Application of Experimental Design in Optimization of the Separation Condition for Determination of Four Active Components in Cold Medicines by Flow Injection-Capillary Electrophoresis

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Abstract

Orthogonal design (OD) was employed to optimize the separation condition of flow injection-capillary electrophoresis (FI–CE). In order to compare the optimum condition, uniform design and univariate approach were also adopted. The influences of variables such as buffer pH, buffer concentration, acetonitrile (ACN) percentage, and separation voltage were discussed. The optimum separation condition was established. The limits of detection were 1.94×10^{-2}, 6.40×10^{-3}, 1.16×10^{-2} and 1.94×10^{-2} µg/mL for dextromethorphan hydrobromide (Dex), chlorphenamine hydrogen maleate (Chl), pseudoephedrine hydrochloride (Pse), and paracetamol (Par), respectively. The RSDs of peaks areas were less than 2.0%. The results showed the OD was an effective method among experimental designs for optimizing the separation conditions of CE. The optimum condition was used for separation and determination of Dex, Chl, Pse, and Par in cold medicines. The average recovery was between 96.68–101.25%.

Introduction

Capillary electrophoresis (CE) has evolved into a large family of high-resolution separation techniques over the past two decades. The key point in development of CE method is to obtain an optimum separation condition, and sometimes the optimization procedure is complex because a number of factors (e.g., running buffer, organic modifier, applied voltage, temperature, injection condition, etc.) can influence the performance of CE separation (1,2). Therefore, more emphases has been put on the use of rational strategies for the optimization and validation of analytical methods. When dealing with samples, it has the possibility of multiple interferences and matrix effects. In order to detect these effects and to assess their significance, a considerable number of experiments would be necessary. Extensive experiments, however, are expensive and time-consuming, hence it is necessary to minimize the number of experiments and to maximize the information extracted from them. This has led to an increasing use of statistically-based techniques, usually called experimental design (3). It allows a large number of factors to be screened simultaneously and to determine which has a significant effect on the separation. Depending on the design chosen, the resulting response model can show the relationship of each factor towards the response as well as the interactions between the factors. These factors can then be optimized to give the best separation in a relatively low number of experiments, often resulting in a saving both in time and consumables. Experimental design has been used in such areas as pharmaceutical analysis (4,5), biochemistry (6), biology (7), and foodstuff science (8).

There are many parameters to be considered when developing a new CE method and many of these parameters have strong interactions. These interactions often mean that the effect of a change in one variable on the overall separation will not be immediately obvious. The influences of experimental parameters on CE separation were discussed by McLaughlin et al. (9). So far, most CE experiments today are carried out with the "changing one separate factor at a time" approach to find the optimum condition in separation of the complex matrices (10–15). It is the so-called univariate approach. It is well known that this traditional way involves a number of independent analyses, but it fails in common cases because there are some interactions among factors investigated. Therefore, although this approach is flexible and implemented easily, it cannot reflect the real influences of factors exist. So, it has been gradually replaced by experimental design approaches, which provide a strict mathematical framework for changing all pertinent factors simultaneously, and achieve the fewest experimental runs. Uniform design (UD) is an experimental design method which just considers the evenly disperse of experimental points in experimental range. In UD, each level of each factor just has one experiment. So the number of experiments is equal to the number of levels of factors. Orthogonal design (OD) is an experimental design approach with "changing all parameters at the same time". The design allows different experiments to be present in arbitrary sequences. A substantial improvement in efficiency can be achieved by using OD. It has advantages such as equilibrium dispersing, trimness comparability and fewer experiments times. At the same time, it can

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reflect sufficiently the influence of each level of each factor on the experimental results. But many test points are required in OD and are not sufficient in evenly dispersion in order to obtain the trimness comparability. Therefore the test points in UD have better representation and uniformity than OD because UD method takes no account of trimness comparability, thus the experiments in UD are not enough, which is the most difference between UD and OD. The number of experiments increases with the number of levels in UD but increases with the square of levels number in OD. UD is more suitable for the experiments with more levels. However, the application of UD in CE for optimization of the separation conditions is seldom reported because the experimental points from uniform design table are too little and the experimental error is large (16). Oppositely, OD is more suitable for the experiments with lesser levels and has become increasingly popular in recent years (17–20). At present, orthogonal design has been applied abroad in pharmaceutical analysis (17,20). For an experimental response for each electrophoretic result, the chromatographic exponential function (CEF, Eq. 1), an objective function that describes the quality of each experimental run in terms of resolution and time of final elution, has been developed (21).

$$CEF = \{[\sum_{i=1}^{n-1}(1 - e^{a(R_{opt} - R_i)})^2] + 1\}(1 + \frac{t_f}{t_{max}}) \quad \text{Eq. 1}$$

Modified chromatographic exponential function (MCEF) has been reported (22).

$$MCEF = [\sum_{i=1}^{n-1} e^{a(R_{opt} - R_i)} + 1](1 + \frac{t_f}{t_{max}}) \quad \text{Eq. 2}$$

where a is used to adjust the weighting of the resolution term relative to the time term, and R_i is the resolution of the ith peak and $(i + 1)$th peak for each experimental result. The absence of a distinguishable peak is regarded as the peak, having a resolution of zero, and n is the number of the marked peaks, which are difficult to be separated. t_f is the mobility time (or elution time) of the final peak and the R_{opt} is optimum resolution. t_{max} is the maximum acceptable time. The smaller the value of MCEF is, the better the separation efficiency of CE is.

Although CE has the advantage of high resolution capability, the discontinuous sample introduction mode confined the sample throughput and precision. Flow injection (FI) offers an elegant means for sample injection because it can be fully mechanized. Automated procedures yield higher precision compared with the corresponding manual sample injection. The combined FI-CE system solves the problem of discontinuous manipulation of CE, enhances sampling frequency and improves repeatability contrasted with traditional CE injections (23). In this work, three experimental designs, such as OD, UD, and univariate approach, were used for optimizing the separation condition for CE. Because different methods often gain different results, by comparing the electropherograms and other figures of merit obtained under three optimum conditions, a best method for optimization of CE separation condition was obtained. The MCEF was used to evaluate the quality of the separation obtained by each experimental run in OD. Paracetamol (Par), pseudoephedrine hydrochloride (Pse), dextromethorphan hydrobromide (Dex), and chlorphenamine hydrogen maleate (Chl) were used as model compounds. The four active components in cold medicines were determined simultaneously by applying the optimum separation condition combined with head-column field-amplified with large-volume sample stacking technique (24). The optimum separation condition was applied to real sample with reliable results.

Experimental

Chemicals

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 medicine, namely, Anma Meimin Tablets (Lei Meng Xin) was purchased from local drug stores (Lanzhou, China). All the reagents used were of analytical grade and distilled water was used throughout.

Apparatus

A model HPE-100 CE system with 12 kV maximum voltage (BioRad, Hercules, CA) 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 of 75 µm i.d., 375 µm o.d., and 48.4 cm length (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 background electrolyte (BGE, buffer/carrier) and samples. It was composed of a double plunger pump used for delivery of carrier solution, a 16-way auto-switching valve with three Polytetrafluoroethylene (PTFE) solu-

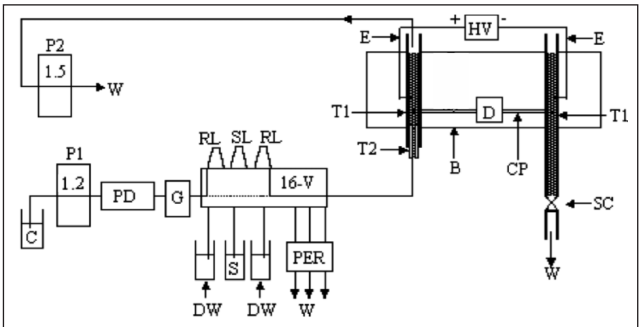

Figure 1. Manifold for the FI-CE (not to scale). Carrier solution (C); sample (S); pumps (P1 and P2); pressure damper (PD); pressure gauge (G); sampling loop (SL); reagent loop (RL); 16-way valve (16-V); peristaltic pump (PER); planar plastic base (B); Tygon tubing (T1); Tygon tubing (T2) (1.2 mL/min);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

tion loops, a peristaltic pump used for delivery of sample solution to the middle PTFE loop (sample loop), and water (or running buffer) to the first (20 µL) and third (20 µL) PTFE solution loops. A 33 cm length PTFE tubing (0.5 mm i.d.) from the valve to the split-flow interface was used as transport line. The charging time and injecting time were set manually. The time period for the injecting sample was defined through man/access mode. The manifold of FI-CE was shown in Figure 1. The detailed description of the H-channel microchip and the schematic diagram of two stages of an FI system with a 16-way auto-switching valve had been given elsewhere (25).

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

Preparation of the reagents and drugs

The carrier solution was freshly prepared and consisted of 55 mM borax-15% (v/v) ACN. The buffer was prepared daily from stock solution of 0.1 M borax, ACN with distilled water, and then adjusted to the desired pH using either 2 M NaOH or 2 M HCl. Stock standard solutions (1000 µg/mL) of Par, Pse, Dex, and Chl were prepared with distilled water. Working standard solutions were then obtained by diluting the corresponding stock solutions to the desired concentration with distilled water or running buffer. All buffer solutions were filtered through a 0.45 µm syringe filter before use. Twelve tablets of Anma Meimin Tablets (Lei Meng Xin) 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. 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.

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, between runs, the capillary was washed with distilled water (2 min), 0.1 M NaOH (3 min), distilled water (2 min), and equilibrated with the fresh running buffer (3 min) to ensure a good repeatability.

Results and Discussion

In this part, three methods were used to optimize the separation conditions in order to obtain the optimum result. The four variables investigated in the study were the buffer concentration (c_{Borax}), buffer pH, ACN percentage (v/v) (cACN) and separation voltage (V). The factors were chosen on the basis of their potential to have a significant impact on the analysis. The boundaries of the experimental domain were based on the preliminary experiments.

Orthogonal design

In the study, the OD with the four variables (pH, c_{borax}, V, c_{ACN}) at three levels was adopted, and the chief corresponding index of the sensitivity was the peak area of Chl. These factors mentioned previously were listed in Table I. All 9 experiments were involved in the matrix of OD. Thus, the design can equally generate information in all directions. MCEF was more suitable than CEF to be used as the fitness function in this optimization according to their representations. The objective function can be considered to represent the 'quality' of the electropherogram, which, in essence, gives equal weighting to all components. In Eq. 2, R_{opt}, a, and t_{max} were set as 1.5, 1, and 10, respectively, for facilitating the calculation. The standard solution was used in each experimental design, and Dex and Chl were used as the marked peaks because they were difficult to be separated. The higher sensitivity and resolution and shorter analysis time were the optimization targets. Experimental designs and the responses were presented in Table I, where $R_{1/2}$ were the resolution of two marked peaks. Because the content of Chl in medicines was the least, the peak area of Chl, the resolution of Dex and Chl (R*1/2*), MCEF and t_f were proposed to evaluate the quality of each experimental run. Table I showed that the performance of separation from the Exp. 2 was much better than that of any other experimental runs because the Exp. 2 presented a higher AChl, a lower MCEF and a shorter t_f.

According to the largest donating rule (26), as far as each factor being investigated, the optimum level of factor should be able to obtain the optimum value of index investigated. In Table I, K_1~K_3 and k_1~k_3 are the summation and the average values of AChl under every level of an investigating variable, respectively.

Table I. Experimental Conditions for OD and Corresponding Values of the Peak Area of Chl (A_{Chl}), $R_{1/2}$, t_f, MCEF, and Other Parameters

Exp.	c_{Borax} (A) (mM)	c_{ACN} (B) (v/v)	pH (C)	Voltage (D) (kV)	*MCEF*	A_{Chl}	$R_{1/2}$	t_f
1	55 (1)	5 (1)	9.0 (1)	5.5 (1)	6.6583	66679	1.56	24.29
2	55 (1)	15 (2)	9.3 (2)	6.0 (2)	6.5869	218398	1.49	22.77
3	55 (1)	20 (3)	9.6 (3)	6.5 (3)	4.4512	189161	2.68	24.05
4	65 (2)	5 (1)	9.3 (2)	6.5 (3)	7.8036	80047	1.06	20.57
5	65 (2)	15 (2)	9.6 (3)	5.5 (1)	5.8260	135235	2.60	33.71
6	65 (2)	20 (3)	9.0 (1)	6.0 (2)	7.5844	207168	1.27	23.58
7	75 (3)	5 (1)	9.6 (3)	6.0 (2)	5.4640	123013	2.30	27.70
8	75 (3)	15 (2)	9.0 (1)	6.5 (3)	7.8333	136490	0.99	19.39
9	75 (3)	20 (3)	9.3 (2)	5.5 (1)	7.0255	87445	2.14	36.00
K_1	474238	269739	410337	289359				
K_2	422450	490128	385890	548579				
K_3	346948	483774	447409	405698				
κ_1 (= K_1/3)	158079	89913	136779	96453				
κ_2 (= K_2/3)	140817	163376	128630	182860				
κ_3 (= K_3/3)	115649	161258	149136	135233				
D_{max}	42430	73463	20506	86407				
Best result	A(1)	B(2)	C(3)	D(2)				

For example, for such the variable as c_{Borax}, it had three levels of 55, 65, and 75 mM, the value of K1 in the same column as the c_{Borax} was the summation of three AChl obtained under the level of 55 mM, (i.e., K_1 = 66679 + 218398 + 189161 = 474238). Similarly, K_2 = 80047 + 135235 + 207168 = 422450, K_3 = 123013 + 136490 + 87445 = 346948. In the light of the largest donating rule, the largest value of K was optimum value. So, the optimum experimental conditions in theory were A(1)B(2)C(3)D(2). The best scheme was very near to the conditions of Exp. 2. In addition, D_{max} was defined as the supreme difference of k in the same column. The value of D_{max} in each column was equal to the absolute difference of the largest D_{max} value and the smallest D_{max} value in the same column. For example, for the 42430 in the c_{Borax} column, it represented the difference of 158079 and 115649 (i.e., 158079 – 115649 = 42430). The larger the Dmax value is, the larger the influence of change in levels on experimental index is. In the Exp. 2, only pH (C) was not the best level and the influence of pH on AChl was the lowest among the four factors because of the least D_{max}. As shown in fact, the AChl in Exp. 2 was maximal. It suggested that the best scheme we obtained agreed with practice.

Table IIA. Experimental Factors and Levels in the UD for Optimizing the Separation of the Analytes U5 (54)

	Levels				
Factors	**1**	**2**	**3**	**4x**	**5**
c_{Borax} (mM)	55	65	75	85	95
c_{ACN} (v/v)	0	5	10	15	20
pH	8.7	9.0	9.3	9.6	9.9
Voltage (kV)	6	6.5	7	7.5	8

Uniform design

In order to compare the optimum conditions previously described, a uniform design (UD) was used to systemically investigate the influence of the concentrations of borax, buffer pH, ACN percentage and separation voltage on the performance of CE separation. A five-level UD was used to optimize the variables. The levels of each factor and responses of experimental runs were presented in Table IIB. From the Table IIB, the optimum condition was 65 mM borax-15% ACN, pH 9.30 and separation voltage 6.0 kV.

Table IIB. Experimental Conditions for the UD and Corresponding Values of the Peaks Areas of Four Analytes

Exp.	c_{Borax} (mM)	c_{ACN} (v/v)	pH	Voltage (kV)	Dex	Chl	Pes	Par
1	55	5	7.5	9.3	38118	56473	90477	119946
2	65	15	9.3	6	104799	151687	199542	164110
3	75	0	9.0	7.5	12539	17494	20999	75884
4	85	10	8.7	6.5	30085	101275	92811	6065
5	95	20	9.9	8	Deposit Appeared			

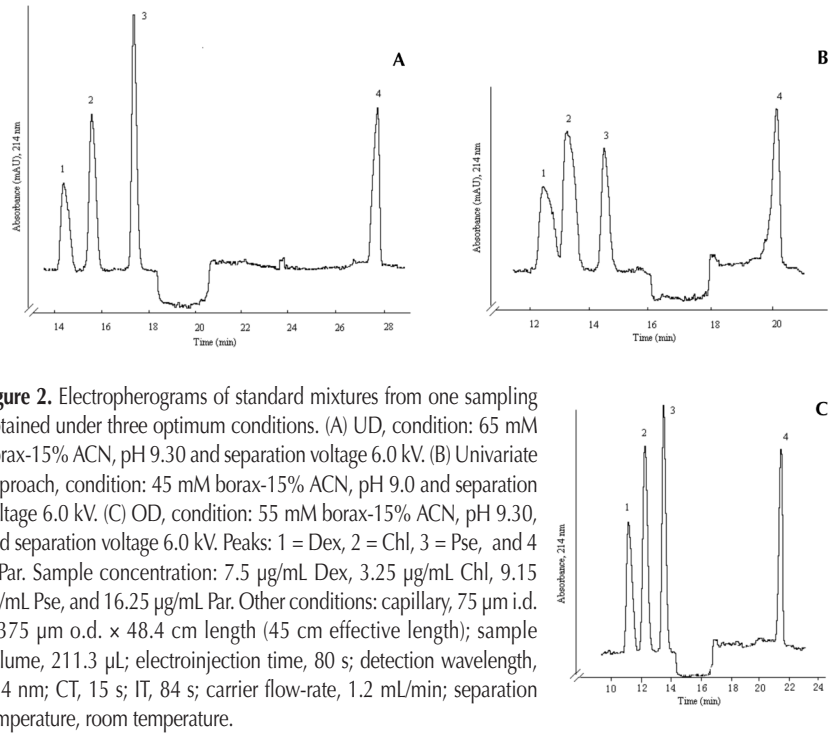

Figure 2. Electropherograms of standard mixtures from one sampling obtained under three optimum conditions. (A) UD, condition: 65 mM borax-15% ACN, pH 9.30 and separation voltage 6.0 kV. (B) Univariate approach, condition: 45 mM borax-15% ACN, pH 9.0 and separation voltage 6.0 kV. (C) OD, condition: 55 mM borax-15% ACN, pH 9.30, and separation voltage 6.0 kV. Peaks: 1 = Dex, 2 = Chl, 3 = Pse, and 4 = Par. Sample concentration: 7.5 µg/mL Dex, 3.25 µg/mL Chl, 9.15 µg/mL Pse, and 16.25 µg/mL Par. Other conditions: capillary, 75 µm i.d. × 375 µm o.d. × 48.4 cm length (45 cm effective length); sample volume, 211.3 µL; 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.

Univariate approach

The univariate approach was different from these two methods previously-mentioned. It was a dynamic optimum method. The bigger peak area and shorter analysis time were the optimum targets. The influences of pH in the range of 8.6–9.6, borax concentration in the range of 35–75 mM, ACN percentage in the range of 10–25% (v/v) and the separation voltage from 5.0 to 7.0 kV on peak area and migration time were investigated. The optimum condition of the method was 45 mM borax-15% (v/v) ACN (pH 9.0), 6.0 kV separation voltage.

Comparison of three optimum conditions

The three optimum conditions were different. The electropherograms obtained under these conditions were shown in Figure 2. In the UD method, there was a bigger peak area of Chl but a longer migration time. The peaks shapes of Dex and Par were broad and terrible. In the univariate approach, the Dex and Chl were not separated and peaks shapes of four analytes were very terrible. In the OD method, the peak area of Chl was much bigger, the peaks figures of analytes were well and had the shorter analysis times. So the optimum condition of OD was adopted. The optimum separation condition was: 55 mM borax-15% (v/v) ACN (pH 9.3), 6.0 kV separation voltage.

Performance of the combined FI-CE system

Calibration graphs were obtained by injecting standard solutions under the optimum condition of OD. The corresponding regression equations, as well as other characteristic parameters for the determination of Dex, Chl, Pse, and Par are shown in Table III. The repeatability (expressed as RSD) was evaluated by using a standard solution containing 7.5 µg/mL Dex, 3.25 µg/mL Chl, 9.15 µg/mL Pse, and 16.25 µg/mL Par. Comparedwith the reference 23, Dex, Chl, and Pse, had approximately 10-fold, 1000-fold, and 100-fold increase in sensitivity, respectively. A significant improvement in precision was also obtained (RSD < 1.91%). But the analysis times of analytes were longer than these of reference 23. The number of theoretical plates of Dex, Chl, Pse, and Par were 6692, 11328, 18648, and 19162, respectively. The electropherogram obtained under the optimum condition was shown in Figure 2C.

Table III. Analytical Performance of FI-CE in Four Components Analysis under Optimized Conditions of OD (n = 5)

	Dex	Chl	Pse	Par	Ref.
LOD	1.94×10^{-2}	6.40×10^{-3}	1.16×10^{-2}	2.84×10^{-2}	
(3 S/N)	0.98	1.19	1.94	–*	23
(µg/mL)	–	0.50	–	0.40	27
	0.22	0.29	0.42	0.70	28
Peak area†	1.01	1.91	0.89	0.92	
	3.50	3.70	2.80	–	23
	–	2.40	–	1.70	29
Peak height†	1.94	3.98	2.66	3.27	
Regression equation‡					
a	–2579.45	–1177.21	4318.88	993.72	
b	22871.41	57110.77	22427.88	9641.83	
Correlation coefficient	0.9989	0.9994	0.9992	0.9990	
Linear range (µg/mL)	0.94–30	0.06–15	0.47–30	0.94–60	

* not reported
† RSD (%)
‡ y = a + bx; y = peak area; x = standard concentration (µg/mL).

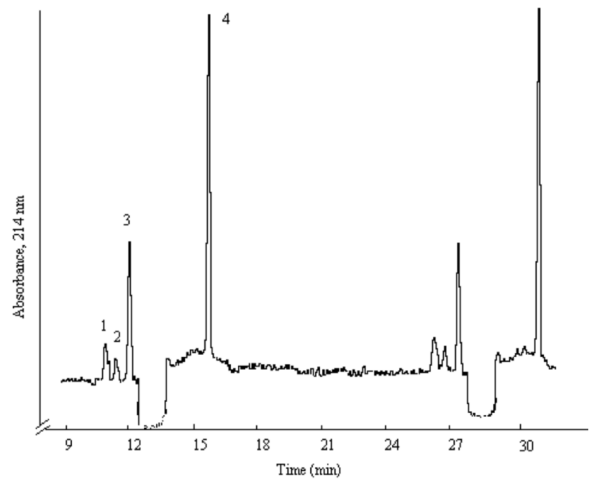

Figure 3. Electropherogram of Anma Meimin Tablets (Lei Meng Xin) under optimum condition of OD from two continuous sampling. Peaks: 1 = Dex, 2 = Chl, 3 = Pse, and 4 = Par. Buffer, 55 mM borax-15% (v/v) ACN (pH 9.30); voltage, 6.0 kV; the other experimental conditions were as Figure 2.

Application

The optimum separation condition from OD based on the FI-CE system combined with stacking technique was applied to the analysis of the commercial medicines containing these analytes. The contents of the analytes found in medicines were given in Table IV. The typical electropherogram of Anma Meimin Tablets (Lei Meng Xin) from two continuous sampling was shown in Figure 3. The peaks were identified by the standard addition method. Accuracy of the method 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 samples. Table IV shows an agreement between the claimed and the found values. 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.

Conclusion

Optimal condition was obtained for the separation of four compounds by applying experimental design. OD could provide an easy and objective procedure for detection and evaluation of mutual interactions of factors and was a most appropriate method for optimization of CE separation condition. The four analytes were determined simultaneously with a higher sensitivity and better figures using the optimum condition [55 mM borax-15% (v/v) ACN, pH 9.3 at a run voltage of 6.0 kV]. The automated FI sample injection provided a high precision (RSD < 1.91%). The frequency of sampling was 5/h. Good accuracy and low limit of detection were obtained by the FI-CE method based on the optimum condition. The OD technique was simple to perform, using conventional instrumentation without complicated procedures. The inherent simplicity and effectiveness of this procedure made it a facile way in FI-CE system.

Acknowledgements

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Table IV. Comparison of the Found with the Labeled in the Lei Meng Xin Under Optimum Conditions

Compounds	Found (mg/tablet)	Labeled (mg/tablet)	Relative Error (%)	Average Recovery (%) (n = 3)
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

References

1. J.P. Schaeper and M.J. Sepaniak. Parameters affecting reproducibility in capillary electrophoresis. *Electrophoresis* **21:** 1421–1429 (2000).
2. B.X. Mayer. How to increase precision in capillary electrophoresis. *J. Chromatogr. A* **907:** 21–37 (2001).
3. C. Altesor, P. Corbi, I. Dol, and M. Knochen. Application of experimental design to the development of a multicomponent derivative spectrophotometric method: simultaneous determination of sulfamethoxazole and trimethoprim. *Analyst* **118:** 1549–1553 (1993).
4. K.D. Altria, B.J. Clark, S.D. Filbey, M.A. Kelly, and D.R. Rudd. Application of chemometric experimental designs in capillary electrophoresis. *Electrophoresis* **16:** 2143–2148 (1995).
5. K.D. Altria and M.T. Kersey. Capillary electrophoresis and pharmaceutical analysis: a survey of industrial applications and their status in the United States and United Kingdom. *LC-GC* **13:** 40–46 (1995).
6. Y.J. Yao, H.K. Lee, and S.F.Y. Li. Optimization of separation of porphyrins by micellar electrokinetic chromatography using the overlapping resolution mapping scheme. *J. Chromatogr. A* **637:** 195–200 (1993).
7. M.S. Nielsen, P.V. Nielsen, and J.C. Frisvad. Micellar electrokinetic capillary chromatography of fungal metabolites resolution optimized by experimental design. *J. Chromatogr. A* **721:** 337–344 (1996).
8. J. Wu, M.K. Wong, H.K. Lee, and C.N. Ong. Orthogonal array design for optimizing the capillary zone electrophoretic analysis of heterocyclic amines. *J. Chromatogr. Sci.* **14:** 139–145 (1996).
9. G.M. Mclaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmierl, K.W. Anderson, S.C. Morris, J.A. Morrison, and T.J. Bronzert. Pharmaceutical drug separations by HPCE: Practical guidelines. *J. Liq. Chromatogr.* **15:** 961–1021 (1992).
10. L. Zhang, Y.H. Liu, and G.N. Chen. Simultaneous determination of allantoin, choline and arginine in Rhizoma Dioscoreae by capillary electrophoresis. *J. Chromatogr. A* **1043:** 317–321 (2004).
11. M.C. Breadmore, R. Theurillat, and W. Thormann. Determination of ribavirin in human serum and plasma by capillary electrophoresis. *Electrophoresis* **25:** 1615–1622 (2004).
12. S. Hillaert and W.V. Bossche. Optimization and validation of a micellar electrokinetic chromatographic method for the analysis of florfenicol. *J. Pharm. Biomed. Anal.* **36:** 437–440 (2004).
13. A. Profumo, B. Cardinali, C. Cuniberti, and M. Rocco. Separation of human fibrinopeptides by capillary zone electrophoresis. *Electrophoresis* **26:** 600–609 (2004).
14. J.R. Flores, J.J.B. Nevado, G.C. Penalvo, and N.M. Diez. Development and validation method for determination of fluoxetine and its main metabolite norfluoxetine by nonaqueous capillary electrophoresis in human urine. *Talanta* **65:** 163–171 (2005).
15. L.H. Liu, X.M. Liu, X.G. Chen, and Z.D. Hu. Separation and determination of tetrandrine and fangchinoline in herbal medicines by flow injection-micellar electrokinetic capillary chromatography with internal standard method. *J. Chromatogr. A* **1098:** 177–182 (2005).
16. *Handbook of Analytical Chemistry*, the first fascicule, Chemical Industry Press, Beijing, China, 1997, pp. 634.
17. T. de Boer, R. Bijma, and K. Ensing. Modelling of conditions for the enantiomeric separation of β_2-adrenergic sympathicomimetics by capillary electrophoresis using cyclodextrins as chiral selectors in a polyethylene glycol gel. *J. Pharm. Biomed. Anal.* **19:** 529–537 (1999).
18. E. Varesio, J.Y. Gauvrit, R. Longeray, P. Lanteri, and J.L. Veuthey. Central composite design in the chiral analysis of amphetamines by capillary electrophoresis. *Electrophoresis* **18:** 931–937 (1997).
19. M. Jimidar, P.F. De Aguiar, S. Pintelon, and D.L. Massart. Selectivity optimization for the separation of chlorophenols in an irregularly shaped experimental region in capillary electrophoresis. *J. Pharm. Biomed. Anal.* **15:** 709–728 (1997).
20. K. Yu, Z.Y. Lin, and Y.Y. Cheng. Optimization of the buffer system of micellar electrokinetic capillary chromatography for the separation of the active components in Chinese medicine 'SHUANGDAN' granule by genetic algorithm. *Anal. Chim. Acta* **562:** 66–72 (2006).
21. V.M. Morris, J.G. Hughes, and P.J. Marriott. Examination of a new chromatographic function, based on an exponential resolution term, for use in optimization strategies: application to capillary gas chromatography separation of phenols. *J. Chromatogr. A* **755:** 235–243 (1996).
22. V.M. Morris, C. Hargreaves, K. Overall, P.J. Mariott, and J.G. Hughes. Optimization of the capillary electrophoresis separation of ranitidine and related compounds. *J. Chromatogr. A* **766:** 245–254 (1997).
23. Y.M. Dong, X.F. Chen, Y. L. Chen, X.G. Chen, and Z.D. Hu. Separation and determination of pseudoephedrine, dextromethorphan, diphenhydramine and chlorpheniramine in cold medicines by nonaqueous capillary electrophoresis. *J. Pharm. Biomed. Anal.* **39:** 285–289 (2005).
24. X.M. Liu, J.S. Zhang, and X.G. Chen. 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. *Anal. Sci.* **24:** 1031–1037 (2008).
25. L.Y. Fan, Y.Q. Cheng, Y.Q. Li, H.L. Chen, X.G. Chen, and Z.D. Hu. Head-column field-amplified sample stacking in a capillary electrophoresis-flow injection system. *Electrophoresis* **26:** 4345–4354 (2004).
26. K.T. Fang. *Applied multivariate statistical analysis [M]*. East China Normal University Press, Shanghai, China, 1989, pp. 120–145.
27. L. Suntornsuk. Separation of cold medicine ingredients by capillary electrophoresis. *Electrophoresis* **22:** 139–143 (2001).
28. X.M. Liu, L.H. Liu, H.L. Chen, and X.G. Chen. Separation and determination of four active components in medicinal preparations by flow injection-capillary electrophoresis. *J. Pharm. Biomed. Anal.* **43:** 1700–1705 (2007).
29. L. Suntornsuk, O. Pipitharome, and P. Wilairat. Simultaneous determination of paracetamol and chlorpheniramine maleate by micellar electrokinetic chromatography. *J. Pharm. Biomed. Anal.* **33:** 441–449 (2003).

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