Online Concentration by Head-column Field-amplified with Large-volume Sample Stacking Using Flow Injection-Capillary Electrophoresis for the Analysis of Four Active Components in Cold Medicines

Xiumei LIU,* Jingshu ZHANG,* and Xingguo CHEN*,**†

*Department of Chemistry, Lanzhou University, Lanzhou 730000, China

**State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, China

A simple, effective, and sensitive online concentration method for the detection of dextromethorphan hydrobromide (Dex), chlorphenamine hydrogen maleate (Chl), pseudoephedrine hydrochloride (Pse) and paracetamol (Par) based on flow injection-capillary electrophoresis (FI-CE) analysis with head-column field-amplified sample stacking and large-volume sample stacking was developed. The background electrolyte (BGE) was a solution composed of 55 mM borate-15% (v/v) acetonitrile (ACN) (pH 9.3). The sample was injected electrokinetically between plugs of water. Under the optimum conditions, about a 30-fold improvement in the concentration sensitivity relative to normal CE methods was achieved, giving low limits of detection (LOD) of 1.94×10^{-5} , 0.64×10^{-5} , 1.16×10^{-5} and 2.84×10^{-5} mg/mL for Dex, Chl, Pse and Par, respectively. The repeatability (defined as RSD) was 1.01, 1.91, 0.89 and 0.92% with the peak-area evaluation and 1.94, 3.98, 2.66 and 3.27% with the peak-height evaluation for Dex, Chl, Pse and Par, respectively. This method has been successfully applied to the analysis of commercial pharmaceutical preparations containing Dex, Chl, Pse and Par.

(Received September 25, 2007; Accepted December 26, 2007; Published August 10, 2008)

Dextromethorphan hydrobromide (Dex), chlorphenamine hydrogenmaleate (Chl), pseudoephedrine hydrochloride (Pse) and paracetamol (Par) are effective components in many cold medicines. Dex can ease the pain of centrum, being applicable for cold, acute and chronic bronchitis, bronchus asthma, tuberculosis *etc.* Chl is an antihistamine, used for allergic diseases.¹ Pse has the function of constricting blood vessels, eliminating mucous membrane congesting and the tumefying of nasopharynx. Par is used as a cure for fever, headaches and neuralgia. Because they are always present in the same drug tablet as active compounds, it is important to determine these four components simultaneously.

Because the contents of the four components in each tablet are very different, the most is 325 mg and the least is only 2 mg, the developed CE methods at present, such as MEKC,² CZE,³⁻⁵ nonaqueous capillary electrophoresis (NACE)^{6,7} and cyclodextrin-modified microemulsion electrokinetic chromatography (CD-MEEKC),⁸ are difficult to analyze simultaneously the four ingredients using a single method. In our previous study, we developed an FI-CZE⁵ method for the separation and determination of Dex, Chl, Pse and Par by the analysis of two different concentrations, which was not in accord with the high efficiency of CE. Therefore, it is necessary to establish a simple and sensitive method to analyze the four components simultaneously for strictly controlling the quantity of drugs.

CE has gained widespread interest as a favorable technique owing to its rapidity, high resolving power, and small amounts

of samples and reagents required.⁹⁻¹¹ However, it suffers from low concentration sensitivity because of the limited optical path length for online UV detection. To solve this problem and to make CE more attractive, a variety of online preconcentration approaches have been developed. The simplest technique for sample stacking is field-amplified sample stacking (FASS). With this mode of stacking, ionic analytes are prepared with a very diluted buffer, or water, and injected electrokinetically into a capillary containing a high-conductivity buffer. When a voltage is initiated, because of the low conductivity of the sample zone, a high electric field strength will be established at the injection point. Then, a large amount of sample ions will be injected into the capillary under this enhanced (amplified) electric field. The sample ions migrate rapidly under the enhanced applied electric field in the sample plug until they reach the concentration boundary. Once they migrate into the high-conductivity buffer region, they slow down and concentrate into narrow bands. However, perhaps because during sampling the boundary at the end of the column is disturbed, and the electric field at the injection point may not be amplified properly, a much smaller than predicted signal enhancement is usually found, and the repeatability of the peak areas and heights is very poor.¹² This is possibly caused by switching the capillary directly from the high-conductivity buffer reservoir to the low-conductivity sample solution during sampling, which causes a disturbance of the buffer boundary at the end of the capillary; thus, the electric field at the injection point might not be amplified properly. In order to solve this problem, Chien and Burgi^{12,13} greatly improved the method by injecting a short plug of water before sample introduction, the so-called head-column FASS (HC-FASS). No special exclusive devices or regulation

† To whom correspondence should be addressed.
E-mail: chenxg@lzu.edu.cn

Fig. 1 Structures and pK_a values of analytes.

mechanisms are required. This sample stacking is based on the presence of a short water plug at the capillary inlet end prior to sample introduction. Also, across which an electric field of up to several hundred-times higher than that employed in normal CE is established, which enables charged analytes to be injected at high velocity. The preinjection water plug cleans the capillary tip, and can prevent contamination of the sample solution with running buffer of high conductivity to ensure proper field amplification at the injection point, and thus to ensure the highest repeatability. HC-FASS in CE has been shown to provide the greatest sensitivity enhancement,^{14–18} and seems to be the most promising approach among the sample-stacking techniques. However, it is inevitable that the hydrodynamic flow (in order to introduce a water plug) induces a disturbance at the sample-buffer zone boundary, which decreases the stacking efficiency. Moreover, the discontinuous sample introduction mode confines the sample throughput and the precision. Recent developments on the coupling of flow injection (FI) to CE demonstrate the potential of achieving efficient continuous sample introduction for CE,^{19–22} including enhanced sampling frequencies and improved repeatability, as compared to conventional sample introduction. Additionally, large-volume sample stacking (LVSS) has been demonstrated to improve the detection limits of charged analytes.^{23,24}

The aim of this work was to develop a simple, rapid and continuous online concentration approach for the analysis of the four components in medicines simultaneously based on FI-CE-HC-FASS, with improved sensitivity by combining the LVSS technique (FI-CE-HC-FASS-LVSS). The advantages of this study over Ref. 5 are as follows: (1) The sensitivity was improved using an online concentration approach. (2) The Chl and Par, whose contents in medicines have large differences, could be determined simultaneously by analysis at one appropriate concentration. (3) The shapes of the peaks and the resolution were improved using an orthogonal design (OD) to optimize the separation conditions. The developed method has been successfully applied to analyze two medicines, including the low level of Chl, with reliable results.

Experimental

Reagents and chemicals

Dex, Chl, Pse and Par were purchased from the National Institute for the Control of Pharmaceutical and Biological

Fig. 2 Manifold for the FI-CE (not to scale). C, Carrier solution; S, sample; P1 and P2, pumps; PD, pressure damper; G, pressure gauge; SL, sampling loop; RL, reagent loop; 16-V, 16-way valve; PER, peristaltic pump; B, planar plastic base; T1, Tygon tubing; T2, Tygon tubing (1.2 mL/min); CP, separation capillary column; E, platinum electrode; W, waste; HV, high voltage; D, detector; SC, screw clamp; DW, distilled water.

Products (Beijing, China). Their structures and pK_a values are shown in Fig. 1. Acetonitrile (ACN) was supplied by Tianjin Secondary Chemical Factory (Tianjin, China). Sodium borate was supplied by Taicang Chemistry Factory (Jiangsu, China). Cold medicines, namely, Anma Meimin Tablets (Lei Meng Xin), Anfen Weima Namin Tablets (Hai Wang Yin De Fei) were purchased from local drug stores (Lanzhou, China). All of the reagents used were of analytical grade, and distilled water was used throughout the study.

Apparatus

A Model HPE-100 CE system with 12 kV maximum voltage (BioRad, Hercules, CA) was used for separation, which was connected to a 486 PC. Data collection was achieved by a Chroma chromatography collection system (BioRad). Uncoated fused-silica capillaries (75 μ m i.d. $\times$ 375 μ m o.d. $\times$ 48.4 cm length and 45 cm effective length) were purchased from Yongnian Optical Fiber Factory (Hebei, China). UV detection was performed at 214 nm.

A K-1000 flow injection analyzer (Hitachi, Japan) was used throughout for transporting the background electrolyte (BGE, buffer/carrier) and samples. It was composed of a double plunger pump used for delivery of the carrier solution, a 16-way auto-switching valve with three PTFE solution loops, a peristaltic pump used for delivery of the sample solution to the middle PTFE loop (sample loop), and water plug (or running buffer plug) to the first (20 μ L) and third (20 μ L) PTFE loops (reagent loops). A 33 cm length PTFE tubing (0.5 mm i.d.) from the valve to the split-flow interface was used as a transport line. The charging time (CT) and injecting time (IT) in the FI system were set by manual acting. A schematic diagram of two stages of an FI system with a 16-way auto-switching valve and detailed description had been given elsewhere.¹⁶

The H-channel interface (Fig. 2) constructed using a planar plastic slide B (30 $\times$ 80 $\times$ 2 mm) as the base plate integrated a separation capillary (75 μ m i.d. $\times$ 375 μ m o.d. $\times$ 48.4 cm length and 45 cm effective length) and two reservoirs. The two reservoirs were produced from Tygon tubing (T1) (3.0 mm i.d., 4.0 mm o.d., 70 mm length for anodic reservoir and 110 mm length for cathodic reservoir), which were first fixed sideways on slide B with epoxy glues, and then connected with the separation capillary having both ends inserted in the center of the Tygon tubing (T1) through holes punctured with a hypodermic stainless-steel needle. Two platinum electrodes that

were used as the anode and cathode were, respectively, inserted into the two reservoirs 30 mm above the capillary tip to avoid entrainment of electrolytically generated oxygen bubbles into the capillary. A 30 mm long, 1.2 mL/min flow rate of Tygon tubing (T2) was push-fitted into the anodic reservoir until 12 mm below the capillary tip, and functioned as the carrier/sample (C/S) inlet. The carrier buffer (or the sample solution) was introduced from the C/S inlet and expelled by forced outflow via an upper outlet. By employing an outflow rate higher than the inflow, the liquid level was maintained constant and identical to that of the capillary outlet, which could decrease the hydrodynamic flow. The lower section of cathodal reservoir (T1) was controlled by a screw clamp, except for filling or changing the electrolyte solution, or for capillary cleaning. While the sample plug passed along the capillary inserted in the split-flow interface, a small fraction of the sample was electrokinetically introduced into the capillary. During this entire sequence, a high, constant voltage was applied across the two platinum electrodes shown in Fig. 2. The time period for the injecting sample was defined through the man/access mode.

The pH values were determined by a PHS-10A pH meter (Xiaoshan Instrumental Factory, Zhejiang, China). The pH meter was calibrated by 0.1 M borate every day.

Preparation of the reagents and drugs

Buffer solutions were prepared by diluting 0.1 M borate stock solutions and ACN with distilled water, and were then adjusted to the desired pH using either 2 M NaOH or 2 M HCl. Stock standard solutions (1000 µg/mL) of Dex, Chl, Pse and Par were prepared with distilled water; working standard solutions were then obtained by diluting the corresponding stock solutions to the desired concentrations with distilled water or running buffer. All buffer solutions were filtered through a 0.45 µm syringe filter before use. Twelve tablets of each preparation of Anma Meimin Tablets (Lei Meng Xin) and Anfen Weima Namin Tablets (Hai Wang Yin De Fei) were weighed exactly and the average weight of each tablet was calculated, then powdered, and a quantity of the powders was weighed exactly, and extracted with 25 mL water for 1 h in an ultrasonic bath, respectively. Prior to analysis, all of the extracts were filtered through a 0.45 µm syringe filter. The solutions were diluted with distilled water, and then injected into the capillary by the 16-way auto-switching valve of K-1000 FIA.

Electrophoretic procedure

Prior to the first use, the new capillary was conditioned with distilled water for 10 min, 0.1 M NaOH for 10 min and distilled water for 10 min, followed by the running buffer for 10 min from the capillary outlet reservoir using a water-circulating vacuum pump. At the beginning of each working day, the capillary was flushed sequentially with distilled water for 5 min, 0.1 M NaOH for 5 min and distilled water for 5 min, followed by the running buffer for 5 min. Simultaneously, the CE instrument was warmed up until a stable baseline was achieved. Moreover, the capillary was washed with distilled water (2 min), 0.1 M NaOH (3 min), distilled water (2 min) and equilibrated with the fresh background electrolyte (BGE) (3 min) by manual handling after each run to ensure a good repeatability. The capillary was left filled with distilled water overnight. Electrokinetic injection was used in the FI-CE system.

Focusing theory

The basic model of sample stacking is illustrated in Fig. 3. A plug of water was injected by electrophoretic flow (EOF) into the capillary to provide a high electric field at the injection

Fig. 3 Schematic illustration of the sample stacking mechanism of HC-FASS in CZE. (A) Sandwich injection, (B) electric field strength augmentation, (C) stacking of sample.

point, the sample was dissolved in a solution that had a lower conductivity than that of the BGE and injected as a long plug compared to that in normal injection, and was injected electrokinetically into the capillary and sandwiched by distilled water (Fig. 3A). The sample and BGE zones were the low- and high-conductivity zones, respectively. When a separation voltage was applied, the electric field strength experienced in the water and sample zone was higher than in the rest of the capillary (Fig. 3B). Because of the high electric field, the sample ions moved rapidly toward the boundary of water and the buffer, and slowed down when they entered the buffer zone with a low electric field. Consequently, the sample ions were focused at the boundary of the two zones (Fig. 3C).

Results and Discussion

Optimization of the separation conditions

In this part, we used orthogonal design (OD) to optimize the separation conditions. We investigated the factors including the buffer pH and concentration, ACN concentration (v/v) and separation voltage. The biggest peak area of Chl, good resolution, better peak figure and shorter analysis time were the optimum targets. The optimum separation conditions were 55 mM borate-15% (v/v) ACN (pH 9.3), 6.0 kV separation voltage. Under the optimum conditions, the elution order of the analytes was Dex, Chl, Pse and Par.

Influence of the volume of sample loop on stacking

The volume of the sample loop in FI was an important factor that determined the amount of sample introduced into the capillary. Because of the diffidence in the FI system, the volume of the sample loop in FI was much larger than that of the separation capillary in CE. For low limits of detection (LOD) of CE, a large volume of the injection fluid was required for samples at low concentration. However, when the injection volume was in excess, broadening of the migration peaks affected the separation adversely. The 58, 115, 211, and 379 µL sample loop volumes were investigated for pH 9.3, 55 mM borate-15% (v/v) ACN, 6.0 kV applied voltage, CT, 15 s, IT, 84 s and 80 s electroinjection time. The peaks areas of Dex, Chl, Pse and Par increased with an increase of the sample loop volume. When the sample loop volume was >211 µL, the peak

Fig. 4 Effect of CT on the peak area. Experimental conditions: buffer, 55 mM borate-15% ACN, pH 9.30; separation voltage, 6.0 kV; sample concentration, 7.5 $\mu\text{g/mL}$ Dex, 3.25 $\mu\text{g/mL}$ Chl, 9.15 $\mu\text{g/mL}$ Pse, 16.25 $\mu\text{g/mL}$ Par; capillary, 75 μm i.d. $\times$ 375 μm o.d. $\times$ 48.4 cm length (45 cm effective length); sample loop volume, 211 μL ; electroinjection time, 80 s (6.0 kV); detection wavelength, 214 nm; IT, 84 s; carrier flow-rate, 1.2 mL/min; separation temperature, room temperature.

Fig. 5 Effect of IT on the peak area. CT, 15 s. Other conditions were as in Fig. 4.

shapes of the analytes gradually became broad. Meanwhile, the theoretical plate numbers were calculated in order to compare well the peak shapes. When the volume of sample loop was 211 and 379 μL , the theoretical plate numbers of Dex, Chl, Pse and Par were 8899, 23922, 12804, 14421 and 5480, 14094, 11113, 12184, respectively. Obviously, the peak shapes of the analytes, when the sample loop volume was 211 μL , were better than those when the sample loop volume was 379 μL , in theory. Therefore, a 211 μL sample loop volume was chosen for further study.

Influence of the charging time (CT) and the injecting time (IT) on stacking

A longer sampling time was needed in order to achieve higher sensitivity.²⁵ To ensure completion of the sample loop and to achieve higher sensitivity, while not consuming much more samples, the effects of CT in the range of 5–21 s (Fig. 4) and IT in the range of 12–84 s (Fig. 5) were studied under the electrophoresis conditions described above. The peak areas

Fig. 6 Effect of the electroinjection time on the peak area. CT, 15 s; IT, 84 s. Other conditions were as in Fig. 4.

almost did not increase when CT was more than 15 s, and sensitivity (peak area and peak height) was augmented with the increase of IT; however, the resolution decreased and the peak figures were broadened when IT was more than 84 s. Because better results could be attained in fitting of IT and CT, 15 s CT and 84 s IT were selected in this analysis.

Influence of the electroinjection time on stacking

The amount of sample introduced is mainly controlled by the electroinjection time. To determine how it affected the sensitivity, we investigated the influence of electrokinetic injection (6.0 kV) on the peak area at between 20 and 100 s under the conditions selected above (Fig. 6). Obviously, upon increasing the electroinjection time, an increase in the response was observed. The reason for this could be due to the fact that the longer was the electrokinetic injection time, the larger was the sample volume injected into the capillary. The peaks areas were proportional to an electroinjection time of between 20 and 80 s, as shown in Fig. 6. However, the longer was the electroinjection time, the longer did it take to stack the samples. A significant peak broadening was obtained for a long electroinjection time, which can be explained by laminar flow. The laminar flow caused peak broadening, which decreased the stacking efficiency. As a compromise, 80 s of electrokinetic injection of the sample with both a high stacking efficiency and a good peak shape was chosen as the optimum condition, and concentration and separation were thus implemented in one step.

Impact of the preinjection water plug on stacking

The data presented in this work demonstrated that the use of a short water plug at the column inlet was essential for improving the reproducibility of FASS with electro-injection, and that the water plug injection volume significantly affected the sensitivity. The sample concentration was further increased by about 2–4 fold by introducing a longer water plug (about 20 μL). When the water plug was longer, laminar flow originated from a mismatch of the EOF velocity of the water plug zone, and the buffer solution became stronger. As a consequence, the peak overload, stacking efficiency decreased, resulting in a decrease in sensitivity.²⁶ Thus, the optimal water plug injection volume was about 20 μL in this study.

In order to validate the effect of stacking, three methods with electrokinetic injection were investigated: normal FI-CE,

Table 1 Stacking enhancement factor (SE) of the peak area and the peak height

Injection method	Peak area				Peak height			
	Dex	Chl	Pse	Par	Dex	Chl	Pse	Par
CE-FI (N_1)	5.8×10^3	5.7×10^3	2.1×10^3	4.4×10^3	1.1×10^2	1.3×10^2	64	80
CE-FI-FASS (N_2)	1.1×10^4	1.0×10^4	8.4×10^3	1.0×10^4	1.8×10^2	1.6×10^2	1.3×10^2	1.4×10^2
CE-FI-HC-FASS-LVSS (N_3)	1.6×10^5	2.2×10^5	2.1×10^5	1.5×10^5	9.1×10^2	1.4×10^3	1.7×10^3	1.4×10^3
SE (N_2/N_1)	1.9	1.8	4.0	2.3	1.6	1.2	2.0	1.8
SE (N_3/N_2)	14.7	21.9	24.6	14.9	5.2	9.0	13.3	9.5
SE (N_3/N_1)	27.3	38.5	99.0	34.4	8.6	11.0	26.2	17.2

Fig. 7 Typical electropherograms obtained by using three different methods. (A) FI-CE with sample dissolved in 55 mM borate-15% (v/v) ACN; (B) FI-CE-FASS with sample dissolved in water; (C) FI-CE-HC-FASS-LVSS with a water plug in front of the sample dissolved in water and electrokinetic injection samples 80 s (6.0 kV). Experimental conditions: buffer, 55 mM borate-15% ACN, pH 9.30; separation voltage, 6.0 kV; sample concentration, 7.5 $\mu\text{g/mL}$ Dex, 3.25 $\mu\text{g/mL}$ Chl, 9.15 $\mu\text{g/mL}$ Pse and 16.25 $\mu\text{g/mL}$ Par; capillary, 75 μm i.d. $\times$ 375 μm o.d. $\times$ 48.4 cm length (45 cm effective length); sample loop volume, 211 μL ; detection wavelength, 214 nm; CT, 15 s; IT, 84 s; carrier flow-rate, 1.2 mL/min; separation temperature, room temperature; peaks, Dex (1), Chl (2), Pse (3) and Par (4).

Table 2 Comparison of the LOD (mg/mL) between this method and that from the literature

	Dex	Chl	Pse	Par	Ref.
FI-CZE- stacking	1.94×10^{-5}	0.64×10^{-5}	1.16×10^{-5}	2.84×10^{-5}	This method
FI-CZE	2.2×10^{-4}	2.9×10^{-4}	4.2×10^{-4}	7.0×10^{-4}	6
CZE	2.5×10^{-3}	—	7.5×10^{-4}	1.2×10^{-3}	5
NACE	9.8×10^{-4}	1.19×10^{-3}	1.94×10^{-3}	—	7
MEKC	—	5.0×10^{-4}	—	4.0×10^{-4}	3

FI-CE-FASS, and FI-CE-HC-FASS-LVSS. In normal FI-CE (*i.e.*, without concentration) samples were prepared in a buffer solution that had the same concentration as that used in the separation. In FI-CE-FASS and FI-CE-HC-FASS-LVSS, samples were prepared in water. The electropherograms obtained using three different methods are shown in Figs. 7A – C. In our study, a water plug introduced by EOF could further enhance the stacking efficiency. It could be explained that this mode of introducing the water plug could avoid the probability of a disturbance induced by hydrodynamic flow at the sample-buffer boundary, which decreased the stacking efficiency. It was evident from the electropherograms that we achieved a large field enhancement at the injection point in FI-CE-HC-FASS-LVSS.

Calculation of the stacking enhancement factor

In order to estimate the extent of the online preconcentration of the test compounds, the stacking enhancement factors (SE) of the peak heights and the peak areas of Dex, Chl, Pse and Par were calculated by using the equations²⁷ $SE_{\text{height}} = H_{\text{stack}}/H$ and $SE_{\text{area}} = A_{\text{stack}}/A$, where the numerator was the peak height or peak area obtained with stacking, and the denominator was the peak height or peak area obtained without preconcentration. The results are shown in Table 1. In order to explain the sensitivity of the developed method, we compared the LODs of this method with those from the literature. The results are given in Table 2. Tables 1 and 2 suggest that the developed method can improve the sensitivity of detection.

Performance of the combined FI-CE system based on the HC-FASS-LVSS stacking technique

Standard solutions were preconcentrated by the HC-FASS-LVSS preconcentration technique in order to obtain a calibration graph. Each point on the calibration graph corresponded to the mean value obtained from four independent peak-area measurements. The corresponding regression equations as well as other characteristic parameters for the determination of Dex, Chl, Pse and Par are given in Table 3. The LODs were estimated from the calibration curve of the peak height *versus* the standard

Table 3 Analytical performance of FI-CE based on the HC-FASS-LVSS stacking technique in the analysis of four components ($n = 4$)

	Dex	Chl	Pse	Par
LOD ($S/N = 3$)/ mg mL ⁻¹	1.94×10^{-5}	0.64×10^{-5}	1.16×10^{-5}	2.84×10^{-5}
Peak area RSD, %	1.01	1.91	0.89	0.92
Peak height RSD, %	1.94	3.98	2.66	3.27
Regression equation ^a				
a	-2.58×10^3	-1.18×10^3	4.32×10^3	9.94×10^2
b	2.29×10^4	5.71×10^4	2.24×10^4	9.64×10^3
Correlation coefficient	0.9989	0.9994	0.9992	0.9990
Linear range/ μ g mL ⁻¹	0.94 – 30	0.06 – 15	0.47 – 30	0.94 – 60

a. $y = a + bx$; y , peak area; x , standard concentration (μ g/mL).

Table 4 Comparison of the contents between found and labeled in different medicines

Sample	Ingredient	Found/ mg tablet ⁻¹	Labeled/ mg tablet ⁻¹	Relative error, %	Average recovery, %
Leimengxin	Dex	15.25	15	1.67	98.89
	Chl	2.02	2	1	96.68
	Pse	29.24	30	-2.5	100.01
	Par	337.21	325	0.32	101.25
Haiwang-yindefei	Chl	2.14	2	7	98.22
	Pse	29.61	30	-1.3	96.72
	Par	320.20	325	-1.48	102.94

concentration, and based on the concentration necessary to yield a net height equal to three-times the standard deviation (SD) of the baseline noise. The baseline noise was evaluated by recording the detector response every 3 s over a period of about 2 min. Because of using FI equipment and the presence of the water plug, the repeatability of the peak area was improved. The repeatability of the FI-CE-HC-FASS-LVSS system was evaluated under the investigated conditions by using a standard solution containing 7.5 μ g/mL Dex, 3.75 μ g/mL Chl, 9.15 μ g/mL Pse and 16.25 μ g/mL Par. The electropherogram of a standard solution obtained by using the HC-FASS-LVSS concentration technique is shown in Fig. 7C. The separation could be achieved within 23 min, and the sample throughput rate could reach up to 4 h⁻¹. Compared with some previous reports, the sensitivity enhancement in this work was not very high. This was caused by the following reasons: (1) within the column FASS, a part of the capillary, or even the whole separation space, was filled with a sample solution. Thus, the sensitivity was based on the sample volume injected, and was limited by the injection volume. Generally, the concentration sensitivity did not exceed 1 – 2 orders of magnitude with a UV detector. (2) In the combined FI-CE system, the injected sample underwent dispersion in the connection lines and split-flow interface during transport to the inlet of the capillary before entering the capillary, mainly owing to convection.

Fig. 8 Electropherogram of Anma Meimin Tablets (Lei Meng Xin). Experimental conditions: capillary, 75 μ m i.d. $\times$ 375 μ m o.d. $\times$ 48.4 cm length (45 cm effective length); buffer, 55 mM borate-15% (v/v) ACN (pH 9.3); voltage, 6.0 kV; sample loop volume, 211 μ L; electroinjection time, 80 s; detection wavelength, 214 nm; CT, 15 s; IT, 84 s; carrier flow-rate, 1.2 mL/min; separation temperature, room temperature; peaks, Dex (1), Chl (2), Pse (3) and Par (4).

Application

The FI-CE-HC-FASS-LVSS method was applied to a simultaneous analysis of commercial medicines containing these analytes. The contents of the analytes found in different kinds of medicines are given in Table 4. The typical electropherogram with twice sampling continuously of Anma Meimin Tablets (Lei Meng Xin) is shown in Fig. 8. The peaks were identified by the standard addition method. The accuracy of the method and the potential matrix effects were established by analyzing the samples. The recoveries of the four constituents were determined by the addition of the standard substances in real samples. It showed an agreement between the claimed and found values. The recoveries of the spiked analytes for these samples were satisfactory, and the method was applicable for the quantification of Dex, Chl, Pse and Par with high accuracy, precision and repeatability.

Conclusions

The continuous online preconcentration approach based on HC-FASS-LVSS in a FI-CE system has been proven to be a feasible and attractive way to improve the sample sensitivity of detection in CE. It was a useful method for the stacking of Dex, Chl, Pse and Par and simultaneous determination of Chl and Par. The preinjection water plug introduced by EOF could avoid a disturbance of the sample-buffer zone boundary, due to pressure-induced flow, and could thus help to maintain field enhancement, and acted as a reservoir to hold the sample. The presence of the water plug also improved the repeatability. This technique further exploited the potential of the FI-CE system. The preconcentration technique was simple to perform, using conventional instrumentation without any complicated procedures. The inherent simplicity and effectiveness of this preconcentration procedure makes it a facile way to enhance sensitivity in the FI-CE system.

Acknowledgements

The project was supported by the National Natural Science Foundation of China (No. 20275014).

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