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Separation and determination of four active components in medicinal preparations by flow injection-capillary electrophoresis

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Abstract

A simple, rapid and accurate method for the separation and determination of paracetamol (Par), pseudoephedrine hydrochloride (Pse), dextromethorphan hydrobromide (Dex) and chlorphenamine hydrogen maleate (Chl) was developed by combination of flow injection and capillary zone electrophoresis for the first time. The analysis was carried out using an unmodified fused-silica capillary (75 mm × 75 μm i.d. × 375 μm o.d., effective separation length of 45 mm) and direct ultraviolet detection at 214 nm, 1.0 kV applied voltage. The optimized running buffer composed of 75 mM sodium borate–15% (v/v) acetonitrile (ACN) (pH* 9.30) was applied for the separation of the four analytes. The separation was achieved in 4.5 min. The sample throughput rate could reach up to 19 h^{-1}. The repeatability (defined as relative standard deviation) was 0.6%, 1.0%, 2.1%, 1.9% with peak height evaluation and 0.7%, 1.8%, 0.7%, 1.1% with peak area evaluation for Par, Pse, Dex and Chl, respectively. The limits of detection (S/N = 3) were 0.22 μg/ml, 0.29 μg/ml, 0.42 μg/ml and 0.70 μg/ml for Par, Pse, Dex and Chl, respectively. The method was successfully applied to determine the four compounds in three cold medicines with recoveries in the range of 97.18–105.15%.

Keywords: Paracetamol; Pseudoephedrine hydrochloride; Dextromethorphan hydrobromide; Chlorphenamine hydrogen maleate; Flow injection-capillary electrophoresis

1. Introduction

Paracetamol (Par), pseudoephedrine hydrochloride (Pse), dextromethorphan hydrobromide (Dex) and chlorphenamine hydrogen maleate (Chl) are effective components in cold curing medicines. Par is one of the major metabolic products of phenacetin and acetanilide, and is widely used to cure the fever, the headache and neuralgia, etc. Pse has the function of constringing the blood vessel, eliminating mucous membrane congesting and tumefying of nasopharynx, alleviating symptom of the nasal congestion. Dex can ease pain of centrum, being applicable for cold, acute and chronic bronchitis, bronchus asthma, tuberculosis, etc. Chl is an antihistamine, used for the allergic disease [1].

High performance liquid chromatography (HPLC) is one of the most useful techniques for the quantification of some of the four compounds [2–7], but the use of HPLC is restricted by long analysis times, peak asymmetry and poor efficiency [6,7]. Proton nuclear magnetic resonance (NMR) [8] and gas liquid chromatography [9] have also been reported for the determination some of these analytes. These methods usually require complicated pretreatment procedures prior to analysis. Though Dex and Chl have been separated by micellar electrokinetic chromatography (MEKC) [10] and capillary zone electrophoresis (CZE) [11], their quantification was not investigated. Pse, Dex and Chl have been separated and determined by nonaqueous capillary electrophoresis (NACE) [12] with 8 min analysis time. Pse, Dex and Par have been analysed by CZE [13], but the peaks of analytes were bad. At present, there is no report on the simultaneous determination of Par, Pse, Dex and Chl. Therefore, a simple and rapid method to determine these components simultaneously is highly desired for controlling drugs quantity validly.

The recent developments on the coupling of flow injection (FI) to CE have shown to be of great value and practical applicability [14–21]. Although CE has the advantages of high-resolution capability, high peak efficiencies and small sample volume, there are some limitations, such as discontinuous mode

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of sample introduction, sampling bias with the electrokinetic mode, low sensitivity and low repeatability in terms of the concentration and complicated sample off-line pretreatment procedures. FI offers an elegant means for sample pretreatment in routine application. It can be fully mechanized, thus avoiding manual handling of hazardous reagents and solvents. Automated pretreatment procedures yield higher precision and can be performed in a shorter time compared with the corresponding manual sample pretreatment. This FI–CE technique solved discontinuous manipulation of CE. The important advantages of the combined FI–CE system over conventional methods included outstanding repeatability in migration time, peak area and peak height; improved sample throughput and the great potential benefits of coupling FI on-line pretreatment techniques to CE.

More recently, the entire FI–CE was miniaturized using a 15 mm × 70 mm microscope slide as the base [22]. This device contained an H-shaped channel with a horizontal, several centimeter long separation capillary connected to two vertical sidearm tubes, one on each end of the slide. The approach is a midway between conventional CE and microfabricated CE on a chip. It approaches high separation speed and efficiency of the latter but is more readily coupled to the FI system. The device is ideal for basic studies of microfluidic systems and can be produced inexpensively without microfabricated equipment [23]. At present, there is no report on a FI–CE simultaneous separation and determination of Par, Pse, Dex and Chl. The purpose of this paper is to establish a simple, inexpensive, rapid and continuous automated sampling FI–CE to separate and determine the four components in medicines.

2. Experimental

2.1. Chemicals and materials

Standards of Par, Pse, Dex and Chl were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 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 tablet (*Leimengxin*), Meixi Weima tablet (*Baijiahei*), Anfen Weima Namin tablet (*Haiwang-yindefei*) were purchased from local drug stores, Lanzhou, China. All chemicals were of analytical reagent grade and were used as received.

2.2. Solutions and sample preparation

The carrier solutions (functioning also as electrophoretic buffer) were composed of 75 mM sodium borate and 15% (v/v) ACN (pH* 9.30), and were prepared daily from stock solution of 0.1 M borate and ACN, and their desired pH values were adjusted by 1 M HCl or 1 M NaOH. All buffer solutions were filtered through a 0.45 μm syringe filter and degassed under vacuum before use. All solution and buffer were prepared in distilled water.

A stock solution of 1.0 mg/ml of Par, Pse, Dex and Chl were prepared in distilled water, and were stored at 4 °C to reduce evaporation and equilibrated to room temperature before use. Working standard solutions were obtained by diluting the corresponding stock solutions with distilled water to the desired final concentration.

Twenty tablets of each preparation of Anma Meimin tablet (*Leimengxin*), black tablets (*Heipian*) of compound Meixi Weima tablet (*Baijiahei*) and Anfen Weima Namin tablet (*Haiwang-yindefei*) were weighed exactly and the average weight of each tablet was calculated, then powdered, and a quantity of the powder 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 capillary by the 16-way auto-switching valve of K-1000 FIA.

2.3. Apparatus

A model HPE-100 CE system with 12 kV maximum voltage (BioRad, Hercules, CA, USA) was used for the separations, which was connected to a 486 PC. Data collection was achieved by a Chroma chromatography collection system (BioRad). Uncoated fused-silica capillaries (75 μm i.d., 375 μm o.d.) were purchased from Yongnian Optical Fiber Factory (Hebei, China). UV detection was carried out at 214 nm.

A K-1000 Flow Injection Analyzer (Hitachi, Japan) was used throughout for transporting background electrolyte (BGE, buffer/carrier) and samples. It was composed of a double plunger pump, a 16-way auto-switching valve with three sample loops of about 20 μl each and a peristaltic pump used for delivery of sample solution to sample loops. A 33-cm length Polytetrafluoroethylene (PTFE) tubing (0.5 mm i.d.) from the valve to the split-flow interface was used as transport line. In charge stage, BGE was pumped by two plungers at a constant flow-rate. Simultaneously, three sample loops were filled with sample solutions by switching on the peristaltic pump. In injection stage, sample solutions that were stored in the three sample loops were injected into the BGE stream simultaneously by switching on the valve and passed through the inlet of capillary in this device.

The H-channel interface (Fig. 1) constructed using a planar plastic slide B (30 mm × 80 mm × 2 mm) as the base plate integrated a separation capillary (75 μm i.d. × 375 μm o.d. × 75 mm length, and 45 mm 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 respective, 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 lower section of cathodal reservoir (T1) was controlled by a

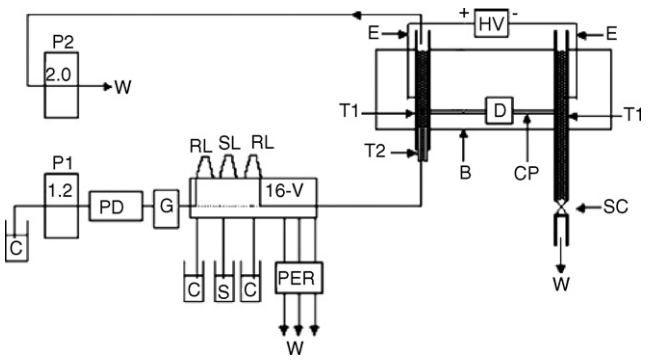

Fig. 1. Manifold for the FI–CE (not to scale). C, carrier solution; S, sample; P1 and P2, pumps; PD, pressure damper; G, pressure gauge; SL, sampling loop; RL, reagent loop; 16-V, 16-way valve; PER, peristaltic pump; B, planar plastic base; T1, Tygon tubing; T2, Tygon tubing (1.2 ml/min); CP, separation capillary column; E, platinum electrode; W, waste; C/S, carrier/sample; HV, high voltage; D, detector; SC, screw clamp.

screw clamp except for filling or changing of electrolyte solution, or for capillary cleaning.

The apparent pH (pH*) values were determined by a PHS-10A pH meter (Xiaoshan Instrumental Factory, Zhejiang, China).

2.4. Operation procedures

For the CE operations, an unmodified fused-silica capillary was used for all analysis. In order to maintain the capillary in good working condition, the beginning of each working day, the capillary was flushed sequentially with distilled water (5 min), 0.1 M NaOH (5 min), and distilled water (5 min) again, followed by running buffer (5 min) from the capillary outlet reservoir using a water-circulating vacuum pump. Simultaneously the CE instrument was warmed up until a stable baseline was achieved. Moreover, the capillary was flushed between analysis with distilled water (2 min), 0.1 M NaOH (3 min), distilled water (2 min) and fresh BGE (3 min) for optimizing the repeatability.

For the FI the operations, the carrier stream was driven by the plunger pump, sample solution was delivered to a sample loop and two reagent loops (volume: 20 μl, respectively) in the 16-way injection valve by the peristaltic pump, respectively. The sample solution was transported through the connecting conduit into the T2, where the flow was split and a fraction of the sample zone injected by FI system was introduced into the separation capillary by electrokinetic means. A series of samples were injected continuously without interrupting the high voltage.

3. Results and discussion

To achieve good sensitivity and satisfactory separation, the optimization of separation conditions was of primary importance. In this work, the separation conditions were optimized by a univariate approach taking the peak areas and migration time as the principal figures of merit. The peak sequence of the four compounds was Dex, Chl, Pse and Par. The concentration

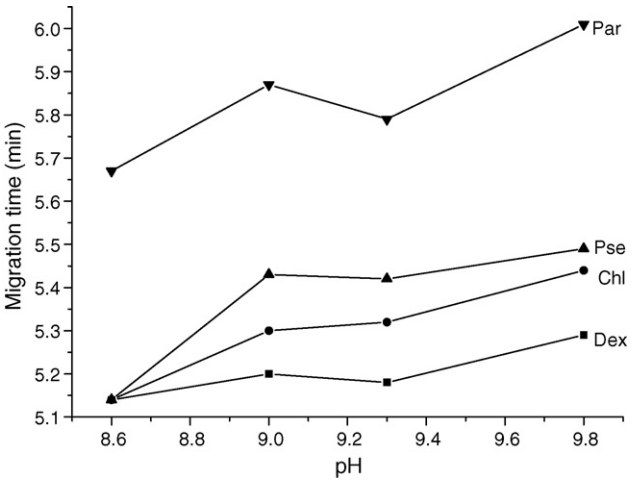

Fig. 2. Effect of pH on migration time. CE conditions: uncoated separation capillary, 75 μm i.d. × 375 μm o.d. × 75 mm length (45 mm effective length), voltage, 1.0 kV, detection wavelength, 214 nm. Conditions of K-1000 FIA: man/access mode, CT = 3 s, IT = 9 s, sample volume, 60 μl, carrier flow-rate, 1.2 ml/min, frequency of injecting sample, 3 min, separation temperature, room temperature, buffer, 75 mM sodium borate–15% (v/v) ACN. Sample: 15 μg/ml Dex, 40 μg/ml Chl, 30 μg/ml Pse and 65 μg/ml Par.

of Dex, Chl, Pse and Par in optimization studies were 15 μg/ml, 40 μg/ml, 30 μg/ml and 65 μg/ml, respectively. The identities of the recorded peaks were confirmed by independent injection of the pure compounds. The sequence of the peaks was invariable when the condition was changed.

3.1. Influence of the pH* value of the buffer on separation

In this study, the effect of buffer pH* on the migration times in the pH* range of 8.60–9.80 with 50 mM borate solution–15% (v/v) ACN, and 1.0 kV applied voltage was investigated (Fig. 2). When the buffer of pH* 8.6–8.8 was used, the peaks of the Dex, Chl and Pse could not be separated. When pH* > 9.0, good baseline resolution was achieved. With concurrent consideration in resolution, peak shape and migration time of these analytes, pH* 9.30 was chosen.

3.2. Influence of BGE concentration

Buffer concentration has obvious influence on the separation because it can affect the electroosmotic flow (EOF) and the viscosity of the electrolyte. The effect of the borate concentration at pH* 9.30 on these separations was studied in the range of 40–80 mM, with 15% (v/v) ACN and 1.0 kV applied voltage (Fig. 3). Varying the buffer concentration did not result in changes in the migration orders of the test mixtures, but it had a significant effect on the resolutions and migration times. The resolutions between Pse, Par and reversal peak were poor at low buffer concentrations. However, increasing buffer concentration caused long separation times. In order to obtain a higher resolution and better peak figures, while avoiding the generation of excessive Joule heating, a buffer concentration of 75 mM was considered to be a good compromise between migration time and resolution.

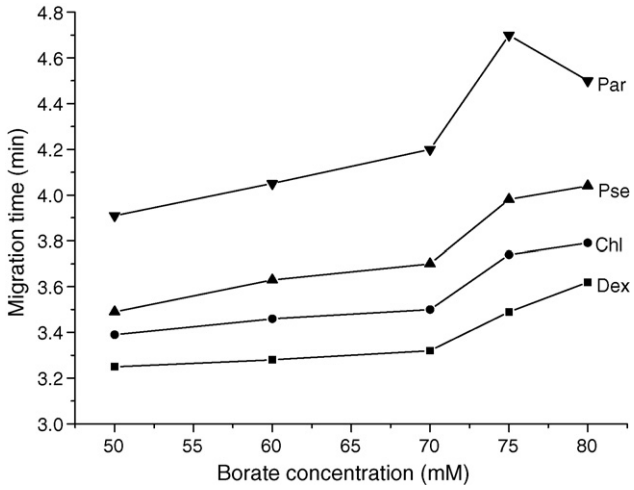

Fig. 3. Effect of borate concentration on the separation. Experimental conditions: pH* 9.30; the other experimental conditions were as in Fig. 2.

3.3. Influence of ACN concentration in buffer on separation

The use of organic solvents such as ACN in the buffer could improve selectivity, reduce electroosmotic velocity, and thus would expand the migration window [24]. The effect of the addition of ACN to the buffer electrolyte on the separation of the analytes was studied in the range of 0–20% (v/v). When the concentration of ACN was increased from 0 to 20% (v/v), there was an increase in the migration time due to the decrease in EOF, and the resolution between each of the four analytes was increased. The compounds were completely resolved upon the addition of 15% (v/v) ACN to the buffer electrolyte at 1.0 kV with 75 mM sodium borate. It should be noted that, although further addition of ACN gave better resolution, peaks figures were worse and sensitivity decreased. Therefore, 15% (v/v) ACN was chosen for further study.

3.4. Influence of applied voltage on separation

The effect of varying the separation voltage from 0.8 to 1.5 kV was investigated under the conditions selected above. The higher voltage was necessary for rapid analysis, which could reduce molecular diffusion and improve column efficiency. When there was a lower voltage, the migration of the compounds became slow and the bands also broadened due to diffusion effects, and Joule heating decreased. To ensure better separation and effective dissipation of Joule heating, a voltage of 1.0 kV was adopted.

3.5. Influence of BGE flow-rate

The influence of the BGE flow-rate of K-1000 FIA in the range 0.80–2.4 ml/min was studied under the electropheresis conditions described above. With fixed sample injection volumes, the carrier flow-rate determined the length of the sample plug entering the capillary and therefore the time available for electrokinetic split-sampling into the capillary [25]. A lower flow-rate ensured the better sensitivity and used fewer reagents, but deteriorated the resolution and theoretical plate number. Considering of different factors, a flow-rate of 1.20 ml/min was selected as optimum.

3.6. Influence of charging time (CT) and injecting time (IT)

CT and IT influenced the sensitivity of analytes. To ensure completion of three sample loops and would not consume much more samples, 3 s CT was selected in this experiment. Sensitivity (peak area and peak height) was augmented with the increase of IT, however, the resolution was decreased and the bands were broadened when IT was more than 12 s. Better results could be attained in fitting of IT and CT, so 9 s of IT was selected in this analysis.

The optimum separation condition was: 75 mM sodium borate–15% (v/v) ACN buffer (pH* 9.30), 1.0 kV voltage, 60 μl sample volume, 214 nm UV detection, 1.2 ml/min buffer flow-rate, CT 3 s and IT 9 s.

3.7. Linearity, repeatability, and detection limits

Calibration graphs were obtained by injecting standard solutions at seven different concentrations. Each point on the calibration graph corresponded to the mean value was obtained from three independent peak area measurements. The corresponding regression equations, as well as other characteristic parameters for the determination of Dex, Chl, Pse and Par were listed in Table 1. And the standard deviations at the regression coefficients (s_a and s_b) were worked out [26]. The LODs (S/N = 3) and LOQs (S/N = 10) were also given. The proposed method allowed the determination of Dex, Chl, Pse and Par at low levels. The peak areas were employed for quantification. The repeatability of FI–CE was

Table 1
Analytical performance of FI–CE in four components analysis ($n = 5$)

	Dex	Chl	Pse	Par
LOD (S/N = 3) (μg/ml)	0.22	0.29	0.42	0.70
LOQ (S/N = 10) (μg/ml)	0.73	0.95	1.40	2.33
Peak area R.S.D. (%)				
Intra-day	0.7	1.8	0.7	1.1
Inter-day	1.0	3.1	2.1	3.8
Peak height R.S.D. (%)				
Intra-day	0.6	1.0	2.1	1.9
Inter-day	8.3	8.5	9.7	3.1
Migration time R.S.D.				
Intra-day	0.7	1.2	0.3	2.3
Inter-day	3.1	2.5	2.0	3.2
Regression equation[a]				
a	−968.24	−792.79	−959.28	−633.92
b	494.20	379.22	252.42	332.20
s_a	±655.7	±328.6	±506.6	±279.3
s_b	±8.0	±4.0	±5.8	±3.0
Correlation coefficient	0.9984	0.9993	0.9974	0.9997
Linear range (μg/ml)	1.6–200	1.6–200	3.1–200	6.2–200

[a] $y = a + bx$; y, peak area; x, standard concentration (μg/ml); s_a, standard deviation of a; s_b, standard deviation of b.

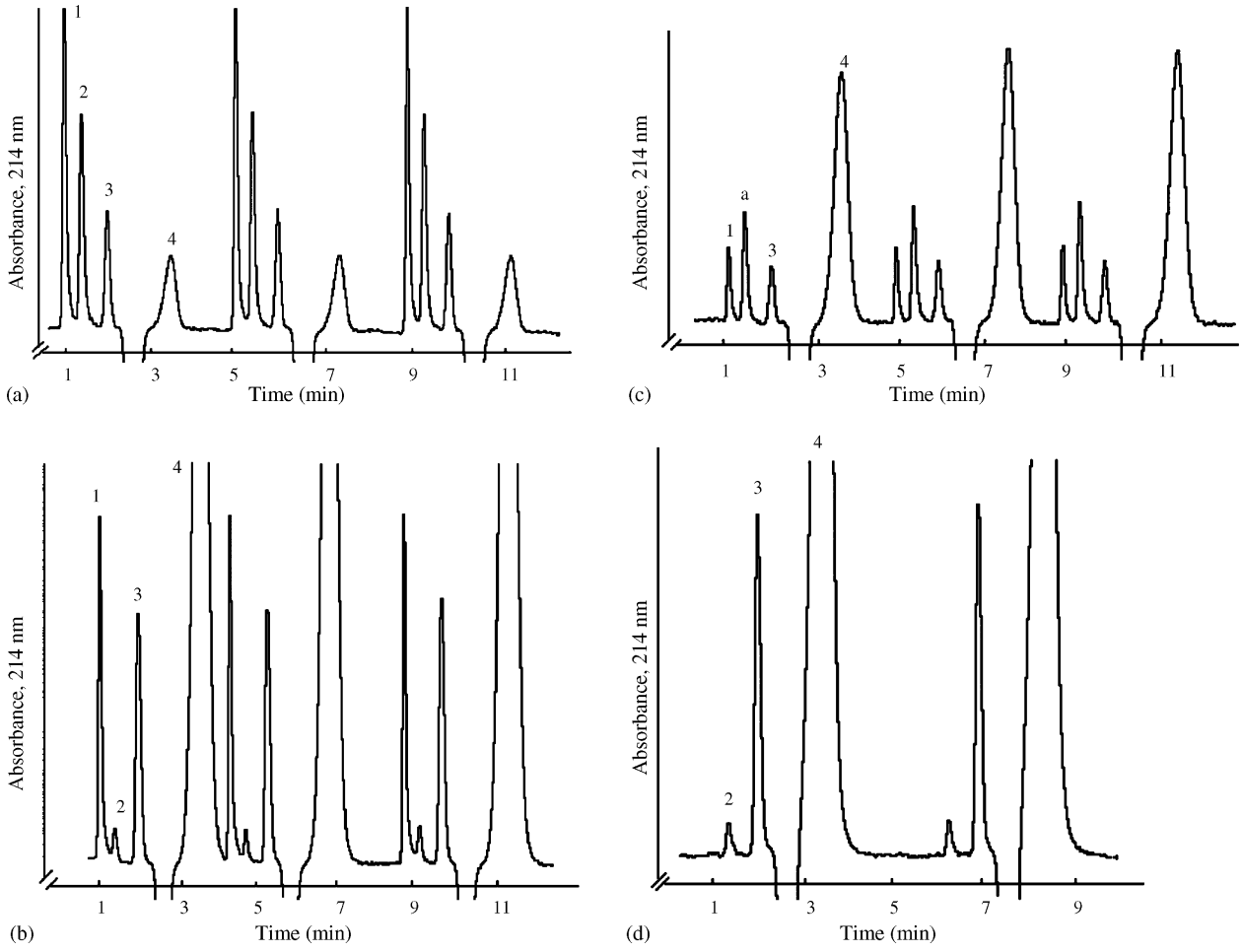

Fig. 4. Electropherograms of the standard mixture solution and the real samples: (a) standard mixture solution. Concentrations of the analytes: 15 μg/ml Dex, 40 μg/ml Chl, 30 μg/ml Pse and 65 μg/ml Par, (b) *Leimengxin*, (c) black tablets (*Heipian*) of compound Meixi Weima tablet (*Baijiahei*), (d) *Haiwang-yindefei*. Peaks: 1 = Dex, 2 = Chl, 3 = Pse, 4 = Par, a = unknown. Separation condition: buffer, 75 mM sodium borate–15% (v/v) ACN (pH* 9.30); capillary, 75 μm i.d. × 375 μm o.d. × 75 mm length (45 mm effective length); applied voltage, 1.0 kV; sample volume, 60 μl; UV detection, 214 nm; flow-rate, 1.2 ml/min.

evaluated under optimized conditions using a standard solution containing 100 μg/ml Dex, Chl, Pse and Par. The analytes were repeatedly injected into the CE system every 3 min. The electropherograms obtained were shown in Fig. 4. The repeatability was expressed as the R.S.D. values of the peak heights and peak areas obtained for five injections (given in Table 1).

3.8. Applications

Quantitative analysis was performed under the optimum conditions obtained from the experiments described above. The method was applied to the analysis of the commercial medicines containing these analytes. Because the difference in content of Chl and Par were very large, two different concentration solutions were used for determination Chl and Par. The contents of the analytes found in different kinds of medicines were given in Table 2. In addition, the compare of the Par between current results and previous studies were given in Table 3. The typical electropherograms of black tablets (*Heipian*) of compound Meixi Weima tablet (*Baijiahei*), *Leimengxin* and *Haiwang-yindefei* were shown in Fig. 4. The peaks were identified by the standard addition methods. Accuracy of the methods and the potential matrix effects were established by analyzing samples. The recoveries of the four constituents were determined with the addition of the standard substances in real samples, with results ranging from 97.18% to 105.15%. It showed that the method was applicable for the quantification of Dex, Chl, Pse and Par with high accuracy, precision and repeatability (Table 4).

Table 2
Compare of the contents between found and labeled in the different medicines

Samples	Compounds	Found (mg/tablet)	Labeled (mg/tablet)	Relative error (%)
Leimengxin	Dex	15.21	15	1.4
	Chl	2.15	2	7.5
	Pse	29.50	30	−2
	Par	331.55	325	2
Baijiahei (heipian)	Dex	15.40	15	3
	Pse	30.18	30	0.6
	Par	324.06	325	−0.29
Haiwang-yindefei	Chl	2.09	2	4.5
	Pse	29.39	30	−2
	Par	314.50	325	−3.2

Table 3
Recoveries of four analytes ($n = 3$)

Sample	Ingredient	Added (μg)	Average recovery (%)	R.S.D. (%)
Leimengxin	Dex	84, 168, 252	105.15	3.43
	Chl	84, 168, 252	102.00	5.18
	Pse	84, 168, 252	101.46	2.97
	Par	14, 19, 38	99.32	2.90
Baijiahei	Dex	50, 100, 150	99.02	2.40
	Pse	50, 98, 196	103.50	3.97
	Par	98, 196, 294	99.79	2.88
Haiwang-yindefei	Chl	10, 15, 30	103.84	4.31
	Pse	10, 10, 15	97.18	4.28
	Par	10, 10, 15	103.20	2.74

Table 4
Compare of the results between current and previous studies involving Pse

Mode	Recoveries (%)	LOD (μg/ml)	Linear range (μg/ml)	Ref.
Previous				
FI-CZE	92.6–107.3	–[a]	50–1000	[27]
CZE	103	0.75	2.5–300	[13]
FI-SPE-CZE	–	0.03	0–3	[28]
FI-SPE-CZE	92–101	1.2×10^{-4}	0.025–1.00	[29]
Current				
FI-CZE	97.2–103.5	0.42	3.1–200	

[a] Not reported.

4. Conclusions

The coupling of an FI system with CE equipment has been successfully used to analyze the Dex, Chl, Pse and Par for the first time. The result indicated that the proposed FI–CE system was suitable for the determination of principle components in the samples. With synergistic coupling to FI sample introduction through a split-flow interface, a sample (standard solution) throughput rate up to $19\,h^{-1}$ was achieved in this work with baseline separation of four analyte peaks. The low-cost FI–CE may be a valuable technique for the drug quality control in terms of repeatability and accuracy, and may be useful for routine analysis, process analysis and monitoring. The speed of separation significantly exceeded those achievable by conventional CE and other methods. This system represented a new contribution in the field of analytical methods.

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