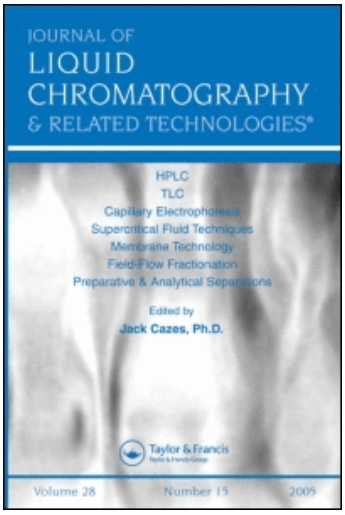

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Capillary Electrophoretic Method for Determination of Matrine in Caco-2 Cell Medium

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ABSTRACT

A capillary electrophoretic (CE) method has been developed for the analysis of matrine in a biological medium. The optimized run buffer solution contained 200 mM *tris*(hydroxymethyl)aminomethane, 40 mM sodium dihydrogen phosphate, and 20% 2-propanol, adjusted to pH 5.8 using phosphoric acid. A voltage of 25 kV was applied across a capillary (50 μm × 40 cm, distance to detector: 10.2 cm) for CE separation. Each analysis was completed in less than 5 min. Linearity of matrine calibration in the concentration range of 3.2–185 μg/mL (at the detection wavelength of 214 nm) was excellent ($R^2 = 0.9994$). The run-to-run repeatability ($n = 3$), as expressed by the relative standard deviation (RSD), was found to be better than 4.0%. The limit of detection was

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estimated to be 3.2 μg/mL. This CE method was employed to determine matrine in Caco-2 cell media after transport of matrine through the cells.

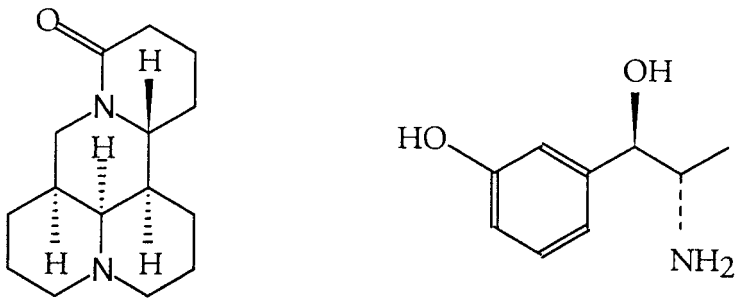
Key Words: Capillary electrophoresis; Matrine; Caco-2 cell.

INTRODUCTION

Matrine is a naturally occurring alkaloid and its chemical structure is given in Fig. 1(a).[1] It is found in the roots of various herbs such as *Sophora tonkinensis* Gapnep (Shandougan), *S. alopecuroides* L. (Kudouzi or Kugancao), and *S. flavescens* Ait. (Kushen).[2,3] This herb-derived compound has been reported to have several pharmacological effects, such as anti-inflammatory,[4] anti-ulcer,[5] anti-nociceptive (pain reduction),[6] and anti-tumor[7–9] activities.

Since matrine can be a potential drug, there is a need to perform transport study of matrine to understand its absorption in the intestines using intestinal Caco-2 cells.[10,11] These cells, which consist of the apical and basolateral sides with respect to the intestinal lumen, can be used to study the transport of drugs in the direction from the apical to the basolateral side (drug absorption), and reverse (drug excretion). Therefore, a bioanalytical method is required to separate and analyze the drug in biological fluids obtained in both sides (apical and basolateral) of the cells.

So far, analysis of matrine in herbal extracts has been carried out using HPLC[12,13] or CE.[14,15] Nevertheless, only HPLC[16] and GC[17] have been employed to analyze matrine in biological fluids, such as human and rabbit



(a) (b)

Figure 1. Chemical structures of (a) matrine and (b) metaraminol.

sera, respectively. However, CE, which has not been used to analyze matrine in biological media, is a desirable method because the aqueous samples can be directly analyzed without sample preparation, such as derivatization or extraction. Accordingly, in this paper, a CE separation method was first developed and the linearity and precision of the calibration were evaluated. Then, the method was applied to analyze matrine in the biological media on the apical side of Caco-2 cells. However, the complete drug transport study is beyond the scope of this paper.

EXPERIMENTAL

Materials

Matrine (CAS# 519-02-8) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard matrine solutions of known concentrations (3.2, 16.1, 64.2, 128.4, 184.6 μg/mL) were prepared in Hanks' balanced salt solution (HBSS) (Gibco-BRL), which was obtained from VWR Canlab (Mississauga, ON, Canada). HBSS, which was supplemented with 25 mM HEPES, adjusted to pH 7.4, was used because it was the same medium used in the subsequent drug transport study. Metaraminol, which was purchased from Sigma–Aldrich (Mississauga, ON, Canada), was used as an internal standard (IS). This compound, as shown in Fig. 1(b), was prepared as a 1 mg/mL stock solution in HBSS.[15] A 5 μL metaraminol solution was added in each standard solution, to be made up to a total volume of 120 μL.

Apparatus

Uncoated fused silica capillary tubes of o.d. 375 μm and i.d. 50 μm were purchased from Polymicro (Phoenix, AZ). The total capillary length used was 40 cm, with the distance between the inlet and detector being 10.2 cm. A CE system purchased from Beckman-Coulter (MDQ, Fullerton, CA) was used for CE analysis. A photodiode array (PDA) detector was employed for detection.

Culture of Caco-2 Cells

Caco-2 cells (HTB-37) were obtained from American Type Culture Collection (ATCC, Rockville, MD). These cells were grown in 12-well plates (Falcon), which were obtained from VWR Canlab. The cell culture

medium used was Dulbecco's modified Eagle's medium (DMEM), supplemented with non-essential amino acid (10 mM) (Gibco-BRL), sodium bicarbonate, fetal calf serum, penicillin–streptomycin (Gibco-BRL). All these materials were obtained from VWR Canlab. The subculturing procedure, which was performed every 7 days was carried out in a Class A/B3 biosafety cabinet (NU-425-400, NuAire, Plymouth, MN). All cell cultures were maintained at 37°C, 5% CO_2, and 95% relative humidity in an air-jacketed CO_2 incubator (NU-5500, NuAire)

After the cells have attained 80% confluence, they were treated with trypsin–EDTA (2.5 g trypsin, 0.38 g EDTA–4Na per liter in HBSS without Ca^{2+} and Mg^{2+}) (Gibco-BRL) to detach the cells. Then, 5 mL of the cell suspension (at a cell density of 1×10^6 per mL) was transferred onto cell culture inserts (Falcon, VWR Canlab).

A volt-ohmmeter (for resistance measurement across the cell layer) equipped with a special "chopstick" electrode was purchased from World Precision Instruments (WPI, Sarasota, FL). With this instrument, the cell integrity was confirmed by noting that the measured resistance increased to a constant value after 13 days.

Development of the CE Separation Method

The standard solutions (in HBSS) were placed in the autosampler of the CE system for analysis. Between runs, the capillary was flushed with water (2 min), 0.1 M NaOH (5 min), water (2 min), and then filled with run buffer (1 min). The optimized run buffer contained 200 mM *tris*(hydroxymethyl)-aminomethane (tris), 40 mM sodium dihydrogen phosphate, and 20% 2-propanol, adjusted to pH 5.8 using phosphoric acid. Sample introduction was carried out hydrodynamically at 0.5 psi for 10 sec. Separation was carried out at 25 kV for 5 min. Electric current was determined to be between 78 and 86 μA. Although UV detection (190–300 nm) was accomplished by a PDA detector, the electropherograms were presented at 190, 214, and 254 nm.

Treatment of Matrine to Caco-2 Cells and Subsequent CE Analysis

Solutions of three different concentrations of matrine (i.e., 199, 398, or 796 μg/mL) were prepared in HBSS for treatment to Caco-2 cells.

To study the transport of matrine from the apical side to the basolateral side (i.e., drug absorption), a matrine solution (0.4 mL) was placed in the top (apical) chamber, and 1.5 mL of HBSS was placed in the bottom (basolateral)

chamber. After 0.5 hr, 150 μL of the solution was sampled from the basolateral chamber and put in Eppendoff tubes. Then, 150 μL of HBSS was replenished to the basolateral chamber. After 1, 1.5, 2.5, and 5 hr, this procedure was repeated. All the biological samples were stored frozen for subsequent CE analysis.

To study the transport of matrine from the basolateral side to the apical side (i.e., drug excretion), a matrine solution (1.5 mL) was placed in the bottom (basolateral) chamber, and 0.4 mL of HBSS was placed in the top (apical) chamber. With a similar procedure as described earlier applied to the apical chamber, the biological samples were sampled from the apical chamber at various time intervals, and stored frozen.

Before CE analysis of the biological samples obtained from basolateral or apical chambers, the frozen samples were thawed at room temperature. Then, 40 μL of a sample was taken and mixed with 75 μL of fresh HBSS in a mini-vial, and 5 μL of metaraminol solution (IS) was added. This solution was placed in the autosampler of the CE system for analysis using the method as described in the preceding paragraph.

RESULTS AND DISCUSSION

Validation of the CZE Method

Separation conditions were first established using matrine and metaraminol standards spiked to HBSS media. A run buffer, which contained 200 mM *tris*(hydroxymethyl)aminomethane (tris) and 40 mM sodium dihydrogen phosphate, adjusted to pH 5.8 using phosphoric acid, provided a good starting point for separating the amine-containing compounds. However, the separation time was very long because of the slow electroosmotic flow resulting from the low pH of the run buffer. To alleviate this problem, the short section of the capillary tube was used in order to shorten the analysis time. This was achieved by performing injections at the capillary end near the detector, so that the inlet to detector distance became 10.2 cm. In order to carry out separation commencing at this end, a reverse polarity was used. The separation was completed within 2 min., and the electropherogram was shown in Fig. 2(a). It is observed that matrine (I) and metaraminol (II) co-migrated near 1 min, and they were not separated. In addition, a broad peak appeared and it was attributed to the components in HBSS.

To improve separation, various amounts of 2-propanol were used as an organic modifier in the run buffer in order to resolve matrine (I) and metaraminol (II). Addition of 10% isopropanol improved the situation slightly [Fig. 2(b)], which still did not provide sufficient resolution. The best

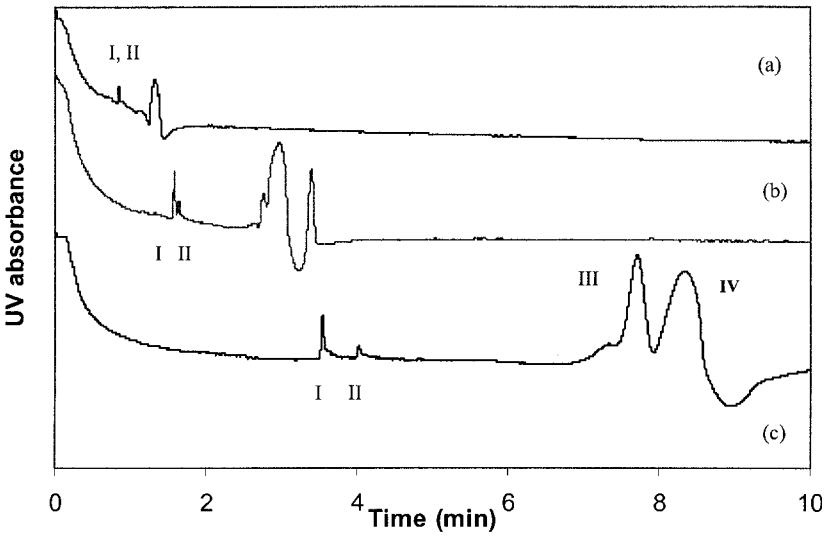


Figure 2. Electropherograms of separation of matrine and metaraminol in HBSS. Capillary: 50 μm × 40 cm; distance to detector: 10.2 cm; run buffer: 200 mM tris and 40 mM sodium dihydrogen phosphate with (a) 0%, (b) 10%, and (c) 20% 2-propanol, pH 5.8; sample introduction: 0.5 psi for 10 sec; voltage: 25 kV for 5 min; wavelength: 214 nm. Peak I: matrine, peak II: metaraminol, peaks III and IV: HBSS reagent peaks.

separation was obtained at 20% isopropanol [Fig. 2(c)]. With this amount of organic modifier present in the run buffer, the matrine and metaraminol were well-resolved under 5 min. Under these CE conditions, the HBSS components were also broadened out. As these components migrated at a later time, they did not interfere with the analysis of matrine. Subsequent CE separations were carried out for 5 min.

Separations using the optimized conditions were carried out. Figure 3(a)–(d) depicts the electropherograms of separation of matrine (at five concentrations) and metaraminol (at one concentration) in HBSS. It is clear that the area of peak I increases as the matrine concentration increases. The matrine peak at its lowest concentration was shown in the inset of Fig. 3(a). The optimal detection wavelength (214 nm) was selected from the complete data set collected from the PDA (190–300 nm). The peak areas of matrine were measured and normalized to those of the IS to produce the peak area ratios. The linearity of the matrine calibration, in the concentration range of 3.2–185 μg/mL, was determined. The linearity was found to be excellent ($n = 15$ and $R^2 = 0.9994$).

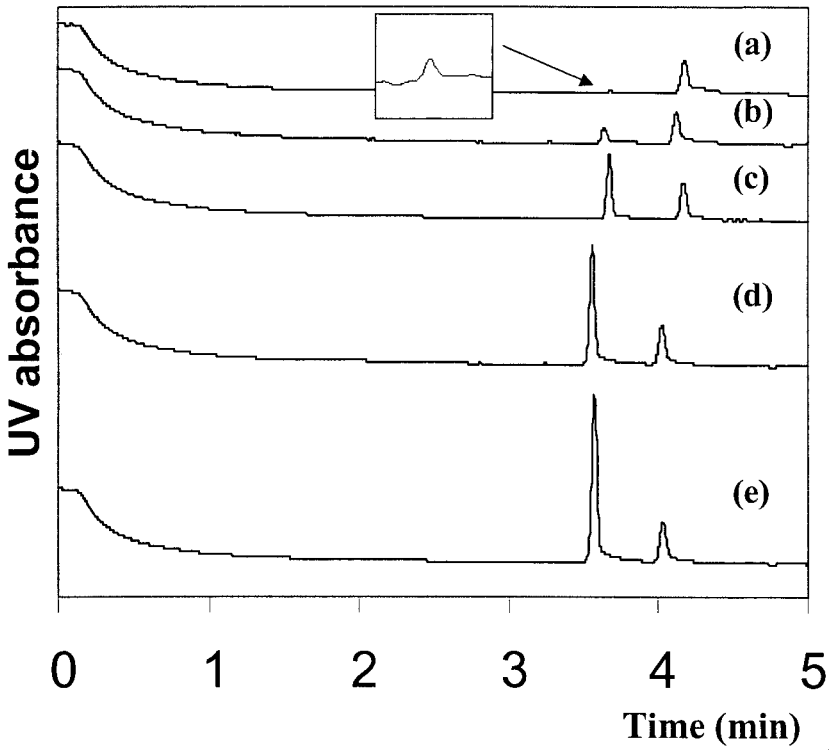


Figure 3. Electropherograms of separation of matrine and metaraminol in HBSS medium. CE conditions are given in Fig. 2: peak I, matrine [(a) 3.2, (b) 16.1, (c) 64.2, (d) 128.4, (e) 184.6 μg/mL]; peak II, metaraminol (41.7 μg/mL). The inset depicts the matrine peak at the lowest concentration.

Precision of the method was evaluated in terms of both run-to-run and day-to-day repeatabilities. In the evaluation, matrine in HBSS at three concentrations at the low, middle, and high ranges was used. In run-to-run repeatability ($n = 3$, i.e., in triplicates), the relative standard deviation (RSD) values were found to be better than 4.0%. In day-to-day repeatability ($n = 3$, i.e., in 3 days), the RSD values were better than 7.4%. It is concluded that the run-to-run and day-to-day repeatabilities of our method are satisfactory, as the analysis has been carried out in biological medium. In addition, as shown in the inset of Fig. 3(a), the matrine peak had a S/N value about 3, and so the detection limit was about 3.2 μg/mL. The developed CE method will then be employed to analyze matrine in the HBSS medium after transport of matrine through Caco-2 cells.

Analysis of Matrine in Caco-2 Cell Medium

After treatment of matrine to one side of the cells, the amounts of matrine in the HBSS medium collected on the other side of the cells were analyzed using the CE method. The electropherogram was similar to those shown in Fig. 2(c), and no metabolite of matrine, such as oxymatrine, was found. In fact, it is known that oxymatrine was converted to matrine, but not vice versa.[18]

First, the concentrations of matrine in the HBSS medium collected in the apical chambers were determined. The determination was significant to establish matrine transport from basolateral to apical sides of Caco-2 cells. Then, the results were used to determine the transport flux (expressed as mass of matrine per unit area). The transport flux for matrine treatments at three concentrations was plotted against the transport time, as shown in Fig. 4. In all the three cases, more matrine was transported to the apical chamber as time went on, and the initial rates of transport were the highest. Moreover, treatment of matrine with a lower initial concentration in the basolateral chamber has resulted in less matrine excreted to the apical chamber and a lower initial rate of transport. These results show that the CE method is useful to analyze matrine in the Caco-2 cell medium. A complete study of matrine transport under various pharmacological relevant conditions is beyond the scope of this paper.

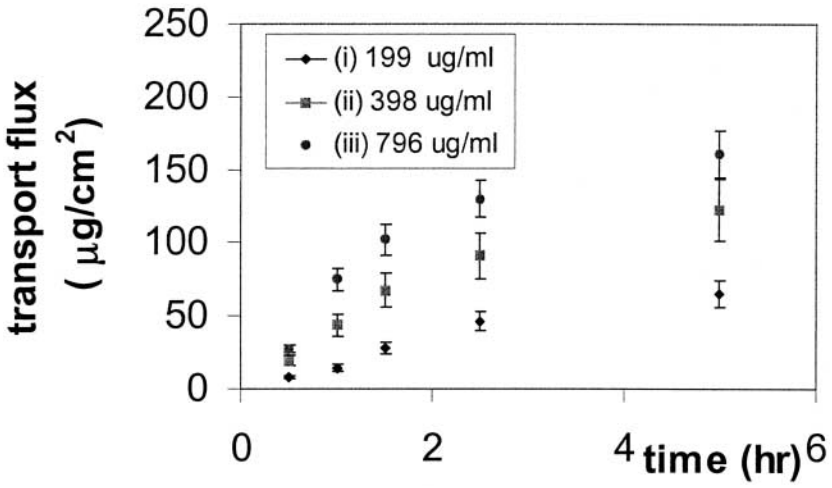


Figure 4. Transport flux of matrine through Caco-2 cells to the apical chamber vs. treatment time. The amounts of matrine for the three treatments were (i) 199, (ii) 398, and (iii) 796 μg/mL.

CONCLUSION

A CE method has been developed to determine the concentration of matrine in Caco-2 cell medium. An IS, metaraminol, was used. Calibration using the developed method provided good linearity and precision. This method was found to be very useful to analyze the amounts of matrine in the apical chambers of Caco-2 cell inserts. The CE method was significant in determining the transport of matrine by Caco-2 cells.

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