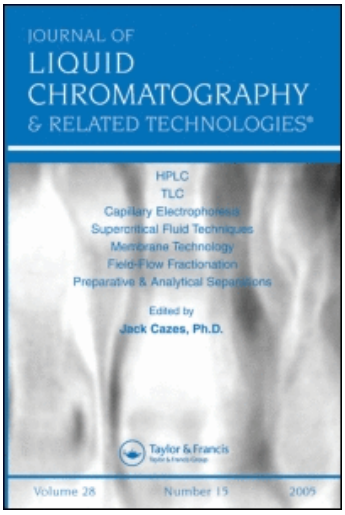

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MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY OF CYCLOSPORIN A IN A COMMERCIAL PREPARATION

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ABSTRACT

A simple micellar electrokinetic capillary chromatography is described for the determination of cyclosporin A in commercial preparation. The analysis of cyclosporin A was performed in a Tris buffer (10 mM; pH 7.1) with sodium dodecyl sulfate (SDS) (30 mM) as an anionic surfactant. Applied voltage was 20 kV and temperature was 25°C. Cefaclor was used as an internal standard and detection set at 200 nm. Several parameters affecting the separation of the drug were investigated, including the pH of the buffer, the concentrations of the buffer and SDS.

The linear range of the method for the determination of cyclosporin A was over 30-500 μM. Application of the method to the determination of cyclosporin A in capsules from a commercial source proved to be satisfactory.

INTRODUCTION

Cyclosporin A is a neutral cyclic peptide composed of 11 amino acids, with a total molecular weight of 1202 daltons (Figure 1). With its potent immunosuppressive activity that selectively affects helper T cells without suppressing the bone marrow, cyclosporin A has been widely used in organ transplantation to prolong the survival of allogenic transplants. Recently, more

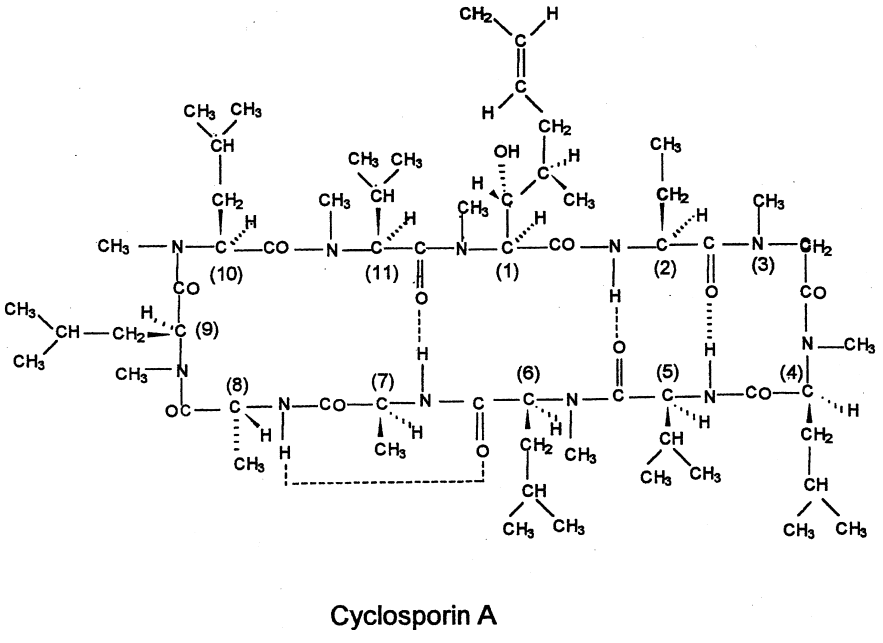

Figure 1. Chemical structure of cyclosporin A.

extensive clinic indications have been found, including rheumatoid arthritis, glomerulonephritis, red cell aplasias, uveitis, inflammatory bowel disease and psoriasis.[1]

Although a relationship has been demonstrated between serum cyclosporin A concentration and therapeutic effect, the difference between subtherapeutic and toxic concentrations of cyclosporin A is narrow. Therefore, it is quite essential to assure the potency and content uniformity of cyclosporin A in the pharmaceutical preparations.

Numerous approaches including high performance liquid chromatography (HPLC),[2-5] radioimmunoassay (RIA),[6-8] and fluorescence polarization immunoassay (FPIA),[9-10] have been established for the determination of cyclosporin A. The RIA and FPIA are sensitive; however, the methods would be inappropriate for determination of cyclosporin A in the potential presence of degradation product of this cyclic peptide in bulk and pharmaceutical preparation.

The HPLC method has been commonly used to assay the cyclosporin A in pharmaceutical formulation, but the method need to be operated at temperature

as high as 80°C. The rigorous performance condition is apt to damage the chromatographic column and cause poor reproducibility. This is the cardinal reason and motivation for us to develop a simple, more accurate, and analytical method for cyclosporin A in commercial preparation.

In this work, a simple micellar electrokinetic capillary chromatography (MEKC) is developed for the quantitation of cyclosporin A in commercial capsules. The drug was analyzed on an uncoated capillary with Tris buffer (10 mM; pH 7.1) and sodium dodecyl sulfate (SDS)(30 mM) as a background electrolyte. Application of the method to the determination of cyclosporin A in capsules was demonstrated and proved to be satisfactory.

EXPERIMENTAL

Reagents and Solution

Cyclosporin A was from Research Biochemicals International (Natick, MA, USA). Sodium dodecyl sulfate (SDS) and cefaclor were supplied by Sigma (St. Louis, MO, USA). Sodium hydroxide, hydrochloric acid, and Tris (hydroxymethyl)aminomethane were from E. Merck (Darmstadt, Germany), HPLC grade acetonitrile was supplied by Tedia (Fairfield, OH, USA). All chemicals were used without further purification.

Deionized water was obtained from a Milli-Q system (Millipore MA, USA). The running buffer consisting of 10 mM Tris (pH 7.1) and 30 mM SDS was prepared in deionized water. The cefaclor solution (5 mM) (internal standard, I.S.) was prepared in 50% acetonitrile solution (acetonitrile-water, 1:1, v/v).

CE System

A Beckman P/ACE system 2200 (Fullerton, CA, USA) equipped with a filter UV detector and a liquid-cooling device was used. MEKC was performed in an uncoated fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 37 cm ×50 μm I.D.(effective length 30 cm). The sample was injected by pressure for 1s, equivalent to a volume of about 1.78 nL for determination (according to specification of manufacturer).

The system was operated at 25°C in running buffer with an applied voltage of 20 kV and current not exceeding 20 μA. Detection was carried out by the on-column measurement of UV absorption at 200 nm (cathode at the detection side). Electropherograms were recorded with IBM computer using a Gold software system.

Reference and Sample Solutions

Reference standard cyclosporin A and cefaclor (I.S.) were prepared in 50% acetonitrile solution for study. Solutions of cyclosporin A at various concentrations (30-500 μM) with a fixed concentration of cefaclor (5 mM) were prepared for the determination. A sample solution for the content uniformity of cyclosporin A was prepared by dissolving suitable amount of each capsule of cyclosporin A with 50% acetonitrile solution in 50-mL volumetric flask.

The sample solution for assay of cyclosporin A was prepared as follows: twenty capsules of cyclosporin A were weighed and a suitable amount of the content, equivalent to about 30 mg of cyclosporin A was transferred to a 50-mL volumetric flask and diluted with 50% acetonitrile solution to the volume. The sample solution was used for quantitation of cyclosporin A in commercial capsules.

RESULTS AND DISCUSSION

Preliminary tests of standard cyclosporin A by capillary zone electrophoresis (CZE) was briefly studied at 20 kV with Tris buffer (10 mM) over the pH range 7.1-9.0 and phosphate buffer over the pH range 5.8-7.0 in the absence of SDS. A broader peak of cyclosporin A was observed under the various tested pH of phosphate buffer, but a sharp and symmetric peak of cyclosporin A was obtained in Tris buffer.

It seems the more hydrophobic and cationic Tris molecule may be able to bind tightly to the silica surface[11] to provide effective protection the cyclosporin A against the interaction with the surface of capillary tube. However, we applied the simple Tris buffer (CZE mode) for the determination of cyclosporin A in commercial preparation (capsule) and unsolved peaks appeared in the electropherogram as shown in Figure 2. This indicates that a simple separation mode of CZE, based mainly on the differences of charge to mass ratios of the analytes in the tested conditions, is difficult to differentiate the cyclosporin A from the other components that coexist in the commercial capsules.

Therefore, MEKC with SDS as a micellar source was used to separate the analyte from the other components in commercial preparations. As a consequence, simple parameters affecting the MEKC of the drug were studied, including pH of buffer and concentrations of buffer and SDS. In addition, optimal absorption wavelength for monitoring of the drug at Tris buffer was also briefly investigated.

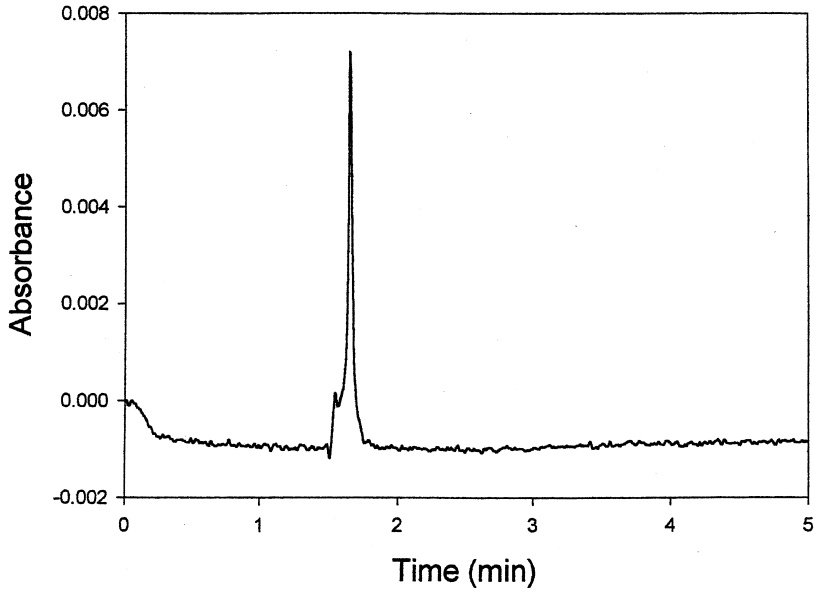

Figure 2. Electropherogram for CZE mode of cyclosporin A in commercial capsules. CE condition: Tris buffer,10 mM, pH 7.1; applied voltage, 20kV (detector at cathode side); uncoated fused-silica capillary, 30 cm (effective length) × 50 μm I.D.; sample size, 1s by pressure.

Concentration of SDS

The effects of SDS at a concentration range of 0-40 mM in Tris buffer (10 mM; pH 7.1) on the separation are studied. The results indicate that electrophoresis of the drug in the absence of SDS result in no resolution of cyclosporin A from coexisting components in capsule. MEKC of the cyclosporin A in commercial sources at lower levels of SDS (5-10 mM) gives resolved peaks, but the peak of cyclosporin A is in extreme tailing. At higher SDS concentration (20-40 mM), a more sharp and symmetric peak of the drug was obtained. The effects of SDS on the migration time of cyclosporin A and cefaclor (I.S.) are shown in Figure 3.

The retention and chromatographic behaviors of cyclosporin A and cefaclor with the same running buffer (10 mM Tris buffer and 30 mM SDS) at different pH value (7.1-9.0) was also examined. The peak shape of cyclosporin A has no significant changes at various pH value, while the migration time of cyclosporin A are slightly shorter with increasing pH as shown in Figure 4.

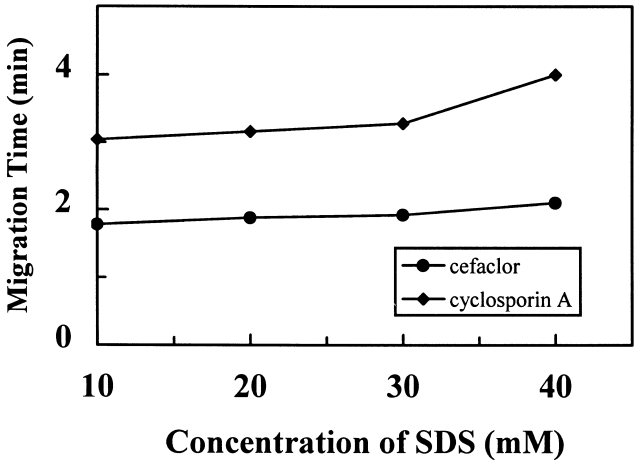

Figure 3. Effect of SDS concentrations (10-40 mM) on the migration of cyclosporin A and cefaclor in Tris buffer (10 mM, pH 7.1); applied voltage, 20kV (detector at cathode side); uncoated fused-silica capillary, 30 cm (effective length) × 50 μ m I.D.; sample size, 1s by pressure.

This can be explained by considering the effect of pH on the electroosmotic flow (EOF). The higher EOF was obtained at higher pH value of the running buffer. The migration time of cyclosporin A in pH 9.0 buffer solution was shorter among the tested pH conditions.

It has been reported that the silica is unstable in alkaline solution. Therefore, MEKC of the cyclosporin A in Tris buffer (10 mM) with SDS (30 mM) at pH 7.1 was selected for the analysis.

Wavelength for Detection

After MEKC separation of cyclosporin A and cefaclor in Tris buffer (10 mM; pH 7.1) with SDS (30 mM), the eluted compounds were monitored at 200, 214, or 254 nm. From the results we note that a higher response and better sensitivity can be obtained with detection of the drugs at 200 nm and, therefore, monitoring the drugs at 200 nm was selected.

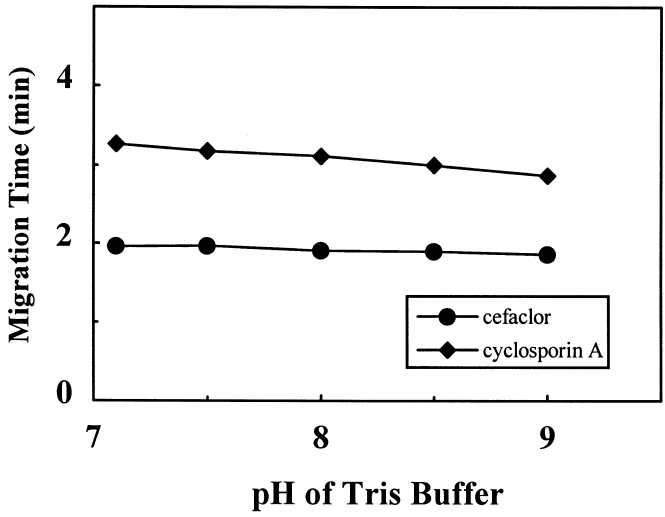

Figure 4. Effect of pH of Tris buffer with 30 mM SDS on the migration of cyclosporin A and cefaclor. See Figure 3 for other CE condition.

Concentration of Tris Buffer

The retention behavior of cyclosporin A in Tris buffer (pH 7.1) at a concentration range of 10-40 mM with constant SDS level (30 mM) was studied. Figure 5 shows the migration time of cyclosporin A and cefaclor in 30 mM SDS with different concentrations of Tris buffer. The symmetry and sharp peaks of cyclosporin A and cefaclor were obtained under the tested varied concentrations of the buffer. It indicated that the concentration of Tris buffer did not effect the peak shape of both.

From the results of SDS and the buffer effects on the MEKC mode for analysis of the cyclosporin A, optimization of the CE conditions was formulated as reported in the Experimental section, and the typical electropherogram is shown in Figure 6(A). The method is simple, specific, and rapid.

Analytical Calibration

For evaluating the quantitative applicability of the method, five different concentrations of cyclosporin A in the range 30-500 μM were analyzed, using

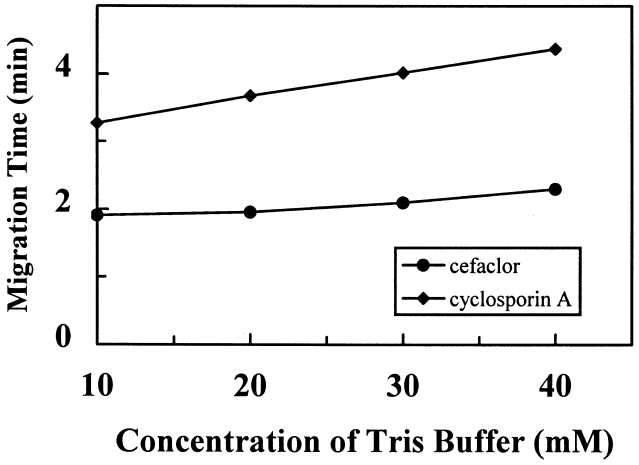

Figure 5. Effect of Tris buffer concentrations (10-40 mM) (pH 7.1) with 30 mM SDS on the migration of cyclosporin A and cefaclor. See Figure 3 for other CE condition.

cefaclor 5 mM as an I.S. The linearity between the peak-area ratios (y) of cyclosporin A to I.S. and the concentration (x, μM) of analyte was investigated. The linear regression equation (n = 5) obtained was y = (0.0213±0.0181) + (0.0037±0.0001) x (r = 0.998). The results indicate that high linearity between y and x is attainable over the range studied.

The relative standard deviation (R.S.D.) of the method based on the peak area ratio for replicate determination of cyclosporin A at 500, 200, and 50 μM was studied. The results in Table 1 indicate that the intra-day R.S.D.s (n=5) of the analyte at three concentration levels were all below 3%; in parallel, the inter-day R.S.D.s (n=5) for the analyte at three concentration levels were all below 4%.

Application

Application of the method to analysis of cyclosporin A in capsules was studied, including both the content uniformity test and the assay usually required by an official pharmacopoeia. The results of the content uniformity test is given in Table 2; all the analytical values from 10 capsules fall within the labeled amount of 85%-115% required by the United States Pharmacopoeia

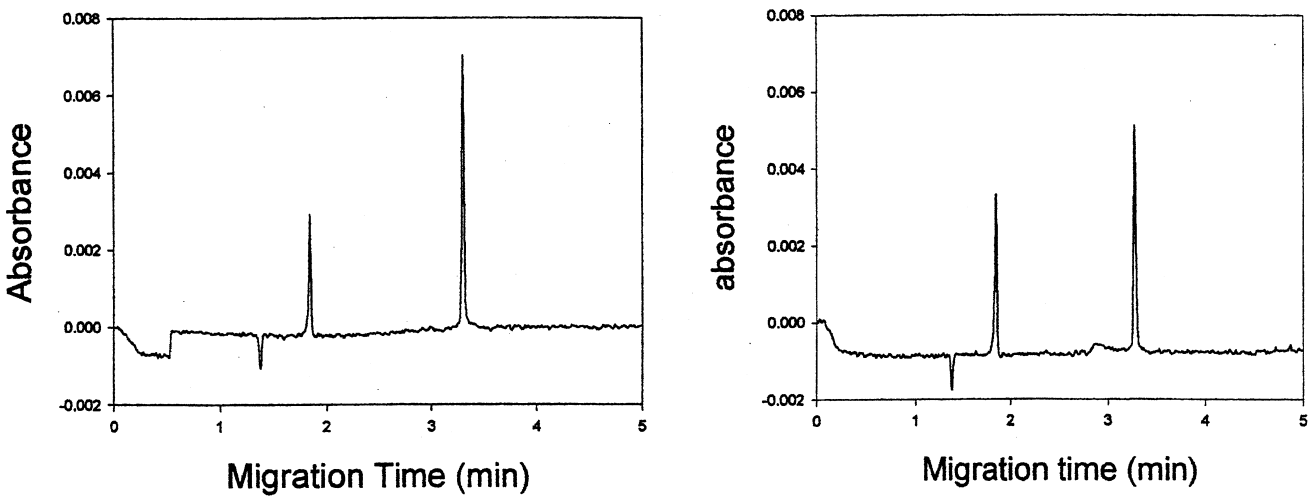

Figure 6. Electropherograms for MEKC of cyclosporin A and cefaclor: (A) reference standard and (B) from commercial source. Peaks: 1, cefaclor; 2, cyclosporin A. MEKC conditions: buffer, 10 mM Tris buffer (pH 7.1) with 30 mM SDS; applied voltage, 20 kV (detector at cathode side); uncoated fused-silica capillary, 30 cm (effective length) × 50 μm I. D.; sample size, 1s by pressure.

Table 1

Precision for Determination of Cyclosporin A

Concentration Known (μM)	Concentration Found (μM)	R.S.D.(%)
Intra-day* (n=6)		
500	508.1±6.1	1.20
200	203.3±2.6	1.27
50	51.1±1.5	2.93
Inter-day* (n=8)		
500	511.3±7.8	1.52
200	209.3±3.6	1.72
50	52.4±2.1	4.00

* Intra-day data were based on six replicate analyses and inter-day data were from eight consecutive days.

Table 2

Analytical Results for Content Uniformity of Cyclosporin A Capsules Obtained from Commercial Source

Capsule[a]	Amount Found[b] (mg)	Percentage of Claimed Content[c] (%)
1	97.2±0.2	97.2
2	101.1±0.2	101.1
3	103.2±0.3	103.2
4	99.8±0.2	99.8
5	97.8±0.1	97.8
6	104.6±0.3	104.6
7	105.3±0.3	105.3
8	99.7±0.2	99.7
9	96.8±0.2	96.8
10	101.6±0.3	101.6

[a] Labeled amount of cyclosporin A in capsule is 100 mg. [b] Mean ± S.D. of five replicate analyses. [c] Content uniformity test is used to check the variation of cyclosporin A in each capsule.

Table 3

Assay Results of Cyclosporin A in Capsules Obtained From Commercial Source

Sample[a]	Amount Found[b] (mg)	Percentage of Claimed Content (%)
1	104.2±0.4	104.2
2	103.1±0.3	103.1
3	97.3±0.2	97.3
4	98.8±0.1	98.8
5	97.2±0.1	97.2
6	103.6±0.3	103.6

[a] Labeled amount of cyclosporin A in capsule is 100 mg. [b] Mean ± S.D. of five replicate analyses.

[USP];[2] and those analytical results of the assay (Table 3) also pass USP requirement based on the content range 90-110% of labeled amount.

A typical electropherogram for the analysis of cyclosporin A in capsule is shown in Figure 6(B). The recoveries of cyclosporin A at three concentration levels (500 μM, 200 μM and 50 μM) were studied, based simply on a known amount of reference cyclosporin A added to the content of capsule. All the recoveries are above 95%.

In conclusion, a simple and rapid MEKC method has been developed for the quantitative of cyclosporin A in commercial preparation. The method is simple and practical. It can be applicable to the quality control of cyclosporin A in bulk and in pharmaceutical preparations.

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