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ORIGINAL

Development and Evaluation of Quantitative Capillary Electrophoresis with a 4-nL Internal Loop Injector

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Abstract A quantitative capillary electrophoresis (qCE) apparatus has been developed and evaluated in order to improve the accuracy and precision of the currently commercially available CE. A four-port nano-valve with a 4-nL internal loop is employed in the apparatus to precisely inject the amount of sample. A micro-fluidic device based on a syringe pump provides a stable and mild flow to ensure the stability and reliability of the instrument. The separation capillary has an inner diameter of 50 μm i.d. and 40-cm effective length. Run-to-run reproducibility in terms of the relative standard deviation lower than 1 % for the peak area and 0.5 % for retention time were respectively achieved using a test sample of dimethylsulfoxide. Six synthetic adulterants, namely fenfluramine, sibutramine, pseudoephedrine, amfebutamone, clenbuterol and norepinephrine, were used to test the feasibility of the qCE system under the following experimental condition: an electrolyte containing 20 mM NaH_2PO_4/H_3PO_4 at pH 2.0 with a thermostat temperature of 15 °C and voltage at 15 kV.

Keywords Quantitative capillary electrophoresis · Nanoliter injector · Electric decoupler · Adulterant

Introduction

Capillary electrophoresis (CE) has the advantages of high efficiency, high resolution, low consumption of sample and reagent, and simple instrumentation compared with other conventional separation methods, such as high-performance liquid chromatography (HPLC). However, the limitations of sample introduction make the commercially available CE instruments hard to use broadly in quantitative analysis, for example, quality control (QC) or quality assurance (QA).

Conventional sample introduction in CE mainly includes electrokinetic injection and hydrodynamic injection, but both have some drawbacks [1–7]. The former is prone to sample discrimination, and the latter is not suitable for high-viscosity samples and buffer. Moreover, both the above-mentioned methods need a capillary inlet dip into the sample vial, then move back to the buffer vial. This "dip in" method has the ollowing natural defects: first, there is no way to accurately know how much sample has been injected; second, the precision (reproducibility) is not as good as the injection by a fixed loop valve as in HPLC. In addition, the movement of the capillary inlet between the sample and buffer not only causes cross contamination, but also disturbs the equilibrium of the electrochemistry in the separation capillary and therefore sacrifices the accuracy and reproducibility in quantitative analysis. The rotary valve injector has already been generally utilized as a sample injection method for HPLC, which has a sample volume on the order of microliters. However, the injection volume in CE and capillary electrochromatography (CEC) should be lower than 20 nL for a capillary with an inner diameter smaller than 100 μm. Apparently, it is not possible to use an HPLC rotary valve injector directly for CE and CEC. Several groups have attempted to change the status quo with the introduction of a nano-injector. Tsuda et al. [8] first put forward the concept of a nano-injector in CE in 1987; an injector with a rotor and stator made of ceramic material was used for sample introduction, but the injection amount of 350 nL was too large. Takeuchi and Ishii [9] studied a 20-nL injector for use in micro HPLC. They used a very

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thin rotor (0.7 mm) made of stainless steel. The use of the rotor has two drawbacks: first, extremely sophisticated technology is needed in the production process; second, a thin rotor is unstable. In 2003, Tsuda et al. [10] modified the rotary sampler and designed a 6–24-nL sampling valve for use in CE. They could make successive injections without interruption of the applied voltage but were not able to accurately control the amount of sample introduced.

Our group has been committed to research on quantitative sample introduction by using a nano-rotary-type injector for CE and CEC. We designed a CE apparatus including a nano-rotary-type injector for quantitative analysis in 2009 [11]; the dragging force of EOF was used to provide supplementary flow for 20-nL nano-valves. However, experiments were often interrupted because of the high buffer viscosity. In this work, we built a new quantitative CE (qCE) system by using a rotary-type injector with 4-nL volumes. A syringe pump coupled to a splitting tee provided supplementary pressurized flow. An electric decoupler was made to isolate the injection valve outside the electric field. With optimized conditions, run-to-run reproducibility in terms of a relative standard deviation (RSD) lower than 1 % for the peak area and 0.5 % for the retention time, respectively, was achieved using a test sample of dimethylsulfoxide (DMSO). This device can be used routinely for QC and QA in the pharmaceutical industry.

Weight loss is a hot topic, and all kinds of weight loss products continue to emerge. Individual manufacturers illegally add adulterants to weight loss products to achieve stimulating effects in a short time period. The adulterants mainly consist of anorexics and energy consumers such as fenfluramine, sibutramine and pseudoephedrine, etc. These adulterants can cause great harm to human health. Many countries have prohibited their use in all weight loss products. Therefore, it is necessary to establish a rapid and accurate separation and detection method for illegal additives. A number of HPLC and high-performance capillary electrophoresis (HPCE) methods have been reported for determination of the illegal additives. Wang et al. [12], using a liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) method, simultaneously determined six synthetic adulterants, namely fenfluramine, phenolphthalein, *N*-di-desmethyl sibutramine, *N*-mono-desmethyl sibutramine, sibutramine and orlistat. Li et al. [13] simultaneously determined seven adulterants in slimming functional foods by HPCE. In this work, we evaluated the qCE system for simultaneous determination of six synthetic adulterants including fenfluramine, sibutramine, pseudoephedrine, amfebutamone, clenbuterol and norepinephrine in weight loss products.

Experimental

qCE Apparatus and Capillary Arrangement

A schematic of the qCE system is shown in Fig. 1. A syringe pump (pc2 70-2219, Harvard, Holliston, MA, USA) and glass syringe (1002TLL, Hamilton, CH-7402, Bonaduz, Switzerland) were used to provide a flow, usually in a range of 0.25–2 μL min^{-1}, and split with a tee connector (P-727 PEEK Tee, Upchurch, WA, USA) coupled to a

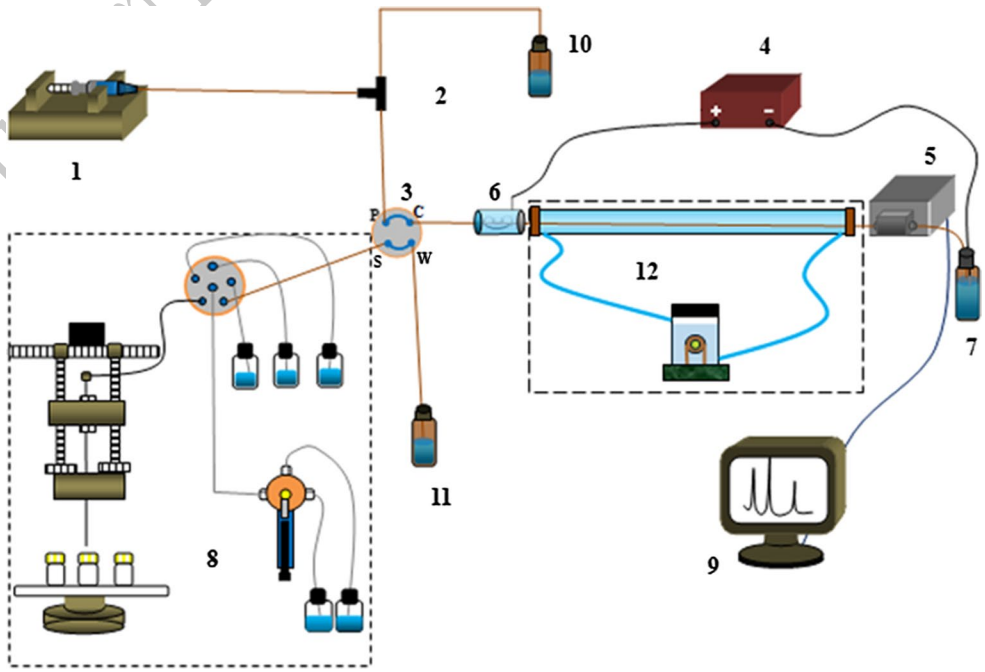

Fig. 1 Schematic overview of the qCE system. *1* Syringe pump (glass syringe). *2* Tee. *3* Four-port nano-valves. *4* High-voltage power supply. *5* UV detector. *6* Electrical decoupler. *7* Outlet buffer reservoir. *8* Autosampler. *9* Data handling system. *10–11* Waste reservoir. *12* Thermostat

piece of capillary with a certain i.d. and length (Polymicro Technologies, Phoenix, AZ, USA).

The splitter functions were used in such a way that they would change the splitting ratio during the operation to compensate for the electroosmotic flow (EOF) induced by the applied voltage. The split flow was introduced by a piece of capillary in the four-port nano-valves of 4 nL (C4N-4344-004 Valco, Houston, TX, USA) and drove the samples in the loop into the separation capillary. A ±30-kV high-voltage power supply and variable-wavelength UV/Vis absorbance detector (Unimicro Technologies, Pleasanton, CA, USA) were used. The two electrodes for the high-voltage power were placed in the outlet buffer reservoir and an electrical decoupler. A chromatography workstation (Unimicro Technologies, Pleasanton, CA, USA) was used for data acquisition. A laboratory-made autosampler was used for sample introduction. A laboratory-made thermostat with cooling capacity was used to control the temperature in the capillary.

Chemicals and Reagents

Fenfluramine hydrochloride, sibutramine hydrochloride, amfetamone hydrochloride, pseudoephedrine hydrochloride, clenbuterol hydrochloride and norepinephrine bitartrate were purchased from the National Institues for Food and Drug Control (Beijing, China). Adenine was purchased from Shanghai Bio Science (Shanghai, China). Cellulose acetate (CA), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and sodium borate were obtained from Sinopharm Chemical Reagents (Shanghai, China). Acetonitrile of HPLC grade was purchased from Tedia Co. (Fairfield, OH, USA). All other chemicals were of analytical grade. The SPE column (SCX, 500 mg, 3-mL column volume) was purchased from ANPEL Scientific Instruments (Shanghai, China). Three types of real samples including weight loss capsules, weight loss milk tablets and weight loss teabags were purchased from a local drug store. All the samples and buffer solutions were filtered by a 0.22-μm filter and degassed by a sonifier.

Preparation of Electrical Decoupler

A small scratch was made on the wall of a capillary at about 5 cm from one end; then, the capillary with the scratch was fixed on a plastic support by epoxy glue. Pressure was exerted on the scratch to make it fracture, then the fractured section was coated with a 12 % cellulose acetate solution (in acetone) in the cleft. After the acetone had evaporated, an electricity-conductive membrane was formed on the fracture region. Finally, this membrane-coated capillary section was put into a sealed container filled with 2 mL buffer solution, and the high-voltage electrode was also inserted into the container. The sealed container was actually an Eppendorf tube. We cut it at the bottom and used another cap to seal it; a small hole (about the same as the capillary o.d.) was drilled in both caps to allow the capillary to go through. When the membrane-coated capillary section was finished, we took off the caps on both sides and put the section into the Eppendorf tube, then threaded the capillary through the caps.

Sample Preparation

Preparation of Standard Solution

Six chemical drug standards were dissolved in water to 1 g l^{-1}, respectively, and diluted into eight concentration levels of 5.0, 10.0, 25.0, 50.0, 75.0, 100, 250 and 500 mg L^{-1}. Linear regression analysis of the peak area ratios with the above concentrations was made.

Preparation of Real Samples

Tablets, teabags and capsule contents were ground into powders, a 0.5-g portion of homogenized powders was accurately weighed and dissolved with 10 mL of 1 % formic acid solution and ultrasonic extraction for 30 min, then centrifuged at a rate of 3,000 r/min; the supernate was filtered by a 0.45-μm filter as stock solutions. The stock solutions (5 mL) were loaded onto an SPE column (SCX, 500 mg, 3-mL column volume) conditioned with methanol (5 mL), water (5 mL) and 1 % formic acid solution (5 mL), respectively. The SPE column was eluted with mixed solvent CH_2Cl_2–isopropanol–NH_4OH (78:20:2, v/v/v, 10 mL). The eluent was collected and dried under nitrogen and dissolved with 1 mL of 0.01 mol L^{-1} hydrochloric acid; after centrifuging at a rate of 10,000 r min^{-1}, the supernate was filtered by a 0.22-μm filter and used as a sample solution.

Sample Analysis Process

A piece of 60-cm-long fused-silica capillary (50 μm i.d., 370 o.d.) was inserted through an electrical decoupler and with the short end (about 5 cm) connected to the C port of the nano-valve as shown in Fig. 1. At 20 cm from the other end, a detection window of about 2-mm length was made by burning off the capillary coating. This end passed through the UV detection flow cell and stretched into the outlet buffer reservoir. The buffer in the glass syringe was driven by the pump with a piece of 100 μm i.d. capillary into a PEEK tee, then split and connected to the P port of the nano-valve. The S port and W port of the nano-valve connected to the

autosampler and waste reservoir, respectively. After a proper ratio of pressurized flow to applied voltage had been selected, the instrument was allowed to reach the steady state. Prior to the first use, the new capillary was conditioned by rinsing with methyl alcohol for 5 min, deionized water for 5 min, 0.1 M hydrochloric acid for 5 min, 0.1 M sodium hydroxide for 5 min and finally by background buffer (BGE) for 5 min. Thereafter, the capillary was rinsed with BGE for 2 min before each run. The separation capillary was thermostated at the desired temperature.

Results and Discussion

Study of the Syringe Pump

In our previous study [14], a dragging force of EOF was used to provide supplementary flow for the 20-nL nanovalves, but when lower volume valves of 10 nL with higher viscosity buffer were used, the liquid was hard to suck into the capillary. We then tried to use the gravity of the buffer in a bottle fixed at 1 m height to provide a gentle flow. However, the system was not reliable and stable as sometimes bubbles were generated inside the injector and stagnated the flow. Another method was to use a syringe pump to provide a consistent pressurized flow. After passing through a splitter, the flow into the capillary could be as low as dozens of nL/min and remained stable and reliable. Therefore, the syringe pump coupled with the splitter was chosen as the device to provide supplementary flow for the qCE system.

Optimization of Pressurized Flow and EOF

According to previous research [15–17], the profile of the EOF is plug-like, and the pressurized flow is parabolic. Therefore, column efficiency in CE is much higher than that in HPLC. In our case, pressurized flow is superimposed on the EOF; consequently, the column efficiency in qCE should be somewhat lower than that in CE, and, theoretically, the lower the pressurized flow, the higher the column efficiency. However, in practice, we have to carefully balance the EOF and the pressurized flow because of the complicated influence of the domination of the two driving forces on separation efficiency. We gradually reduced the flow rate of the syringe pump under a certain voltage (12 kV) to study the column efficiency of adenine. The results are shown in Fig. 2.

As we can see, the separation efficiency increased with the decreasing pressurized flow, reaching a maximum point when the pressurized flow reached 44 nL min^{-1} (EOF was 100 nL min^{-1}), where the separation efficiency was 210,000 plates/m for adenine. However, after passing the maximum efficiency point, when the pressurized flow was further reduced, the efficiency was decreased instead. The reason for this phenomenon may be that, after the optimum point, the pressurized flow was too low to play the role of providing enough quantity of buffer for the qCE system, resulting in the dragging force produced by the EOF, which may make the flow pattern an “inverted parabolic” profile, which consequently damaged the column efficiency. The key is to find the optimal ratio of EOF to pressurized flow. In our experience, the optimum ratio is about 2.3:1. In other conditions with different capillary i.d.s or different buffers,

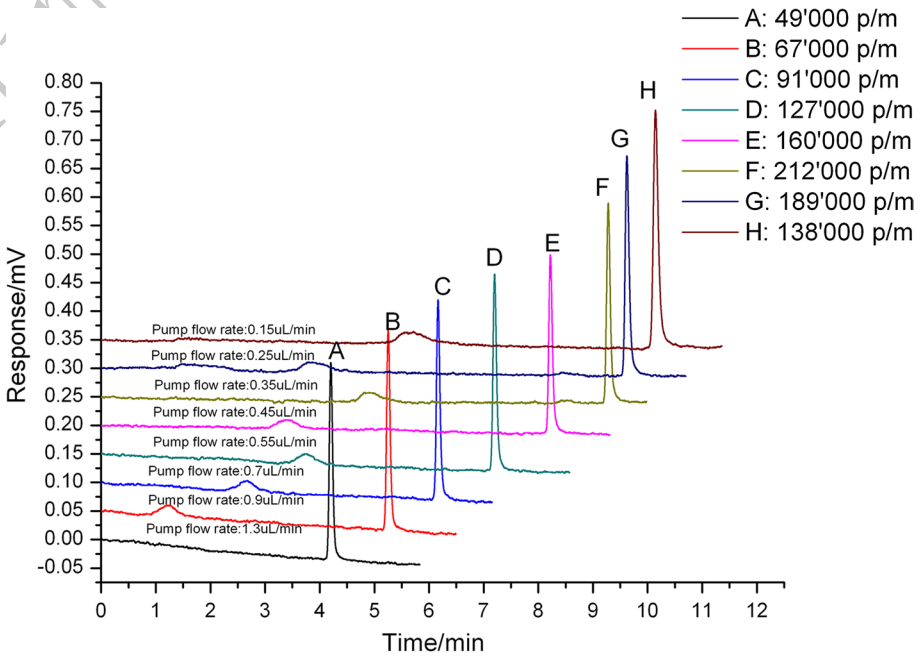

Fig. 2 Effect of flow rate of the syringe pump on efficiency at constant voltage. Capillary: 40 cm effective length, 50 µm i.d., 370 µm o.d.; voltage: 12 kV; wavelength: 220 nm, buffer: 30 mM sodium borate (pH 9.20); split ratio: 10:1 (no electric field); test sample: adenine

we measured the EOF first and then regulated the pressurized flow to an optimal range, roughly in the range of 2.3:1 (EOF to pressurized flow). According to the resolution of the separation, one can also adjust the ratio to achieve the desired separation time. For example, one can shorten the analysis time by increasing the pressurized flow when the resolution is much higher than needed. Therefore, the balance is not a fixed point for a specific separation, but a flexible range depending on the resolution and migration time. We also performed conventional CE with the same capillary at the same voltage with the same condition, and the column efficiency for adenine was 254,000 plates/m. Thus, the loss of column efficiency in this case was about 17 % in the qCE system compared with the conventional CE.

Electric Decoupler

In our experience, the qCE device does not function properly when the injector is located within the electrical field, probably due to the fact that the internal structure of the injector is too complicated to allow the electric current to pass smoothly. We had to apply the voltage somewhere from outside of the capillary after its connection to the injector using an "electric decoupler" (see Fig. 3). This decoupler serves two purposes: first, to isolate (or decouple) the injector from the electrical field; second, to apply voltage (current) from outside into the capillary, at the same time without allowing the buffer to leak out of the capillary.

Because of the electrolytic reaction on the electrode in the decoupler, the buffer concentration and pH may affect the reproducibility of the system. In this work, three buffer solutions of NaH_2PO_4 (pH 4), NaH_2PO_4–Na_2HPO_4 (pH 7) mixture and Na_2HPO_4 (pH 10) were chosen to represent acidic, neutral and alkaline solutions, respectively, and each buffer was set to three concentrations of 5, 20 and 100. We measured the current, and the results are shown in Table 1. The current at higher concentration (100 mM) is obviously higher than that at low concentration (5 mM). The exterior and interior of the capillary in the electric decoupler form an equivalent resistance. The resistance decreases with increasing concentration, leading to the increased current. In this experiment, the buffer concentration in the capillary was constant. The change in the solution concentration within the electric decoupler only played a small role in conducting the whole electric current, so the current intensity only increased slightly with the change of the solution concentration in the decoupler. The current under alkaline conditions (pH 10) was slightly higher than that under acidic conditions (pH 4). We know that when the positive electrode is placed in the decoupler, an electrode reaction occurs: $2H_2O \rightarrow 4e^- + 4H^+ + O_2$. The reaction is promoted under alkaline conditions and inhibited under acidic conditions. In our experiment, the variation of the current would have had a certain influence on reproducibility, so a neutral buffer was chosen as the solution in the decoupler.

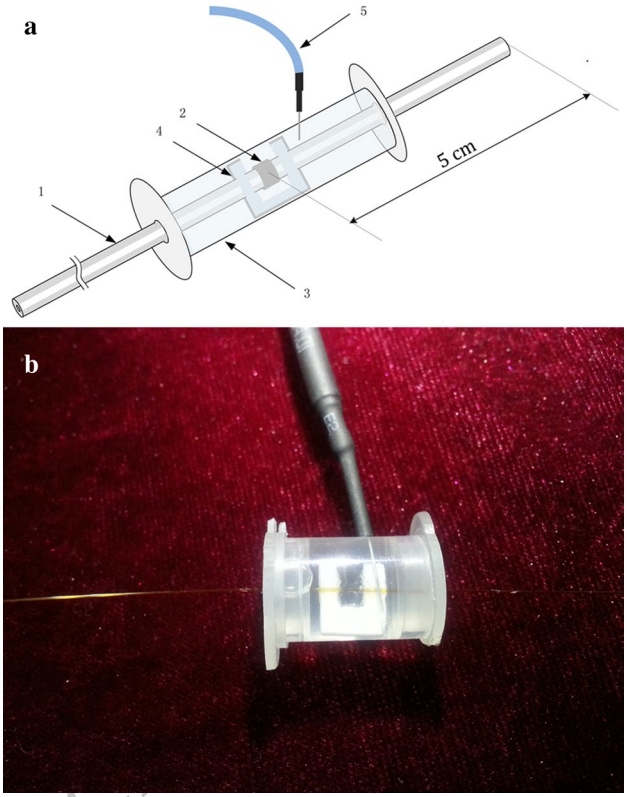

Fig. 3 **a** Schematic of the electrical decoupler. *1* Capillary. *2* Electricity-conductive membrane. *3* Sealed container. *4* Plastic support. *5* Electrode. **b** Photo of the electrical decoupler

Table 1 Effect of buffer pH and concentration on the current

	NaH_2PO_4 (pH 3) (μA)	NaH_2PO_4/Na_2HPO_4 (pH 7) (μA)	Na_2HPO_4 (pH 10) (μA)
5 mM	16.8	17.8	20.5
20 mM	17.2	18.5	21.5
100 mM	19.0	20.3	23.0

Temperature Control

Joule heat is produced in the process of running CE as well as in the qCE system [18–20]. Joule heat makes the temperature in the capillary increase and results in changes in the mobile phase viscosity, zeta potential and dielectric constant, leading to changes in the electroosmotic velocity. Besides, the diffusion coefficient of the solute, the ionic strength and pH of the buffer were also affected by temperature. In our work, a laboratory-made thermostat with a cooling capacity was built to control the temperature in the

Table 2 Temperatures effect on qCE performance

Temperatures (°C)	Column efficiency (plates/m)	Time (min)	RSD of time (%)	RSD of peak area (%)	Current (μA)
25[a]	75,000	5.4	2.81	2.80	32.5
20	77,000	6.2	1.37	2.02	28.0
15	79,000	6.4	1.02	2.64	26.5
10	81,000	6.9	1.05	1.02	24.2
5	83,000	7.5	0.73	0.58	22.4

[a] Parameters at 25 °C were actually measured at room temperature without a thermostat

capillary. The parameters were measured at different temperatures including 20, 15, 10 and 5 °C. DMSO was chosen as the sample for this experiment, and the results are listed in Table 2. In general, as the temperature decreased, the column efficiency increased, the migration time was prolonged, the current was reduced, and the reproducibility became better. The increase of column efficiency is mainly due to the reduction of solute longitudinal diffusion and Joule heat. The electrical current reduction mainly results from the reduction of the ionic activity. In general, the temperature control, especially the cooling capacity, is favorable for the performance of the qCE system.

Real Sample Analysis in qCE

Optimization of the Separation

In a previous study [21], a micellar electrokinetic chromatography method was used to determine seven synthetic adulterants. In our study, three commonly used buffers, namely sodium tetraborate, sodium dihydrogen phosphate and disodium hydrogen phosphate, were tested at different concentrations and pHs. In the case of 20 mM sodium tetraborate at pH 9.30 and 20 mM disodium hydrogen phosphate at pH 9.50, we found that the peak of norepinephrine was significantly broadened, and sibutramine could not be detected. Besides, the resolution of fenfluramine and amfebutamone was bad in the case of disodium hydrogen phosphate. The situation improved by using 20 mM sodium dihydrogen phosphate at pH 3.60. For this instance, fenfluramine, pseudoephedrine, amfebutamone and norepinephrine were successfully separated, but sibutramine and clenbuterol overlapped. The situation improved after adding some 0.1 mol L^{-1} phosphoric acids, which made the solution pH value decrease to 2.0. The six synthetic adulterants were completely separated at 20, 40, 60 and 80 mM of NaH_2PO_4/H_3PO_4 (v/v, 95/5) concentrations, but a shorter run time was obtained with 20 mM NaH_2PO_4/H_3PO_4. Finally, 5 % acetonitrile was added to further improve the resolution. The experiments were conducted at 5, 10, 15, 20 and 25 °C, respectively. The resolution became better but the migration time prolonged as the temperature decreased. Finally, 15 °C was chosen as the separation temperature.

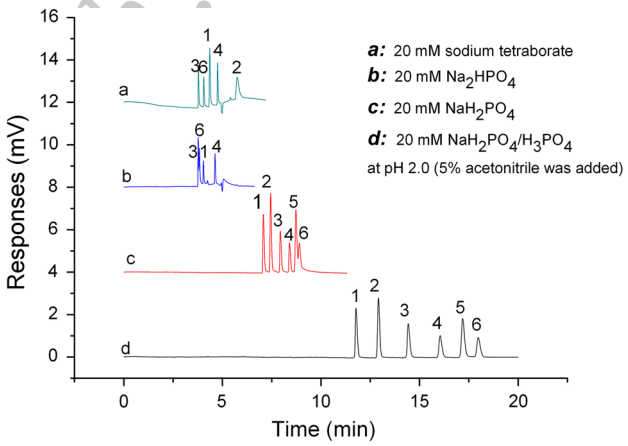

Fig. 4 Electropherogram of six synthetic adulterants. Experimental conditions: capillary: 40 cm effective length, 50 μm i.d., 370 μm o.d.; voltage: 15 kV; wavelength: 195 nm; temperature: 15 °C; pressurized flow rate by syringe pump: 0.6 μL min^{-1}; split ratio: 10:1 (no electric field); buffer: *a* 20 mM NaH_2PO_4/H_3PO_4 at pH 2.0 (5 % acetonitrile was added), *b* 20 mM NaH_2PO_4, *c* 20 mM Na_2HPO_4, *d* 20 mM sodium tetraborate; analytes: *1* pseudoephedrine, *2* norepinephrine, *3* fenfluramine, *4* clenbuterol, *5* sibutramine, *6* amfebutamone

Table 3 Regression equations and detection limits of six synthetic adulterants

Samples	Regression equation	*r*	Detection limit (μg mL^{-1})	RSD %
Pseudoephedrine	$y = 314.61x - 705.68$	0.999	0.5	1.27
Norepinephrine	$y = 381.35x + 0.408$	0.999	1.0	0.63
Fenfluramine	$y = 249.32x - 191.18$	0.999	1.0	1.48
Amfebutamone	$y = 227.39x - 998.24$	0.997	1.5	1.09
Sibutramine	$y = 431.69x - 1{,}127.2$	0.999	0.5	1.98
Clenbuterol	$y = 272.5x - 862.4$	0.996	1.0	1.58

y peak area, *x* concentration

The optimal condition comprises an electrolyte containing 20 mM NaH_2PO_4–0.1 mol L^{-1} H_3PO_4 (v/v, 95/5) at pH 2.0 with the capillary temperature at 15 °C, 15-kV voltage and 195-nm detection wavelength. The syringe pump flow-rate was set at 0.6 μL min^{-1} with a split ratio of 10:1 (in the absence of the electric field). The split ratio would be slightly decreased to about 9:1 because of the supplementary EOF when an electric voltage of 15 kV was applied. In this experimental condition, the EOF was 125 nL min^{-1}, and the pressurized flow rate was 54 nL min^{-1}. Consequently, the ratio of EOF to pressurized flow was about 2.3:1. Figure 4 presents electropherograms showing the separation of these adulterants at different experimental conditions.

Linearity, Detection Limits and Reproducibility

Under the optimum conditions, calibration curves for fenfluramine, sibutramine, pseudoephedrine, amfebutamone, clenbuterol and norepinephrine were established by preparing eight different concentrations of standard solutions. Each concentration was injected six times; the regression equations of the six curves (*x*-axis as concentration, *y*-axis

Table 4 Determination of real sample with the qCE system ($n = 3$)

Sample	Compound	Added amount (μg)	Founded amount (μg)	Recovery	
				Recovery (%)	RSD (%)
Teabags	Pseudoephedrine	50.0	50.4	100.8	2.38
		25.0	24.6	98.4	3.14
	Norepinephrine	100.0	98.2	98.2	3.65
		50.0	47.9	95.8	0.35
	Fenfluramine	100.0	100.5	100.5	2.22
		50.0	48.9	97.8	5.04
	Amfebutamone	200.0	197.8	98.9	4.46
		100.0	98.3	98.3	0.65
	Sibutramine	50.0	47.6	95.2	2.53
		25.0	23.8	95.2	5.48
	Clenbuterol	100.0	98.7	98.7	1.14
		50.0	48.4	96.8	2.37
Capsules	Pseudoephedrine	50.0	50.7	101.4	2.68
		25.0	25.7	102.8	3.91
	Norepinephrine	100.0	98.7	98.7	0.90
		50.0	48.5	97.0	4.47
	Fenfluramine	100.0	101.2	101.2	4.01
		50.0	50.0	100.0	2.63
	Amfebutamone	200.0	198.2	99.1	1.54
		100.0	98.8	98.8	1.18
	Sibutramine	50.0	48.1	96.2	2.54
		25.0	24.3	97.2	1.83
	Clenbuterol	100.0	99.2	99.2	0.90
		50.0	48.9	97.8	4.68
Tablets	Pseudoephedrine	50.0	49.9	99.8	2.78
		25.0	24.1	96.4	1.84
	Norepinephrine	100.0	98.7	98.7	1.66
		50.0	47.9	95.8	3.27
	Fenfluramine	100.0	98.6	98.6	1.66
		50.0	48.6	97.2	5.41
	Amfebutamone	200.0	197.6	98.8	3.23
		100.0	98.6	98.6	2.78
	Sibutramine	50.0	47.4	94.8	0.65
		25.0	23.1	92.4	1.65
	Clenbuterol	100.0	98.1	98.1	1.92
		50.0	49.1	98.2	3.65

as peak area) and their correlation coefficients (r) are shown in Table 3. The detection limits (signal-to-noise ratio of 3) for the six synthetic adulterants are: 0.5 μg mL^{-1} for pseudoephedrine and sibutramine, 1.0 μg mL^{-1} for norepinephrine, fenfluramine and clenbuterol, and 1.5 μg mL^{-1} for amfebutamone, respectively. The results showed that the curves are linear in the concentration ranges studied with acceptable correlation coefficients, and the sensitivities and reproducibility are satisfactory.

Application for Analyses of Capsules, Tablets and Tea Bags

A 1 % formic acid was chosen for sample extraction considering they are weakly alkaline. An SPE column (SCX) was used to remove impurities in the samples thoroughly. The recovery rates of the six adulterants in the real sample were investigated in three replicate runs at two concentration levels, and the determination results are listed in Table 4. (The average recoveries of these real samples were between 92 and 103 %.) From the table, we can see that the method is highly reliable for the determination. Fenfluramine was detected in two real samples, 0.002 mg g^{-1} in tablets and 0.014 mg g^{-1} in capsules.

Conclusions

A quantitative capillary electrophoresis apparatus has been developed and evaluated. Experimental parameters, including the ratio of EOF to pressurized flow, the choice of buffer in the electric decoupler and the temperature effect, were investigated. The reproducibility in terms of the relative standard deviation (RSD) of the peak area lower than 1 % for DMSO was achieved. Finally, the system was evaluated by the analysis of six synthetic adulterants and the real sample. The feasibility and versatility of the qCE were demonstrated.

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References

1. Jorgenson JW, DeArman Lukacs K (1981) J High Resolut Chromatogr 4(5):230–231
2. Jorgenson JM, Lukacs KD (1981) Anal Chem 53:1298–1302
3. Tsuda T, Nomura K, Nakagawa G (1981) J Chromatogr 248:241–247
4. Tsuda T, Nomura K, Nakagawa G (1983) J Chromatogr 264:385–392
5. Burton DE, Sepaniak MJ, Maskarinec MP (1986) Chromatographia 21:583–586
6. Smith RD, Udseth RH, Loo JA, Wright BW, Ross GA (1989) Talanta 36:161–169
7. Fishman HA, Amudl NM, Lee TT, Scheller RH, Zare RN (1994) Anal Chem 66:2318–2329
8. Tsuda T, Mizuno T, Akiyama J (1987) Anal Chem 59:799–800
9. Takeuchi T, Ishii D (1981) J High Resolut Chromatogr 4:469–470
10. Izuka E, Tsuda T, Munesue M, Samizo S (2003) Anal Chem 75:3929–3933
11. Li MJ, Zhou JY, Gu X, Wang Y, Huang XJ, Yan C (2009) J Sep Sci 32:267–274
12. Wang J, Chen B, Yao S (2008) Food Addit Contam 25:822–830
13. Li Y, Hu JT, Shi Y (2011) Food Anal Methods 4:505–516
14. Li MJ, Zhou JY, Gu X, Wang Y, Huang XJ, Yan C (2009) J Sep Sci 32:267–274
15. Saeid M, Reza K, Mohammad E, Amir K (2013) Comput Fluid Dyn 27:599–616
16. Mirzo K, Coraline R, Leonid T, Cherney W, Sergey N (2012) Anal Chem 84:149–154
17. de Stephanie J, Sergey N (2012) Anal Chem 84:453–458
18. Guo FC, Ulrich T, Andreas S, Zhang YK (2004) J Chromatogr A 1044:287–294
19. Tang GY, Yang C, Gong HQ, Chai JC, Lam YC (2006) Anal Chim Acta 561:138–149
20. Michael U, Musheev R, Yuri F, Sergey N (2010) Anal Chem 82:8692–8695
21. Li Y, Hu JT, Shi Y, Sun AM, Sun CJ (2011) Food Anal Methods 4:505–516

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