)		Inter-day R.S.D. ($n = 10$)	
	Migration time	Peak area	Migration time	Peak area
Control MF	0.33	2.22	0.37	2.79
Commercial MF	0.43	3.10	0.39	3.31
Lab-MF	0.39	2.13	0.33	2.93

MF: mangiferin.

3.8. Quantitation and accuracy

We determined that the purity of our mangiferin was 97.39%. The quantitation repeatability of 20 injections of 375 μg/ml of our mangiferin was 365.2 μg ± 0.016, R.S.D. 3.41%. Furthermore, three different concentrations at 10, 500 and 1000 μg/ml of control mangiferin were determined for accuracy testing of this method ($n = 10$). The accuracies of three concentrations were 99.00, 99.38 and 99.14%, respectively. All R.S.D.s were below 3%.

4. Conclusions

Here, we have developed and validated an optimized CZE method for the analysis of mangiferin extracted from *M. indica* L. bark and from *M. persiciformis* C.Y. Wu et T.L. Ming leaves. Our results show that this method is relatively fast, uncomplicated, less costly, stable, and reproducible. Therefore, it is in our interest to utilize the CZE method in subsequent experiments for mangiferin analysis in clinical biological samples, and both pharmacological and pharmacokinetic studies involving mangiferin.

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