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Original Paper

Quantitative capillary electrophoresis and its application in analysis of alkaloids in tea, coffee, coca cola, and theophylline tablets

A quantitative CE (qCE) system with high precision has been developed, in which a 4-port nano-valve was isolated from the electric field and served as sample injector. The accurate amount of sample was introduced into the CE system with high reproducibility. Based on this system, consecutive injections and separations were performed without voltage interruption. Reproducibilities in terms of RSD lower than 0.8% for retention time and 1.7% for peak area were achieved. The effectiveness of the system was demonstrated by the quantitative analysis of caffeine, theobromine, and theophylline in real samples, such as tea leaf, roasted coffee, coca cola, and theophylline tablets.

Keywords: Caffeine / Quantitative CE / Theobromine / Theophylline

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1 Introduction

CE has been developed into a major analytical tool owing to its high efficiency, high resolution, low consumption of sample and reagent, and simple instrumentation. It has been widely used in various fields such as pharmaceutical, environmental, biotechnology, and chemical analyses. However, there are still some limitations in the technique, especially those associated with sample introduction [1, 2]. The lack of a reliable mode of sample introduction hampers the application of CE in quantitative analysis, thereby limiting its scope of utilization.

There are several modes of sample introduction in CE, among which electrokinetic injection and hydrodynamic injection are frequently used [3]. In an electrokinetic method, the sample size is related to EOF, electrophoretic mobility, potential gradient, and injection time. However, sample discrimination is a great shortage of electrokinetic injection, which makes the analytical results unreliable for a mixture of neutral and charged compounds. For hydrodynamic injection, the sample is introduced into the column by pressure difference between the capillary inlet and outlet. But the pressure is influenced by many factors such as mechanical precision, temperature, and viscosity of the buffer. These factors are difficult to control accurately and especially unfavorable to the accuracy and reproducibility for quantitative analysis. In addition, both electrokinetic and hydrodynamic injections belong to the "dip in" method, and neither is as accurate as the injection method in LC where a rotary type of injection valve with a fixed sample loop is used for sample introduction. During the injection process in the "dip in" method, the applied voltage has to be interrupted, and the system will need a period of time to be stabilized when it is restarted, which also affects the analytical precision.

To overcome the limitations of traditional injection modes, several groups did a great deal of research in valve injection for CE in order to inject an accurate and precise amount of sample into the capillary column. After Tsuda and Zare [4] described a split injector for CE which may be recognized as the first successful coupling of flow injection (FI) to CE, several groups studied the combined FI-CE system. Fang *et al*. [5] used a flow-through reservoir equipped with a conical inlet as an interface to connect the FI system to the capillary inlet. However, the combined system consumed micro-liter samples, which made some sacrifice in detection sensitivity.

Nano-injector for CE was first introduced by Tsuda *et al*. in 1987 [6]. This was the first time to perform quantitative injection with CE, which brought about lots of advantages. The injection amount was accurate. Moreover, while a high electrical field was applied, successive injections were possible by setting a micro-syringe in line with the above passage. However, the sample volume

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Abbreviations: BA, benzoic acid; **CA/TB/TP,** caffeine/theobromine/theophylline; **FI,** flow injection; **qCE,** quantitative CE

(350 nL) was too large to get high performance for CE with a capillary inner diameter lower than 100 μm. For safety reasons, it was necessary for the injector to be operated automatically. Since the sample solution was kept under a high electrical field, there was a possibility that unstable components may decompose if kept in the flow passage for a long period of time. Although the rotary-type injector had some disadvantages, it was more reliable for quantitative determination and easy to operate. Their work was of significant contribution.

After this initial work, several groups put their energy into developing nano-injector for CE. Hanai *et al*. [7] described a slide-type nano-injector for CE. Tsuda *et al*. [8] constructed a sample chamber with a piece of fused-silica capillary in it. Yan *et al*. [9] designed a CE apparatus including a nano-rotary-type injector for quantitative analysis, in which both pressurized flow and EOF were used to drive the BGE solution. The efficiency was enhanced by increasing the contribution of electroosmosis and decreasing the contribution of pressurized flow to the overall flow. These nano-injectors have great advantages such as no capillary movements, no electric field interruption, no cross-contamination, and most importantly, more accuracy and reproducibility. But there was a problem with this technique. The injection valve was integrated into the electric field, which caused the possibility of bubble generation, leading to electric current breakage.

Therefore, there was a desire to develop a novel sample injection technology to circumvent the problems in current CE system. In this work, a new quantitative CE (qCE) system with high-precision was developed, in which an electric decoupler was introduced in order to put the injection valve outside the electric field, thereby avoiding bubble generation. The accurate amount of sample could be injected consecutively without voltage interruption.

Caffeine, theobromine, and theophylline are alkaloids widely existing in drugs and foods. They are of addiction and harm to human body by large dose or long-time use. Too much caffeine makes people restless, anxious, and irritable. It may also keep one from sleeping well and cause headaches, abnormal heart rhythms or other problems. The amount of these compounds needs to be controlled strictly due to their stimulative effects. Several chromatography methods such as HPLC [10], TLC [11], and CE [12, 13] have been reported for the determination of these alkaloids. Among these methods, RP-HPLC is one of the most commonly used technologies. Abuirjeie *et al*. [14] used LC equipped with derivative UV-spectrophotometry to simultaneously determine xanthines in selected food products. Meyer *et al*. [15] simultaneously determined caffeine, theophylline, and theobromine in different food products by LC with amperometric detection. Chen *et al*. [16] used ion chromatography to determine the three xanthines in foods and pharmaceutical preparations.

Despite of the advantages of LC, a new analytical method still needs to be developed for benefiting from full combination of the high-accuracy offered by LC and the high-efficiency provided by CE. However, to our knowledge, till now no report is cited in the literature for using CE with nano-valve injection as a separation analytical tool to determine caffeine, theobromine, and theophylline.

The aim of this work was to develop a qCE system and check its applicability by the separation and analysis of caffeine, theobromine, and theophylline in real samples including tea leaf, roasted coffee, coca cola, and a medicine, theophylline tablets. Various experimental parameters, such as the dimensions of the separation and connection capillaries, the buffer type, concentration and pH, were investigated and optimized.

2 Experimental

2.1 Chemicals and materials

Fused-silica capillaries (75 μm id, 375 μm od) were purchased from Yongnian Optical Fiber Factory (Yongnian, Heibei, China). Cellulose acetate (CA) was obtained from Shanghai Chemicals Company (Shanghai, China). Caffeine, theobromine, and theophylline (Sigma–Aldrich, St. Louis, USA) were better than 99%. All other chemicals were of analytical reagent grade or better. Deionized water (Millopore, Bedford, MA, USA) was used throughout the experiment. All the real samples were purchased from local super market and drug store, and divided into four categories: drinks including canned soft drink and tea drink, tea leaves, roasted coffee, and medicine.

2.2 Sample preparation

2.2.1 Drinks

A 5.0 mL portion of canned tea drink, coca cola, and sports drink was transferred separately into a 50 mL volumetric flask and diluted to volume with buffer (15 mM borax).

2.2.2 Tea leaves and roasted coffee

2 g of accurately weighed ground tea leaves or 1 g of roasted coffee was transferred into a 100 mL conical flask, and 70°C hot water was added. The solution was heated in a boiling water-bath for 60 min, then cooled to room temperature and filtered. Then the filtrate was sonicated and filtered again. Further dilutions (1:100) with buffer (15 mM borax) were carried out just before the analysis.

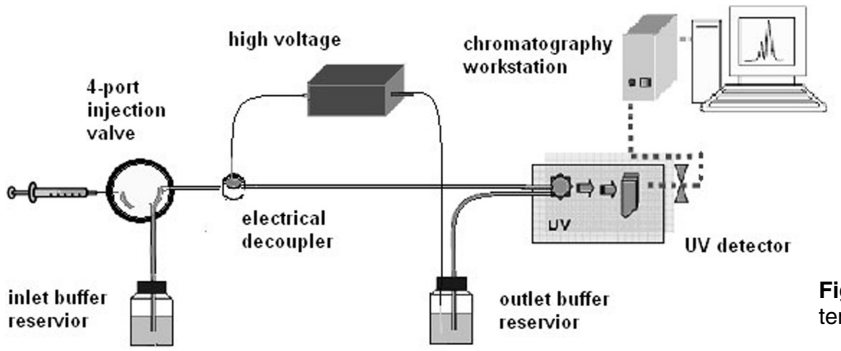

Figure 1. Schematic diagram of the qCE system.

2.2.3 Medicine

Theophylline tablets were analyzed. Twenty tablets were levigated and evenly mixed. A 0.4 g portion of homogenized tablets were accurately weighed and dissolved with 100 mL buffer (15 mM borax), and 1 mL of this solution was diluted to 20 mL with the buffer.

All solutions were filtered through a 0.22 μm membrane filter (MSI, Westboro, MA, USA) and degassed by sonication (Benchtop Cleaners HS3120) before use.

2.3 Quantitative CE apparatus

The electrophoresis was performed on a set of modified TriSep™-2100 system (Unimicro Technologies, Pleasanton, CA, USA) equipped with a high-voltage power supply and a variable-wavelength UV/Vis absorbance detector. Commercially available 4-port nano-valves (C4-0344-02/C4-0004-02) of 20 nL (Valco, Inc., Houston, TX, USA) were used for sample introduction throughout the study. Chromatography workstation was supplied by TriSep-2003 Chromatography Data Handling System (Unimicro Technologies, Pleasanton, CA, USA) for data acquisition.

2.4 Preparation of electrical decoupler

An electrical decoupler, used to provide electrical isolation in CE, was employed to isolate injector from the electrical field in our high-precision qCE system [17, 18]. It was made in-house as follows:

A small scratch was first made on the wall of a capillary at 10 cm distance from the CE capillary outlet-end using a capillary cutter. The capillary was fixed on a plastic holder with epoxy glue on both sides of the joint. After the glue was solidified, the capillary was bent gently at the scratch, and then a small break was formed on the column fracture. Drops of 12% cellulose acetate solution (in acetone) were carefully dripped onto the fracture, avoiding clogging the column. Under a gentle stream of air, a thin film of electricity-conductive membrane was formed uniformly over the fracture region, which not only prevented solvent and sample molecule from leaking out, but also enabled electrolyte to pass to obtain identical voltage exterior and interior the capillary.

Thereafter, a plastic columnar reservoir was fixed onto the plastic holder using epoxy glue for accommodating buffer without any leakage. Finally, the electric decoupler was cured overnight at room temperature.

2.5 qCE experiment

A schematic overview of the qCE system is shown in Fig. 1. The system consists of a high-voltage power supply, a 4-port nano-valve, a home-made electrical decoupler, a 75 μm-id capillary column, two buffer vials and a UV/Vis absorbance detector.

A fused-silica capillary (75 μm id, 375 μm od), going through the electrical decoupler, was connected to the sample port of the nano-injector, while another end was immersed in the outlet reservoir containing buffer. A piece of 250 μm id fused-silica capillary of 15 cm length was placed between the pump port of the injector and the inlet reservoir. Because there was no voltage applied on this segment, the liquid flow was sucked into the 250 μm id capillary, then consequently to the whole separation channel, due to the dragging force of EOF in the separation capillary. Considering the friction between the inner-surface and the buffer, a larger bore capillary (smaller surface to volume ratio) was used here to ensure the buffer to be easily drawn into the valve channel.

When the qCE system was running, the CA-membrane-based electrical decoupler was filled with running buffer. Grounding electrode was placed into the electrical decoupler reservoir, while another electrode was put into the outlet reservoir to complete the electrical circuit. In the load position, the internal sample loop was initially filled with the sample by manual injection. The running buffer was sucked into the nano-valve and arrived at the CA-membrane joint, subsequently driven into the separation capillary by EOF. In the inject posi-

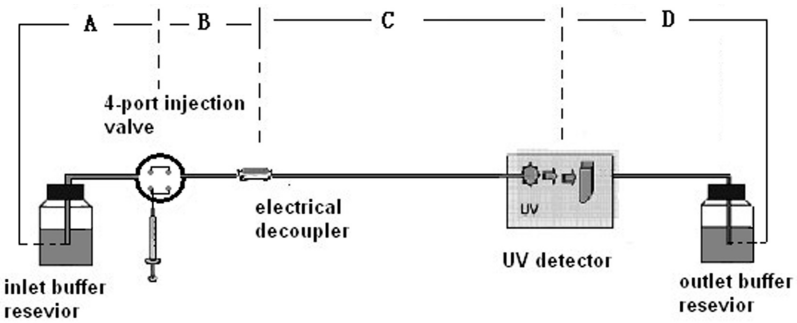

Figure 2. Schematic diagram of the whole capillary, Section A: from capillary end in the inlet reservoir to the nano-valve injector; Section B: from nano-valve injectior to the electrical decoupler; Section C: from the electrical decoupler to detective window; Section D: from detective window to the capillary end in the outlet reservoir.

tion, the sample loop was switched into flow path and the sample was carried by the running buffer to the separation capillary.

3 Results and discussion

In the format of a traditional CE injection, the capillary inlet is moved out from the buffer vial and dipped into the sample vial (dip-in), then, a hydrodynamic force (*e.g.*, pressure, vacuum, and gravity) or electric field was briefly applied to introduce the sample plug. After the injection, the capillary is moved back into the buffer vial, then, electric voltage is applied to start the electrophoresis. During the process, the separation capillary was moved around between the buffer and sample vials and the voltage was also interrupted. When the qCE system is used, it brings more advantages than CE with traditional (dip-in) sample introduction: (i) Accurate and precise amount of sample can be injected with the sample loop design. (ii) The electric field across the separation channel need not be interrupted, thereby ensures a stable electric field condition. (iii) There is no need to mechanically transfer the inlet end of capillary between sample vial and buffer vial, therefore minimizing the error of the injection. (iv) High-throughput continuous analysis becomes feasible, and so does on-line analysis. (v) Valve injection does not cause sample discrimination which happens when the electrokinetic injection is used in the conventional CE.

3.1 Optimization of the qCE system

3.1.1 Position of nano-valve injector

In our previous study, we investigated the feasibility of using the nano-rotary-type injector directly in the electric field without the decoupler [7]. However, the system was not very stable with the electroosmosis-driven flow because of bubbles generated inside the injector. In order to solve this problem, a new electrical decoupler was introduced into qCE system in this study. By this way, the nano-valve injector was isolated from the electric field, and both pressurized flow and EOF were used to drive the BGE.

The electrical decoupler was located between the injector and separation capillary (see Fig. 2), so that an electric field was constantly applied across the capillary section between the electrical decoupler and the outlet reservoir. Therefore, frequent valve-switching does not influence the stability of electric field, then improving analysis precision. Furthermore, it made major contribution to the elimination of bubble formation in the qCE system.

3.1.2 Effect of capillary length and internal diameter

We divided the whole capillary into four sections as showed in Fig. 2 and studied the effect of the capillary length of every section on the separation performance using benzoic acid (BA) as probe solute. Section A, in which the flow was created by the suction of the EOF produced in section C and D, connected the inlet buffer reservoir to the injector. Since the dragging force in section A caused a parabolic flow profile in the system, and consequently lowered the column efficiency, a shorter capillary was preferable. The column efficiency increased 432 plates/meter (p/m) when it was shortened by every one centimeter (data not shown here). Such improvement was more pronounced for section B, on which the efficiency was increased about 7040 p/m for every decreased centimeter. This was because section B served as a connection to transport both the buffer and the sample to the column. A longer capillary in this section would create not only a larger dragging force, resulting in a parabolic flow, but also a dead volume for the sample. Section C was the separation column. Change of its length would affect both the column efficiency and resolution. Therefore, the length of section C should be determined according to the nature of sample. The effect of section D, which was after detection window, was not so obvious to influence the results. Considering the geometry restriction of the connection, the experimentally adaptable capillary length was chosen to be 15 cm for Section A, 7 cm for Section B and 20 cm for Section D.

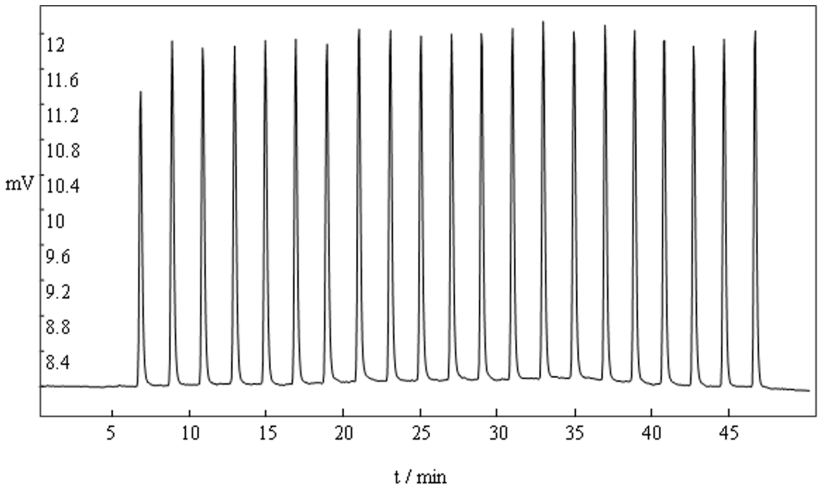

Figure 3. Twenty replicate injections of DMSO, Experimental conditions: Capillary: 75 μm id, overall length 62 cm, effective length 40 cm, Buffer: 10 mM borax buffer at pH 9.2, Voltage: 20 kV, Detection: UV at 220 nm, Injection volume: 20 nL, Sample: DMSO.

Table 1. Comparison of precision and sample discrimination of three injection modes ($n = 7$, A for peak area)

	RSD% of A_{DMSO}	RSD% of A_{BA}	A_{DMSO}/A_{BA}
Gravitational injection	2.56	2.67	0.412 ± 0.006
Electrokinetic injection	1.77	1.87	0.688 ± 0.028
Nano-valve injection	1.46	1.64	0.389 ± 0.005

Theoretically, Joule heat could be reduced by employing narrow-bore capillary, which makes it possible to exert high voltage in order to get high separation efficiency and speed. Unfortunately, the column efficiency of employing 50 μm id capillary as separation channel turned out to be poor. The reason was that the volume of nano-valve was 20 nL and rather large with respect to 50 μm id (or smaller) separation column. The length of the sample plug of 20 nL in the 50 μm id capillary was calculated to be about 10 mm, which would lead to significant peak broadening. For a compromise, a 75 μm id capillary was selected as the separation capillary.

3.2 Performance of quantitative CE

3.2.1 Reproducibility

The reproducibility of the qCE system was evaluated by performing five replicate analyses of mixed standard solution, and the results were summarized in Table 1.

The results showed that the injection precision of nano-valve injection in the qCE system was better than that of commonly used "dip-in" injections including both gravitational and electrokinetic methods in CE. The RSD of DMSO for peak area was 1.46% by nano-valve injection, better than 2.55% by gravitational injection and 1.76% by electrokinetic injection. Additionally, there was no obvious sample discrimination in nano-valve injection mode, which can be seen from the peak area ratio of DMSO to BA (A_{DMSO}/A_{BA}). In the electrokinetic injection mode, BA was negatively charged and moved against the EOF under the electrical field with positive polarity. Therefore, the peak area of BA in the electrokinetic injection was obviously smaller than those of both nano-valve and gravitational injection modes (See Table 1).

3.2.2 Consecutive analysis

One of the advantages of this qCE system is that consecutive analysis could be performed without interrupting the electric field during run-to-run, thereby making the system more stable during an operation.

To test the repeatability of the consecutive analysis for qCE system, 150 sample injections of DMSO were repeatedly and consecutively made at the interval of 120 s. The RSDs of DMSO were 0.67% and 1.26% for the migration time and the peak area, respectively. Fig. 3 shows a part of the migration diagrams of the consecutive injections in this experiment.

3.2.3 Column efficiency

The theoretical plate number of three injection methods measured by BA was respectively 66 290 using nano-valve injection, 124 408 using electrokinetic injection, and 116 923 using gravitational injection. The possible reasons for the lower efficiency of qCE than that of conventional CE may be elucidated by following aspects. First, the volume of the 20 nL nano-valve chosen in our qCE system was still too large. The sample plug in the separation capillary with 75 μm id was about 4.5 mm in length, which contributed, to some extent, to the band-broadening. The way to solve the problem was to employ a nano-valve with extremely small volume. Secondly, the dead volume of the nano-valve was also too large. The diameter of the port in the injector, which connects the sample loop to the column inlet, was 250 μm and resulted in a 78 nL dead volume. This dead volume caused dramatic peak broadening when the 20 nL sample went through

it. Thirdly, the column efficiency decrease was also associated with the parabolic flow profile created by the partial application of the electric field over the whole capillary as shown in Fig. 2. The driven force in the qCE system is entirely from the EOF existing only in section C and D, which sucked the buffer and sample into the separation capillary. This would inevitably cause a parabolic flow, consequently leading to lower column efficiency.

3.3 Application of qCE for real sample analysis

3.3.1 Optimization of experimental conditions

The separation conditions for caffeine, theobromine, and theophylline in real samples such as tea, coca cola, coffee, and a medicine, theophylline tablet were optimized in consideration of the separation resolution, peak shape, and column efficiency. The effects of buffer type, pH, and concentration were investigated in the method development process.

Firstly, the effect of buffer was studied. Five commonly used buffers, namely sodium tetraborate, sodium dihydrogen phosphate, disodium phosphate, sodium bicarbonate, and sodium acetate were tested at different concentrations. At a high concentration of phosphate buffer, a noisy baseline was observed. It was probably caused by Joule heating at high electric current resulted from the large degree of dissociation of the phosphate. With the decrease of buffer concentration, the separation resolution tended to be worse. When using a carbonate buffer, theobromine and theophylline could not be separated completely. In the case of sodium acetate, the resolution of the separation was even worse. A better separation resolution, peak shape, and column efficiency were obtained by using a sodium tetraborate buffer. Buffer concentration influences the diffusion coefficient of the analytes and the thickness of the electrical double layer and the zeta-potential of the inner surface of the capillary. In this study, the influence of buffer concentration ranging from 5 to 50 mM was investigated. With high buffer concentration, the resolution and the separation efficiency were improved, however, the migration time of the analytes was also increased, and the value of the electric current was increased as well, which resulted in instable baseline and band-broadening. When using 30 mM concentration, the current value was close to 100 μA (the limit of our high voltage power supply). A good separation of the three analytes was achieved when the buffer concentration was 15 mM. Therefore, 15 mM borax buffer was chosen as the optimum value for this work.

The buffer pH affects the zeta potential, EOF and the charge status of the analytes, therefore, affects the separation selectivity and resolution. Under the conditions of 15 mM borax at pH from 7.0 to 11.0, the effect of buffer pH on the separation was explored. As shown in Fig. 4, the migration time of three analytes generally prolonged with the increase of the buffer pH. This was due to the dissociation of the hydroxyl groups, which resulted in increased electrophoretic mobility opposite to the EOF. Meanwhile, the resolution of the analytes improved greatly. Additionally, electric current increased with the increase of pH. When buffer pH was 9.2, good separation for three analytes and stable baseline was achieved. When pH was higher than 10.0, increased Joule heat made the baseline unstable. Therefore, 15 mM borax buffer at pH 9.2 was chosen as the running buffer.

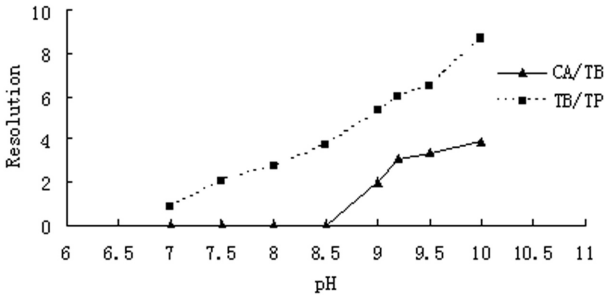

Figure 4. Effect of buffer pH on the resolution, Experimental conditions: Capillary: 75 μm id, overall length 72 cm, effective length 50 cm, Buffer: 15 mM borax buffer, Voltage: 15 kV, Detection: UV at 274 nm, Injection volume: 20 nL, Sample: caffeine, theobromine, and theophylline.

3.3.2 Quantitative analysis of caffeine, theobromine, and theophylline

The repeatability of the system was examined by seven replicate injections of the standard solution containing 0.12 mg/mL caffeine, 0.1 mg/mL theobromine, and 0.08 mg/mL theophylline. The results showed that the RSDs for migration time and peak area were less than 0.6 and 2.8%, respectively.

The linearity, LOD, and LOQ were studied. Under the optimized experimental conditions, all three alkaloids showed good linearities between the concentrations and peak areas. The linearities were evaluated by performing five replicate analyses of nine concentration levels of mixed standard solutions. The linear correlation equations (y is the ratio of peak area, x is the concentration) and coefficients (r) were respectively: Caffeine, $y = 60\,165x - 96.642$ ($r = 0.996$); theobromine, $y = 95\,764x - 53.052$ ($r = 0.998$); theophylline, $y = 202\,759x - 1001.6$ ($r = 0.998$). The LOD and LOQ were calculated to be 3.0 and 10.0 μg/mL for caffeine, 2.1 and 7.0 μg/mL for theobromine, 1.6 and 5.3 μg/mL for theophylline, respectively, on the basis of a S/N of 3 and 10.

3.3.3 Application to real sample analysis

As shown in Fig. 5, three alkaloids in real samples were well separated and detected. Qualitative analysis of the

Table 2. Determination of real sample with qCE system[a)]

Sample	Analyte	Original		Added (mg/mL)	Found (mg/mL)	Recovery	
		Found (mg/mL or mg/g)	RSD (%)			Recovery (%)	RSD (%)
Coca Cola	CA	0.15	1.00	0.074	0.217	97.1	4.85
				0.148	0.289	97.3	2.08
				0.223	0.369	99.1	1.46
	TB	ND		0.009	0.009	95.4	2.37
				0.019	0.018	95.1	1.42
				0.028	0.026	94.2	1.98
	TP	ND		0.009	0.009	103.5	2.27
				0.017	0.017	98.6	0.35
				0.025	0.025	98.4	0.27
Tea drink	CA	0.124	0.33	0.068	0.197	102.8	1.14
				0.135	0.267	103.0	0.83
				0.198	0.336	104.3	0.56
	TB	0.002	0.78	0.003	0.005	97.5	3.65
				0.006	0.008	104.4	5.35
				0.009	0.011	97.4	4.46
	TP	0.008	5.92	0.009	0.017	96.0	3.27
				0.018	0.025	96.1	0.65
				0.027	0.035	99.7	1.50
Tea leaves	CA	34.30	1.63	25.9	60.98	101.3	2.03
	TB	2.87	2.01	2.23	4.98	97.7	2.53
	TP	2.64	4.40	6.92	9.37	98.0	5.48
Roasted coffee	CA	15.58	0.97	25.00	41.17	101.5	1.83
				31.25	46.25	98.8	3.45
				41.67	57.73	100.8	2.68
	TB	1.83	4.94	1.50	3.26	98.0	7.08
				1.88	3.79	102.3	2.64
				2.50	4.21	97.4	2.34
	TP	ND		2.00	2.09	104.5	1.92
				2.50	2.51	100.5	1.48
				3.33	3.28	98.4	0.90
Medicine theophylline tablets	CA	37.13	0.76	25.00	62.28	100.2	2.35
				31.25	68.53	100.2	2.78
				41.67	78.38	99.5	1.66
	TB	62.28	1.75	15.00	77.22	99.9	4.05
				18.75	81.40	100.5	3.93
				25.00	86.33	98.9	1.66
	TP	62.77	1.00	20.00	83.01	100.3	2.26
				25.00	87.51	99.7	2.27
				33.33	96.85	100.8	1.84

[a)] mg/mL for cola and tea drink; mg/g for tea leaves, roasted coffee and medicine theophylline tablets, ND = not detected.

alkaloids in real sample was carried out according to the retention time of individual component. The results showed that there was no theophylline in coffee. There was only caffeine in coca cola. All three alkaloids existed in other real samples.

Quantitative analysis was carried out according to the peak area of individual component. The recovery test was performed in five replicate runs at three concentration levels as shown in Table 2. The average recoveries of these real samples were between 94 and 105%.

4 Concluding remarks

A high-precision qCE system with accurate sample introduction was developed and evaluated in real samples analysis. There was no need to interrupt the applied voltage during operation as in the traditional CE system, which enormously improved the system stability. An electrical decoupler was employed to isolate the injection valve from the electric field, so that the sampling could be conveniently coupled with on-line sample pretreatment. Meanwhile, the system was evaluated by the

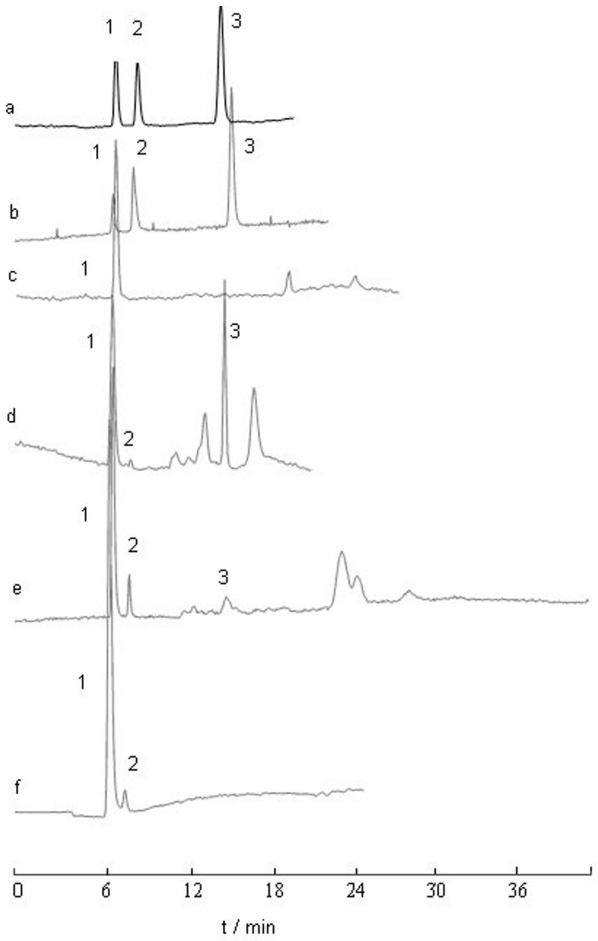

Figure 5. Electropherogram of real samples, Experimental conditions: Capillary: the same as in Fig. 4, Buffer: 15 mM borax (pH 9.2), Voltage: 20 kV, Detection: UV at 274 nm, Injection volume: 20 nL, Sample: (a) standard sample (0.05 mg/mL caffeine, 0.03 mg/mL theobromine, and 0.04 mg/mL theophylline), (b) medicine theophylline tablets, (c) coca cola, (d) tea drink, (e) tea leaves, (f) coffee. Analytes: (1) caffeine, (2) theobromine, (3) theophylline.

analysis of caffeine, theobromine, and theophylline in real samples and the feasibility and versatility of the qCE were demonstrated.

Although there were still problems and difficulties associated with separation efficiency, the successful achievement of quantitative injection by this qCE system holds considerable promise for applications in both quality control and quality assurance.

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