

Separation and Determination of Ephedrine and Pseudoephedrine by Combination of Flow Injection with Capillary Electrophoresis

Hongli Chen¹, Xingguo Chen^{1,*}, Qiaosheng Pu¹, Zhide Hu¹, Zhengfeng Zhao², and Martin Hooper²

¹Department of Chemistry, Lanzhou University, Lanzhou 730000, P.R. China and ²School of Applied Sciences, Monash University, Monash University Gippsland Campus, Churchill, Vic. 3842, Australia

Abstract

A simple, rapid, and accurate method for the separation and determination of ephedrine and pseudoephedrine using direct UV absorbance detection has been developed by the combination of flow injection with capillary electrophoresis for the first time. The buffer solution used is a 40mM borate solution with the pH adjusted to 9.5 using a 2M NaOH solution. The linear calibration range is 50 to 1000 µg/mL ($r = 0.9996$) for both analytes, and the recoveries are 91.2–108.2% for ephedrine and 92.6–107.3% for pseudoephedrine, respectively. The relative standard deviation of the peak area is 1.6% for ephedrine and 1.3% for pseudoephedrine ($n = 6$) at a concentration of 500 µg/mL, respectively. A series of samples is injected repeatedly without current interruption and subsequent rinsing, and the contents of these two alkaloids in three marketed drugs and the medical plant, *Ephedra sinica*, are determined with satisfactory results by this method.

Introduction

Following the early contributions to capillary electrophoresis (CE) made by Mikkers et al. (1), the technique has evolved into a major analytical tool for the separation of charged compounds within the past 20 years. In recent years, much attention has been focused on the application of this technique for the separation and identification of the active compounds in some traditional Chinese medicines (2). Although there are multiple and outstanding advantages of CE as a separation technique, there are some disadvantages such as discontinuous mode of sample introduction, sampling bias with the electrokinetic mode, low sensitivity in terms of the concentration, and complicated sample off-line pretreatment procedures. These are its generally recognized limitations and affect its broad acceptance in routine applications.

Making use of excellent sample pretreatment ability (3) of flow injection (FI), Karlberg's group and Fang's group inde-

pendently and successfully took up a study in 1997 on the combination of FI-CE (4,5) in which samples having complicated matrix compositions were directly introduced into CE equipment by an FI system. The important advantages of the FI-CE system over the conventional methods include outstanding reproducibility in retention time, peak area and height, enhanced sample throughput, and large potentials in coupling FI on-line preconcentration techniques to CE. Thus far, the coupling system has been applied to real samples, including on-line sample filtration (6), dialysis (7), gas diffusion (8), column sorption (9), and others.

High sample throughput is an interesting strongpoint of the FI-CE system, especially for large numbers of samples and for quality control in pharmaceutical plants. However, to our knowledge, hitherto no application of the FI-CE system in the traditional Chinese herbal preparations has been reported. The plant genus *Ephedra sinica* (known by its Chinese name "Ma-huang") is a botanical source of ephedrine alkaloids and commonly used Chinese herbal drug intended for diaphoretic purposes. Among these alkaloids, ephedrine and pseudoephedrine are considered as indices for the estimation of quality of Chinese traditional medicine, *Ephedra sinica* (10). In the past three decades, many methods (11,12) have been reported for the separation and determination of ephedrine and pseudoephedrine. In this study, we have developed a sensitive, automated, and high-frequency procedure based on the FI-CE system, and we have applied the procedure to determine ephedrine and pseudoephedrine in three marketed drugs and the medical plant, *Ephedra sinica*.

Experimental

Apparatus

A model HPE-100 CE system with maximum voltage of 12 kV (Bio-Rad, Hercules, CA) was used for the separations, and it was connected to a PC 486 with a Chroma chromatography collection system (Bio-Rad) for integration and data treat-

* Author to whom correspondence should be addressed: email chenxg@lzu.edu.cn.

ment. An uncoated silica capillary column of 75- μm i.d. \times 375- μm o.d. 50-cm length (35-cm effective length) (Yongnian Optical Fiber Factory, Hebei, China) was used throughout the work. UV absorption measurements were made at 215 nm. The applied voltage across the capillary was 10 kV, unless stated otherwise.

An FIAstar 5020 analyzer (Tecator, Höganäs, Sweden) was used in all studies, which included two 4-channel peristaltic pumps that are controlled by a microprocessor. The transport line from the valve to the split-flow interface of the FI-CE system was 0.5-mm-i.d. 32-cm-length PTFE tubing. A sample loop of 100 μL is made from PTFE, and Teflon pump tubes were used for the delivery of all solutions. The time period for the injecting sample was defined through thumbwheel settings.

The manifold for the FI-CE system used in this study was shown schematically in Figure 1. The 40-mm section of the locally made conical plastic tip (with a 0.5-mm i.d. at the lower end and 50-mm i.d. at the upper end) was used as the flow-through cell of the anode. The inlet of the separation capillary was inserted into the center of the flow cell and positioned 2.0 mm above the bottom. A platinum electrode was inserted into the flow cell to serve as the anode, and its tip was positioned 8 mm above the inlet of the capillary to avoid entrainment of electrolytically generated oxygen bub-

bles into the capillary. One of the 0.5-mm-i.d. PTFE tubings that served as the transport line from the FI system was connected to the bottom of the flow cell, and the other, which was used to draw out the waste solution from the flow cell, was fixed on the outlet of the flow-through cell. The vertical position of the flow cell was adjusted to keep the same liquid level as that in the cathode reservoir.

The sample was transported into the split-flow interface cell by the buffer electrolyte, where the flow was split, a small fraction of the sample zone was electrokinetically introduced into the capillary, and an on-line separation was performed. The separation was carried out from the positive to the negative electrode. A series of samples was injected repeatedly without current interruption and subsequent rinse.

Reagents and chemicals

Ephedrine and pseudoephedrine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and were used without further purification. All the chemicals used were of analytical reagent grade. Distilled water was used in all of the experiments.

Stock standard solutions of ephedrine and pseudoephedrine were prepared at a concentration of 10.0 mg/mL in water. The working standard solutions were obtained by diluting the corresponding stock solutions with a 2mM borate solution to the desired final concentration. Buffer solutions were prepared by diluting 0.1M borate stock solution with water, and their desired pH values were adjusted by the addition of 2M HCl or 2M NaOH. All buffer solutions were filtered through a 0.45- μm syringe filter and degassed under vacuum before use.

Extraction of real samples

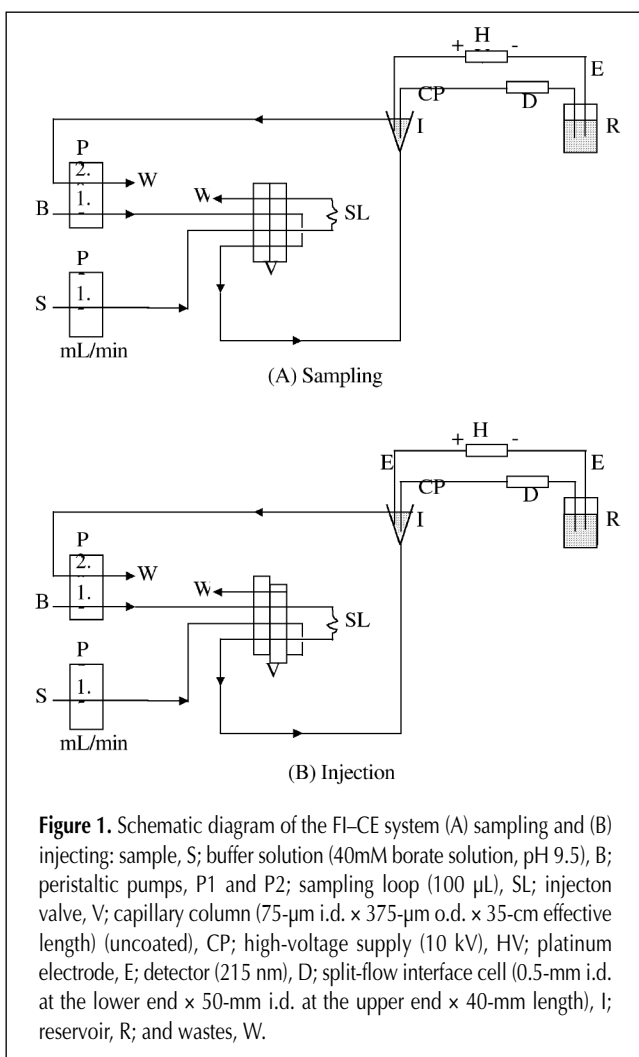
An appropriate amount of each of four samples was powdered and extracted with 25 mL of the 2mM borate solution for 30 min in an ultrasonic bath and then centrifuged at 2500 rpm for 10 min. The extract was filtered through a 0.45- μm syringe filter, degassed under vacuum, then directly injected into the CE equipment by the FI system.

Operating conditions

The running buffer used was a 40mM borate solution at pH 9.5 (adjusted with NaOH). The applied voltage was 10 kV, the analytical temperature was 24°C, and the selected wavelength was 215 nm. In the FI system, the selected flow rate was 1.5 mL/min and the sample volume was 100 μL .

In order to maintain the capillary under good working conditions, its surface was regenerated once a day by consecutive rinsing with distilled water (5 min), 0.1M NaOH (5 min), and distilled water (2 min), followed by the running buffer (10 min). Moreover, the capillary was flushed between analyses with 0.1M NaOH (1 min), distilled water (2 min), and fresh buffer (2 min) for optimizing the migration time and peak shape reproducibility.

The elution order of ephedrine and pseudoephedrine was first ephedrine followed by pseudoephedrine. The identity of the recorded peaks was confirmed by independent injection of the pure compounds.



Results and Discussion

Investigation of CE separation conditions

In order to develop an online CE system for the separation and determination of ephedrine and pseudoephedrine, the investigation of CE separation conditions was aimed at producing a system capable of completely resolving the two analytes with the shortest separation time and sufficient sensitivity.

In the FI-CE system, the sample carrier of the FI system also functions as the running buffer for CE separation. The influence of buffer pH on the CE separation of ephedrine and pseudoephedrine was investigated in the pH range of 8–11 with 10mM borate solution. The results showed that at pH 9.5 the baseline separation of two components was obtained. Therefore, pH 9.5 was chosen as the optimum value.

The effect of buffer concentration on migration time, peak height, peak area, resolution, and theoretical plate number in the range of 5–60mM was also investigated. It was found that the peaks of ephedrine and pseudoephedrine were partially overlapped with only 5mM of borate. Simultaneously with

an increase in borate concentration, the peak height, peak area, resolution, and theoretical plate number of the two analytes were found to increase, which was owed to enhanced sample stacking effects at higher buffer concentrations in the carrier. However, the analysis time was found to be prolonged from 5 to 12 min. On the other hand, the higher buffer concentration led to more Joule heating, which might be deleterious for maintaining stable electrokinetic conditions over extended monitoring periods. In order to obtain higher column efficiency while avoiding the generation of excessive Joule heating, a borate concentration of 40mM was used for further studies.

A high voltage was necessary for rapid CE analysis. The satisfactory resolution of the two analytes was achieved in 40mM borate buffer (pH = 9.5) within the voltage range of 6–12 kV, with the separation speed increasing at higher applied voltages. Nevertheless, when the highest applicable voltage of 12 kV was used, the current increased as much as 85 μ A, causing a baseline drift in the beginning of the operation period. The lowest voltage that could avoid this effect was 10 kV, and this was adopted in the separation studies.

Investigation of FI parameters

The operational parameters of the combined FI-CE system (including carrier flow rate and sample volume) were investigated. With fixed sample injection volumes, the carrier flow rate determines the residence time of the sample zone within the flow-through cell and, therefore, the time available for electrokinetic split-sampling into the capillary. Figure 2A

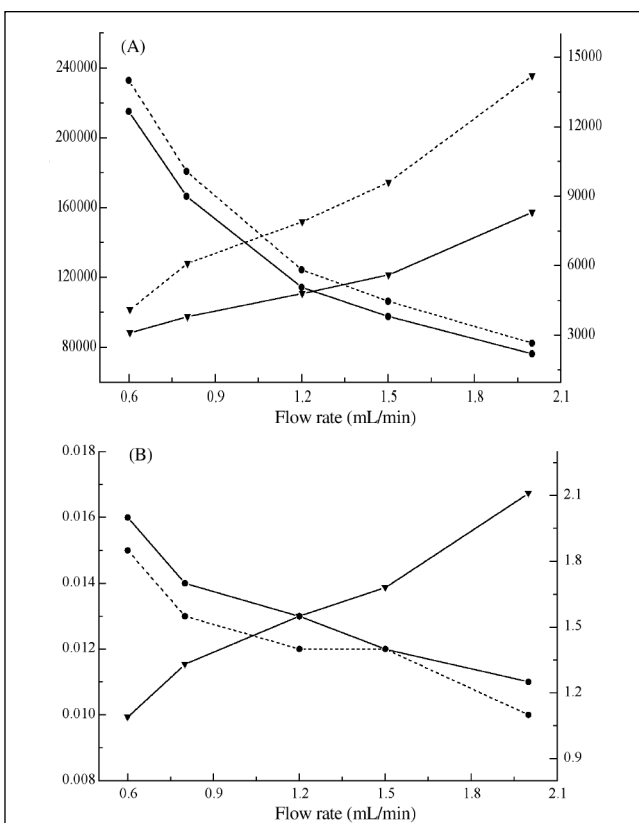


Figure 2. Effect of carrier (separation buffer) flow rate on (A) theoretical plate number and peak area [peak area (●), theoretical plate number (▲), ephedrine (–), and pseudoephedrine (– –)], and (B) peak height and resolution [peak height (●), ephedrine (–), pseudoephedrine (– –), and resolution (▲)]. The sample was 500 μ g/mL ephedrine and pseudoephedrine in 2mM borax. The CE conditions included a 40mM borate solution buffer (pH 9.5), 10 kV voltage, 215-nm detection wavelength, and 75- μ m i.d. \times 375- μ m o.d. \times 50-cm length (35-cm effective length) capillary column (uncoated). The FI conditions included a sample volume of 100 μ L and the interface gap width was 2 mm.

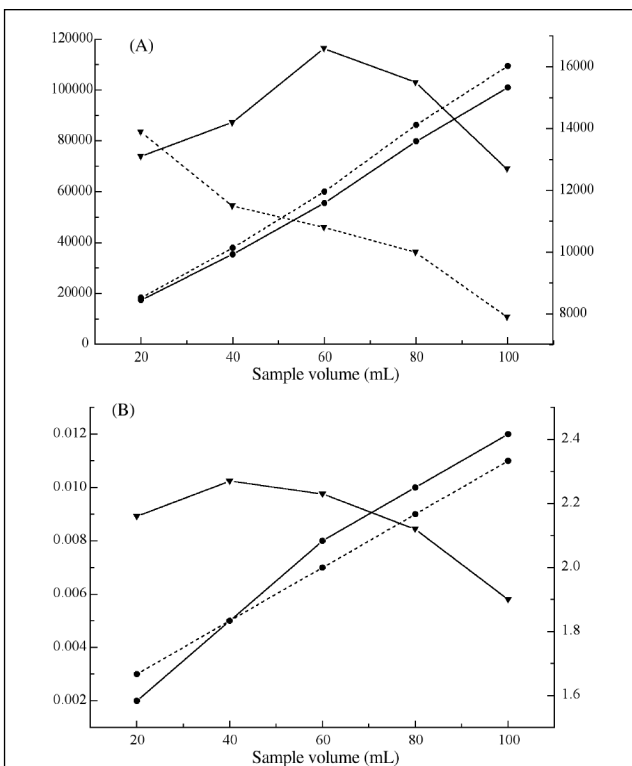


Figure 3. Effect of FI sample volume on (A) theoretical plate number and peak area and (B) peak height and resolution. The carrier flow rate was 1.5 mL/min. The symbol definitions are the same as in Figure 2.

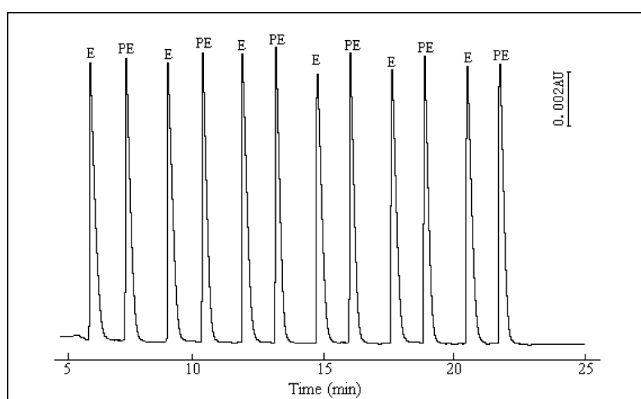


Figure 4. Recordings of six consecutive injections of a standard mixture of ephedrine and pseudoephedrine. The peaks are ephedrine, E, and pseudoephedrine, PE. The CE conditions included a 40mM borate solution buffer (pH 9.5), 10 kV voltage, 215-nm detection wavelength, and 75- μm i.d. \times 375- μm o.d. \times 50-cm length (35-cm effective length) capillary column (uncoated). The FI conditions included a 1.5-mL/min buffer flow rate, 100- μL sample volume. The interface gap width was 2 mm and the sample was 500 $\mu\text{g}/\text{mL}$ of ephedrine and pseudoephedrine in 2mM borate solution.

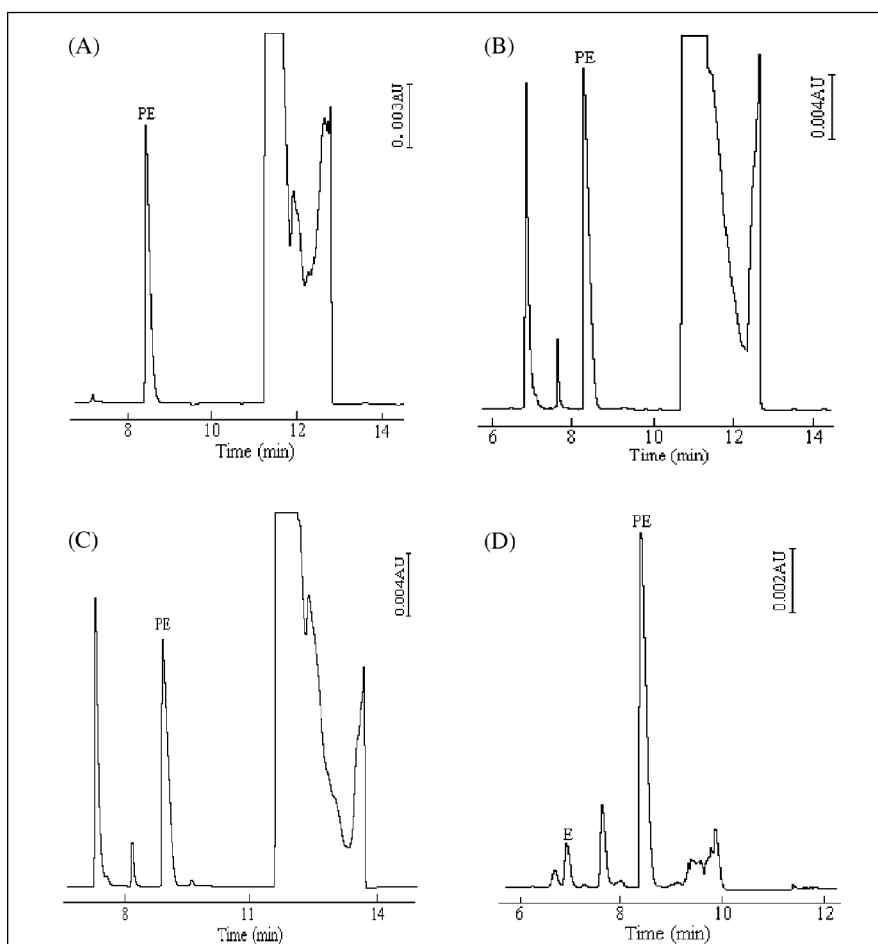


Figure 5. Capillary electropherogram of three marketed drugs and medicinal plant *Ephedra sinica*: (A) compound terfenadine tablets, (B) compound pseudoephedrine hydrochloride tablets, (C) Tylenol Cold, and (D) medicinal plant (*Ephedra sinica*). The peaks included ephedrine, E, and pseudoephedrine, PE. The sample included extracts of three marketed drugs and the medicinal plant in 2mM borate solution. The experimental conditions are the same as in Figure 4.

shows the relationship between the peak area, theoretical plate number, and carrier flow rate in the 0.6 to 2.0 mL/min range. It was found that the peak area decreased nearly in proportion to the increase in carrier flow rate and the plate number increased drastically with increasing flow rate, which apparently was caused by a decreased sample zone resulting from the smaller sample volume injected into CE in the higher carrier flow rate. This was also reflected in the relationship between peak height, resolution, and carrier flow rate (Figure 2B). It can be concluded that the dispersion contribution from sample transporting decreases with the increase of carrier flow rate. Simultaneously considering the sensitivity, resolution, and theoretical plate number, the carrier flow rate of 1.5 mL/min was selected for further studies.

At the fixed carrier flow rate, the injected sample volume determines the amount and length of sample introduced into the capillary, which affects the peak width and, therefore, the plate number of the separation system. Figures 3A and 3B show the peak area, peak height, theoretical plate number, and resolution obtained with different sample volumes at 1.5 mL/min carrier flow rate. The peak height/sample volume relationship was quite typical of those obtained in other FI systems,

indicating the rather limited dispersion within the injection, transport, and interfacing system. In contrast, the peak area increased almost linearly with sample volume, resulting in an increase in peak width and degradation of the theoretical plate number. It was obvious that the larger sample volume resulted in better sensitivity and worse resolution between ephedrine and pseudoephedrine. In our study, for achieving higher sensitivity with sufficient column efficiency and resolution for real samples, a sample volume of 100 μL was considered to be optimum.

Performance of the combined FI-CE system

The relative standard deviation ($n = 6$) of the peak area measurements was 2.1% for ephedrine and 1.7% for pseudoephedrine at a concentration of 500 $\mu\text{g}/\text{mL}$, respectively. The result was illustrated by the recording of a series shown in Figure 4 in which 6 standard samples were introduced repeatedly without intermediate column rinsing. Although the complete analysis time of standard solution of ephedrine and pseudoephedrine was approximately 8 min, a sampling frequency of 20 h^{-1} was achievable using the FI-CE system. However, owing to the complexity and multicomponent of sample matrix, the sampling frequency for sample solutions of three marketed drugs and the medicinal plant (*Ephedra sinica*) only reached approximately 5 h^{-1} .

Table I. Contents of Ephedrine and Pseudoephedrine in Three Marketed Drugs and the Medicinal Plant *Ephedra sinica* (n = 4)

Sample*	Found content			Labeled content	Relative error (%)
	Ephedrine	Pseudoephedrine	Total		
1 [†]	— [‡]	15.1 mg/tablet	15.1 mg/tablet	15 mg/tablet	0.7
2 [§]	— [‡]	29.4 mg/tablet	29.4 mg/tablet	30 mg/tablet	-2.0
3 ^{**}	— [‡]	31.6 mg/tablet	31.6 mg/tablet	30 mg/tablet	5.3
4 ^{††}	0.15 mg/g	3.23 mg/g	3.38 mg/g	— ^{‡‡}	

* Names and compositions of the Ephedrae Herba containing Chinese herbal formulations.
[†] Compound terfenadine containing 15 mg terfenadine, 15 mg pseudoephedrine HCl, and 162.5 mg acetaminophen.
[‡] Content not contained in these Chinese herbal preparations.
[§] Compound pseudoephedrine hydrochloride tablets containing 325 mg acetaminophen, 30 mg pseudoephedrine HCl, 15 mg dextromethorphan HBr, and 2 mg chlorpheniramine maleate.
^{**} Tylenol Cold containing 325 mg acetaminophen, 30 mg pseudoephedrine HCl, 15 mg dextromethorphan HBr, and 2 mg chlorpheniramine maleate.
^{††} Medicinal plant (*Ephedra sinica*).
^{‡‡} No criteria value.

Calibration graphs for ephedrine and pseudoephedrine

Calibration graphs (peak-area (y) vs. concentration (x) in $\mu\text{g/mL}$) were obtained by injecting standard solutions in the range of 50–1000 $\mu\text{g/mL}$. Each point of the calibration graph corresponds to the mean value obtained from four independent peak area measurements. The regression equations of these curves and their correlation coefficients were as follows:

$$\text{for ephedrine, } y = 2953.6 + 179.1x \quad (r = 0.9995) \quad \text{Eq. 1}$$

$$\text{for pseudoephedrine, } y = 2362.3 + 156.5x \quad (r = 0.9996) \quad \text{Eq. 2}$$

Determination of ephedrine and pseudoephedrine in real samples

When water extracts of three marketed drugs and the medicinal plant (*Ephedra sinica*) were injected directly into CE system by FI under the selected conditions, the results were as good as those obtained with pure chemical samples without interference with each peak (Figure 5). The calculated contents of ephedrine and pseudoephedrine given in Table I were obtained. And the recoveries were 91.2–108.2% for ephedrine and 92.6–107.3% for pseudoephedrine, respectively.

Conclusion

The application of the combined FI–CE system to the separation and determination of ephedrine and pseudoephedrine in this work demonstrates the feasibility and precision of performing separations with such a system. The result indicates that the proposed FI–CE method is suitable for the determination of principle components in real samples. Moreover, this

method uses the water extract directly, which is a practical advantage. In addition to its rapid and accurate performance, consecutive injections can be made without current interruption and intermediate column rinsing.

However, in the proposed FI–CE system, the sampling frequency of real samples is approximately 5 h^{-1} , which is much lower than the 20 h^{-1} of the sampling frequency of standard solutions (i.e., it is difficult to obtain a high sampling rate for complicated sample matrices). In order to increase sample throughput, a unique and special extraction method of these real samples should be employed.

References

1. F.E.P. Mikkers, F.M. Everaerts, and Th.P.E.M. Verheggen. High-performance zone electrophoresis. *J. Chromatogr.* **169**: 11–20 (1979).
2. K.D. Altria, M.A. Kelly, and B.J. Clark. Current application in the analysis of pharmaceuticals by capillary electrophoresis. *Trends Anal. Chem.* **17**: 204–14 (1998).
3. *Micro Total Analysis Systems*. A. van den Berg and P. Bergveld, Eds. Kluwer Academic, Dordrecht, The Netherlands, 1995.
4. P. Kuban, A. Engström, J.C. Olsson, G. Thorsén, R. Tryzell, and B. Karlberg. New interface for coupling flow-injection and capillary electrophoresis. *Anal. Chim. Acta* **337**: 117–24 (1997).
5. Z.L. Fang, Z.S. Liu, and Q. Shen. Combination of flow injection with capillary electrophoresis: Part I. The basic system. *Anal. Chim. Acta* **346**: 135–43 (1997).
6. H.W. Chen and Z.L. Fang. Combination of flow injection with capillary electrophoresis: Part 4. Automated multicomponent monitoring of drug dissolution. *Anal. Chim. Acta* **376**: 209–20 (1998).
7. P. Kuban and B. Karlberg. On-line dialysis coupled to a capillary electrophoresis system for determination of small anions. *Anal. Chim. Acta* **69**: 1169–73 (1997).
8. P. Kuban and B. Karlberg. On-line coupling of gas diffusion to capillary electrophoresis. *Talanta* **45**: 477–84 (1998).
9. H.W. Chen and Z.L. Fang. Combination of flow injection with capillary electrophoresis: Part 3. Online sorption column preconcentration capillary electrophoresis system. *Anal. Chim. Acta* **355**: 135–43 (1997).
10. *Dictionary of Traditional Chinese Medicine*, 1st ed. New Medical College of Jiangsu, Shanghai People's Press, 1977.
11. Y.M. Liu and S.J. Sheu. Determination of ephedrine and pseudoephedrine in chinese herbal preparations by capillary electrophoresis. *J. Chromatogr.* **637**: 219–23 (1993).
12. G. Shao, D.S. Wang, F. Wu, S.J. Chen, and X. Luo. Separation and determination of (1)-ephedrine and (d)-pseudoephedrine in plasma by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **18**: 2133–45 (1995).