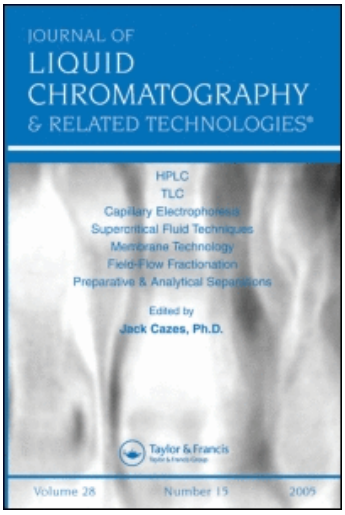

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OPTIMIZATION OF CAPILLARY ELECTROPHORETIC SEPARATION OF CHLORPHENIRAMINE ENANTIOMERS BY A PLACKETT-BURMAN DESIGN. DETERMINATION OF ENANTIOMERIC PURITY OF DEXCHLORPHENIRAMINE

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ABSTRACT

A three-leveled Plackett-Burman experimental design was used for the optimization of enantiomeric separation of chlorpheniramine by capillary zone electrophoresis using hydroxypropyl-β-cyclodextrin as chiral recognition agent. In this study, the effects of five important factors, namely, pH of the buffer, concentration of chiral selector, organic cations composing the background electrolyte, organic solvent and applied voltage upon resolution, migration time, and peak efficiency were investigated. The optimum separation conditions deduced from the effects were used to determine the amount of the (-)-isomer as impurity in a (+)-chlorpheniramine raw substance and two dexchlorpheniramine commercial tablets. It was found that the impurity levels were between 1 and 2 %.

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INTRODUCTION

Chlorpheniramine (Figure 1) and dexchlorpheniramine are antihistaminic drugs given orally for relieving hypersensitive symptoms including urticaria, angioedema, rhinitis, conjunctivitis, and pruritic skin disorders.[1] They are common ingredients of cough and cold preparations. Chlorpheniramine is a racemic mixture while dexchlorpheniramine is the dextrorotatory isomer of chlorpheniramine. Dexchlorpheniramine has approximately twice the activity of chlorpheniramine and, therefore, is administered in half doses.[1]

Capillary electrophoresis, due to its merits of high efficiency, short analysis time, and small amount of reagent consumption (notably for various expensive chiral selecting agents), has been widely used for separation of enantiomeric compounds. For chiral separation of chlorpheniramine using cyclodextrins (CDs) as chiral selectors, several methods have been reported.[2-5] Otsuka and Koppenhoefer used β-CD,[2,3] Crommen used β-CD and hydroxypropyl-β-CD (HP-β-CD),[4] Khaledi used β-CD, HP-β-CD, dimethyl-β-CD (DM-β-CD), and trimethyl-β-CD (TM-β-CD)[5] as chiral selectors.

When faced with many factors which may influence chiral separation, the Plackett-Burman design has been shown to be a very efficient strategy for assessing the number of factors, as compared with the classical single-variable-at-a-time approach.[6,7] This design is a saturated fractional factorial design, which requires only N experiment to be executed to assess the effect of $k = N - 1$ factors, where N is a multiple of 4.[8-10] For example, eight experiments are conducted to assess the effect of seven factors and twelve experiments for eleven factors.

Although the design was proposed originally for ruggedness testing of analytical methods,[11-13] it was also employed to optimize separation of mixtures.[14,15] Chiral separation involves only separation of single pairs of compounds. This feature makes the Plackett-Burman design especially useful as a tool of optimization, because the working process is very simple and straightforward. In this work, various conditions reported in the literature[2-5] were examined, and the fac-

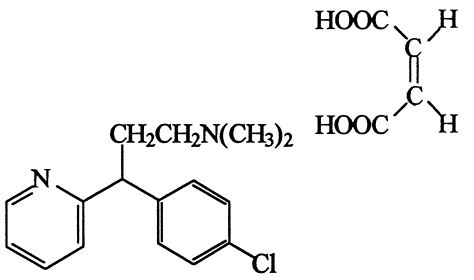

Figure 1. Structure of chlorpheniramine maleate.

tors relevant to separation of chlorpheniramine enantiomers were investigated by the Plackett-Burman design. The enantiomeric purity of dexchlorpheniramine was determined according to the optimized conditions obtained with the design.

EXPERIMENTAL

Apparatus

All experiments were performed on a capillary electrophoretic system consisting of a Lauer Labs Prince programmable injector, a 30-kV high-voltage supplier (Emmen, the Netherlands), and a Dynamax UV-C Absorbance Detector (Rainin, Emeryville, CA, USA) for UV detection. The electropherograms were recorded with a EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA) on a 486 DX2 66 PC with an appropriate ADC card and interface. A fused-silica capillary of 50 μm I.D. and 375 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) was used. A Mettler delta 320 pH meter with an InLab 410 combination electrode (Essex, England) was used for pH measurement.

Chemicals and Reagents

HP-β-CD (MS = 0.6) from Aldrich (Milwaukee, WI, USA), triethanolamine from Wako (Osaka, Japan), tetramethylammonium hydroxide solution (25%) from Riedel-de-Haën (Seelze, Germany), phosphoric acid (85%) and sodium phosphate from Merck (Darmstadt, Germany), methanol and acetonitrile of chromatographic grade from Mallinckrodt (Paris, KY, USA) were used in this work. Water was obtained from a Barnstead Water Purification System (Dubuque, IA, USA).

Racemic (±)-chlorpheniramine maleate and dexchlorpheniramine mLeate ((+)-chlorpheniramine maleate) were purchased from Sigma (St. Louis, MO, USA). Tablets of dexchlorpheniramine maleate of two pharmaceutical companies were acquired from market.

Working Solution Used for Optimizing Separation

A stock solution containing 10 mg of (±)-chlorpheniramine maleate in 10 mL of water was prepared. This was diluted to 100 μg mL^{-1} as working solution for use of optimization of separation.

Test Solutions

(1) Ten milligrams of (+)-chlorpheniramine maleate was dissolved in 10 mL of water to make a solution of 1 mg mL^{-1}. (2) Ten tablets of two commercial dexchlorpheniramine maleates (label claim of 2 mg of dexchlorpheniramine maleate per tablet) were separately weighed and ground to powders. Powders equivalent to 10 mg of active ingredients were dissolved in 10 mL of water and sonicated. The mixture was filtered through a 0.45 mm PTFE membrane (Lida, Kenosha, WI, USA). Each mL of the solution contained 1 mg of (+)-chlorpheniramine maleate according to the label.

Electrophoretic Conditions

For the Plackett-Burman design the experiments were carried out at 15, 20, and 25 kV under ambient temperature (25 ± 1°C). The total and detection lengths of the capillary were 66 and 51 cm, respectively. Samples were injected hydrodynamically at 50 mbar for 1.2 sec, such that the injected volume was about 1.2 nL. The detection wavelength was 210 nm.

The background electrolyte (BGE) solutions were prepared by titrating 100 mM phosphoric acid solutions with 100 mM sodium phosphate, tetramethylammonium hydroxide (25 %), and triethanolamine, respectively, to the desired pHs. The solutions were filtered through 0.45 μm Millipore membranes (Bedford, MA, USA). HP-β-CD was added and the solutions were sonicated. Organic modifiers were added before use.

When a new capillary was used, it was flushed with 1.0 M sodium hydroxide for 10 min, followed by 0.2 M sodium hydroxide for 10 min. Between runs the capillary was flushed with 0.2 M sodium hydroxide for 2 min, then with running buffer for 3 min.

Conditions of Nominal Procedure To Be Optimized

The nominal procedure for chiral separation of chlorpheniramine should be defined before optimization with the Plackett-Burman design. The conditions of the nominal procedure listed below were set with reference to those used in the literature.[2-5]

pH value of the buffer solution was set at 2.5. HP-β-CD was chosen as chiral selector and its concentration fixed at 20 mM. Phosphoric acid/sodium phosphate with concentration of 100 mM was used as buffer. No organic solvent was added to the buffer. A voltage of 20 kV was applied to the system.

Table 1. The 15 Experiments of the Plackett-Burman Design

Factor \ Expt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
pH	−1	−1	−1	0	−1	0	0	0	1	1	1	0	2	−	0
[HP-β-CD]	0	−1	−1	−1	0	−1	0	0	0	1	1	1	0	1	0
Cation	0	0	−1	−1	−1	0	−1	0	0	0	1	1	1	0	1
Org. sol.	−1	0	0	−1	−1	−1	0	0	1	0	0	1	1	1	0
Voltage	0	−1	0	0	−1	−1	−1	0	0	1	0	0	1	1	1
D1	−1	0	−1	0	0	−1	−1	0	1	0	1	0	0	1	1
D2	−1	−1	0	−1	0	0	−1	0	1	1	0	1	0	0	1

The Plackett-Burman Design

A reflected three-leveled Plackett-Burman design was employed in this work for optimization. This required 15 experiments to be executed. Five factors, namely, buffer pH, concentration of HP-β-CD, cation species composing the buffer, organic modifier, and applied voltage were investigated. Two factors were assigned as dummy factors to provide the standard error of the experiments. The design is shown in Table 1, coded numbers -1, 0, and 1 representing the lower, nominal, and upper levels of the factors, respectively. Number 8 of the 15 experiments is the nominal procedure.

The values of the three levels of each factor are listed in Table 2. The factors of cation species and organic modifier were qualitative and their level values were expressed with literal descriptions. All numeric values were set with reference to the conditions in the literature.[2-5] Each level of the dummy factors was

Table 2. Factors To Be Optimized and the Values of Their Three Levels

Factor	Lower Level (−1)	Nominal Level (0)	Upper Level (+1)
pH	2.0	2.5	3.0
[HP-β-CD]	15 mM	20 mM	25 mM
Cation	TMA^+	Na^+	$TEA{\times}H^+$
Organic solvent	ACN (10%,v/v)	not added	MeOH (10%,v/v)
Voltage	15 kV	20 kV	25 kV
Dummy 1	1	2	3
Dummy 2	1	2	3

TMA^+: Tetramethylammonium ion.
$TEA{\times}H^+$: protonated triethanolamine.

arbitrarily allotted a number. Three responses, resolution, migration time, and peak efficiency (plate number) were taken to evaluate the separation.

Resolution between enantiomeric peaks was calculated either by dividing the depth of peak valley with interpolated peak height in the case that the depth of peak valley was less than half of the interpolated peak height, or otherwise by the equation:

$$Rs = 1.18 \times \frac{t_2 - t_1}{w_{0.5(1)} + w_{0.5(2)}}$$

where t_1 and t_2 are migration times, $w_{0.5\,(1)}$ and $w_{0.5\,(2)}$ are peak widths at half-height of the two enantiomeric peaks. The migration time of the second enantiomer was taken. The plate number (N) of each enantiomeric peak was calculated by the equation:

$$N = 5.545 \times \left(\frac{t}{w_{0.5}}\right)^2$$

The N values of the two enantiomeric peaks were averaged to get the overall plate numbers.

The effect of each factor on response was calculated as:

$$E_{x(0,+1)} = \frac{\Sigma Y(+1)}{N/2} - \frac{\Sigma Y(0)}{N/2}$$

$$E_{x\,(0,-1)} = \frac{\Sigma Y(-1)}{N/2} - \frac{\Sigma Y(0)}{N/2}$$

where $E_{x(0,+1)}$ and $E_{x(0,-1)}$ are the effects of factor X on response Y when factor X is changed from level 0 to +1 and 0 to −1, respectively. $\Sigma Y(+1)$, $\Sigma Y(0)$, and $\Sigma Y(-1)$ are the sums of the responses where factor X is at levels +1, 0, and −1, respectively. N is the number of experiments in the design and equal to 8. The calculated effects can be normalized with respect to the averaged response of the 8 experiments and multiplied by 100:

$$\%E_x = \frac{E_x}{\overline{Y}} \times 100\%, \quad \text{where } \overline{Y} = (Y_1 + Y_2 + \ldots + Y_8)/8 \text{ or } (Y_8 + Y_9 + \ldots + Y_{15})/8.$$

The standard error of the experiments is calculated as:

$$\%S.E. = \sqrt{\frac{\Sigma\,(\%E_{Di})^2}{n_i}}$$

Where $\%E_{Di}$ is the normalized effect of dummy factors and n_i is the number of dummy factors. The factor is considered to have significant effect on a response if $|\%E_x| > 2\ \%S.E.$[6,7,9]

RESULTS AND DISCUSSION

The electropherogram obtained with the nominal procedure is shown in Figure 2. The (+)- and (−)-enantiomers were completely separated from each other in ten minutes (resolution = 1.83), the peak efficiency (plate number) being 2.80×10^5.

Figure 3 shows the electropherograms obtained from the 15 experiments of the Plackett-Burman design. Values of the three responses (resolution, migration

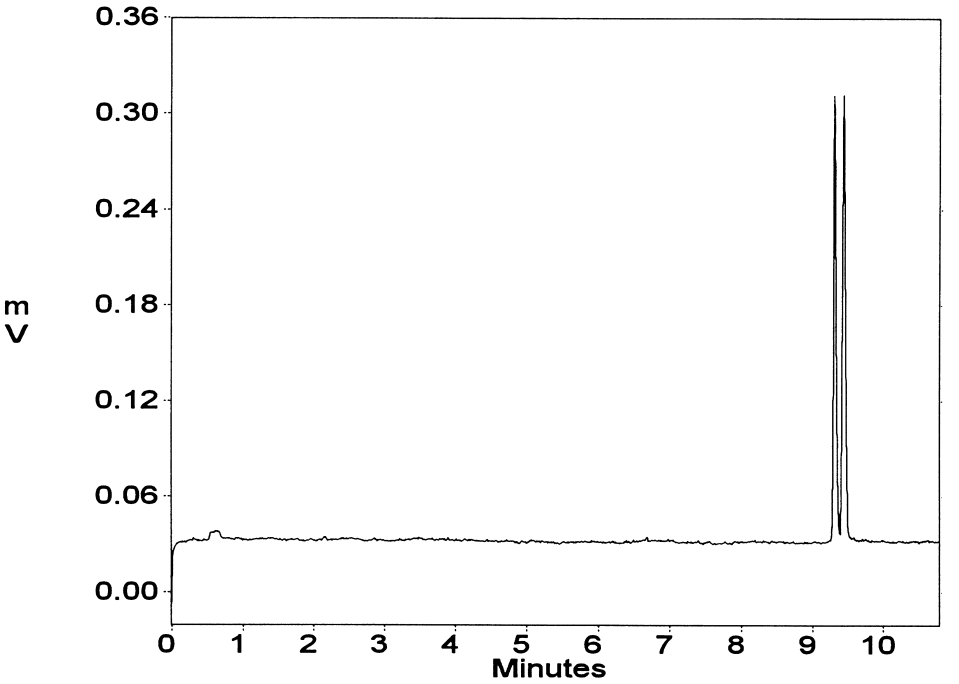

Figure 2. Separation of racemic chlorpheniramine with the nominal procedure. Buffer: 20 mM HP-β-CD in 100 mM sodium phosphate, pH 2.5. Capillary: 50 μm i.d., total length, 66 cm, detection length, 51 cm. Hydrodynamic injection: 50 mbar, 1.2 s. Voltage: 20 kV. Temperature: ambient (25 ± 1°C). Detection: 210 nm. Sample: 100 μg mL^{-1} solution of (±)-chlorpheniramine maleate in water.

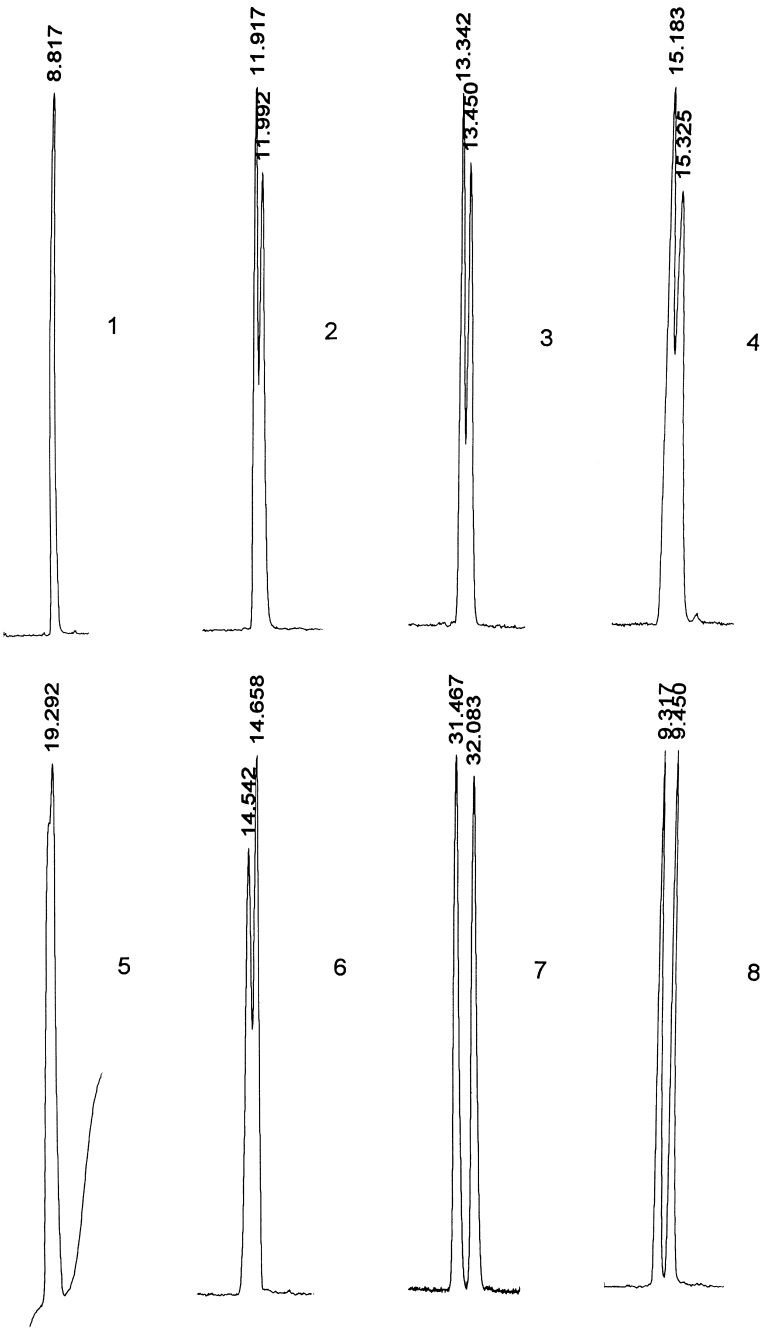

Figure 3. Separation of racemic chlorpheniramine with the conditions of the Experiments 1-15 of the Plackett-Burman design. Sample: 100 μg mL^{-1} solution of (±)-chlorpheniramine maleate in water. Conditions as described in section Experimental. Chromatogram of Experiment 8 is repeatedly shown for the sake of ready comparisons.

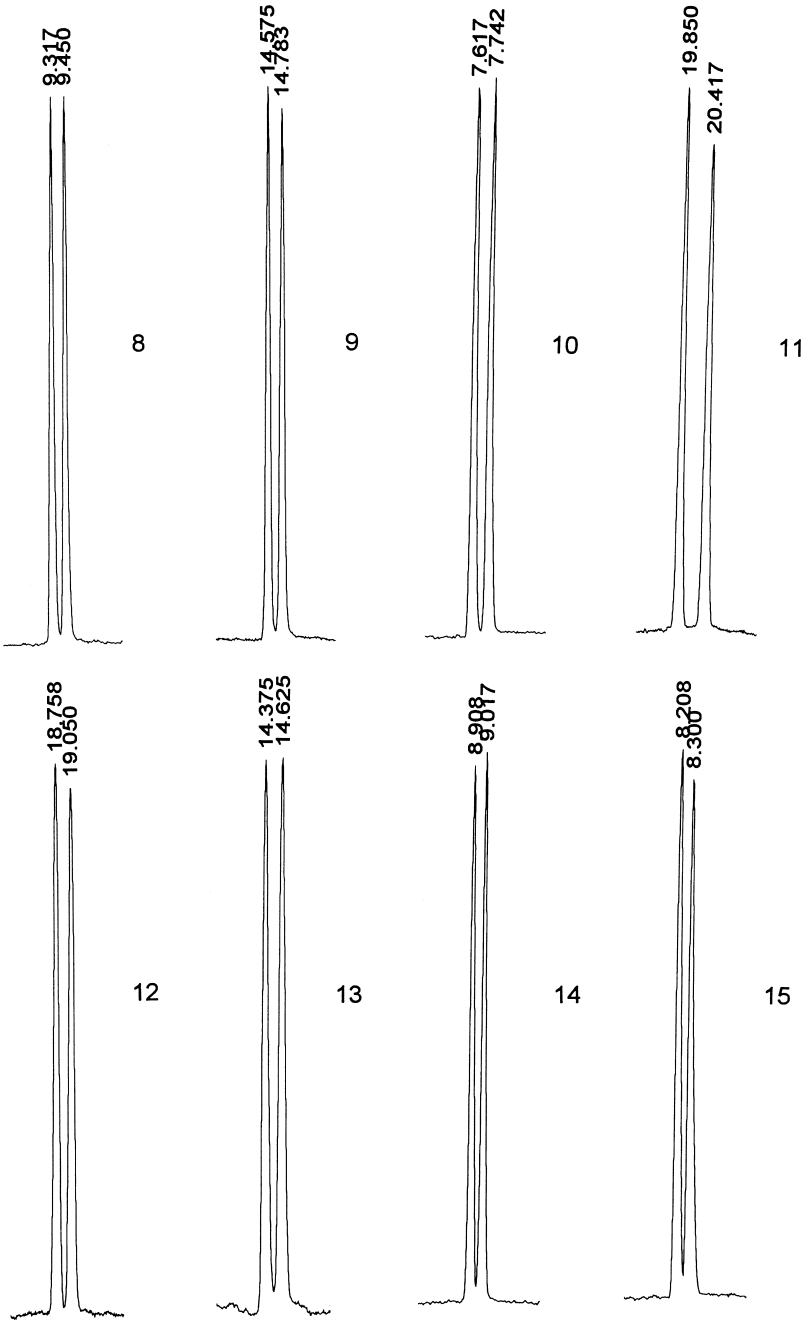

Figure 3. Continued.

Table 3. Resolution (*Rs*) and Migration Time (*t*) Obtained from the Experiments 1-8 of the Plackett-Burman Design

Experiment	*R*s*	*t* (min)
1	0.00	8.70
2	0.51	11.97
3	0.63	13.46
4	0.43	15.22
5	0.00	19.31
6	0.45	14.69
7	1.00	32.05
8	1.00	9.57

**R*s = depth of peak valley/interpolated peak height.

time, and peak efficiency) are listed in Tables 3 and 4. Main effects of factors on responses were calculated and they are indicated in Table 5.

Effects of the factors on the three responses are discussed as follows.

Effect of Buffer pH

Buffers of pH 2.5 and 3.0 had been used in literature for separation of chlorpheniramine enantiomers.[2-5] In this work, pH 2.5 was set as the nominal level of the experiments. From Table 5, it was seen that change to low pH (pH 2.5→2.0) greatly impaired resolution but it had no effect on migration time;

Table 4. Resolution (*R*s), Migration Time (*t*) and Peak Efficiency (N) Obtained from the Experiments 8–15 of the Plackett-Burman Design

Experiment	*R*s*	*t* (min)	N[#]
8	1.82	9.47	2.78×10^5
9	1.83	14.69	2.52×10^5
10	1.89	7.74	2.31×10^5
11	2.91	20.30	1.74×10^5
12	1.63	18.96	1.72×10^5
13	1.50	14.49	1.24×10^5
14	1.49	9.13	2.47×10^5
15	1.13	8.27	1.67×10^5

**R*s = $1.18(t_2\text{-}t_1)/[(w_1)_{0.5}+(w_2)_{0.5}]$.
[#]N = $5.545\ (t/w_{0.5})^2$.

Table 5. Percentage Effect (%E_x) of the Factor Levels on Resolution (*R*s), Migration Time (*t*) and Peak Efficiency (N)

	Percentage Effect (%E_x)		
Level	*R*s	*t*	N
Low pH	−87.00*	−28.95	
High pH	29.21*	22.13*	−10.19
Low [HP-β-CD]	1.00	−22.87	
High [HP-β-CD]	23.03	17.86*	0.49
Low cation (TMA^+)	5.00	56.19*	
High cation ($TEA\times H^+$)	2.25	40.76*	−45.15*
Low organic solvent (Acetonitrile)	−113.00*	−14.61	
High organic solvent (MeOH)	−18.54	22.28*	−6.80*
Low voltage	−5.00	49.73*	
High voltage	−30.90*	−46.20*	−13.11*
Low S.E.	7.00	19.07	
High S.E.	13.35	5.63	6.18

*Indicating the effect is significant.

change to high pH (pH 2.5→3.0) improved resolution slightly, but it had great effect on migration time (migration time being largely increased). pH 2.5 was, therefore, better than pH 2.0 and 3.0 when resolution and migration time were simultaneously taken into account.

Chlorpheniramine has two pK_a's, one (pK_a 4.0) belonging to the less-basic nitrogen on pyridine, the other (pK_a 9.2) to the more-basic nitrogen on aliphatic chain.[16] When buffer pH was changed from 2.5 to 3.0, protonation of nitrogen on pyridine declined, so that part of the doubly-charged molecules became singly charged, the mobility of chlorpheniramine decreased, and the migration time largely increased.

Effect of HP-β-CD

Crommen and Khaledi reported that HP-β-CD and β-CD were more enantioselective than DM- and TM-β-CD toward chlorpheniramine.[4,5] Due to its higher solubility, HP-β-CD was used in this work. Concentration of HP-β-CD at 20 mM was set to the nominal level following the condition of Khaledi.[5] From Table 5, it was seen that a low concentration change of HP-β-CD (i.e., 20 mM→15 mM) affected neither resolution nor migration time; high concentration

change of HP-β-CD (i.e., 20 mM→25 mM) had no effect on resolution, as well as plate number, but it increased the migration time. In theory, resolution should go through a maximum with increasing selector concentration. In this case, resolution remained on a plateau over the whole range of HP-β-CD tested (15 mM ~ 25 mM). Chlorpheniramine has two aromatic moieties attached to the chiral carbon. Although the chlorophenyl group is more lipophilic than the pyridinyl group, the latter should have a chance to enter into the host cavity. The host-guest binding behavior might not be as simple as the one-to-one relationship proposed by Wren.[17] The increase of migration time with a high concentration change of HP-β-CD (20 mM→25 mM) might be explained by the increase of buffer viscosity.

Effect of Organic Cations Composing the Buffer

In chiral separation, inorganic cations composing the buffers were sometimes replaced by organic amines and ammonium ions such as TRIS, triethanolamine, and tetramethylammonium ions. These organic cations can slow down the electroosmotic flow, thus increasing migration time and sometimes enhancing separation. It was also reported that peaks became more symmetrical due to less adsorption of analytes on the wall.[4] Crommen and Khaledi used triethanolamine and tetramethylammonium as buffer cations, respectively, in separation of chlorpheniramine enantiomers.[4,5] In Plackett-Burman design of this work, tetramethylammonium and protonated triethanolamine ions were assigned to the lower and upper levels, respectively, (Table 2) to test their effects on separation.

From Table 5, it was seen that when sodium ion was changed to either tetramethylammonium or protonated triethanolamine ion, migration time was highly increased, however, resolution was not ameliorated at all. In general, decrease of electroosmotic flow helps to enhance resolution, but resolution is also affected by other factors, such as peak efficiency (plate number) and electrophoretic mobilities of the solutes. In these experiments, the awful decay of peak efficiency was associated with the use of triethanolamine (Table 5). This could probably explain why the resolution was not improved by the use of organic cations.

Effect of Organic Modifiers

Organic solvents change viscosity and dielectric constant of buffer solution, as well as zeta potential of both capillary wall and analyte ions. It is difficult to predict the effect of organic modifiers upon separation. Methanol and

acetonitrile are two solvents most often used as organic modifiers and, thus, were tested in this work. From Table 5, it was seen upon addition of acetonitrile (10 % by volume), resolution deteriorated dramatically but the migration time remained unchanged. In contrast to acetonitrile, methanol (also at 10 % by volume) had no effect on resolution but migration time was greatly increased. Acetonitrile affected much more seriously the separation than methanol, probably having something to do with its higher lipophilicity, which favors its binding into the CD cavity.

Effect of Applied Voltage

Increasing voltage (in some appropriate range) shortens migration time of solute and sharpens the peak. Resolution is improved following the increase of plate number. From Table 5, it was seen that low voltage change (20 kV→15 kV) did not improve resolution, but migration time was increased. High voltage change (20 kV→25 kV) damaged resolution and shortened, greatly, the migration time. Peak efficiency was also dropped. Large Joule heating produced at high voltages should be attributed, and it was advisable to set the voltage at nominal level (20 kV) to get an optimum separation.

Optimized Conditions Obtained from the Plackett-Burman Design

As a result of the Plackett-Burman design, the optimized conditions were determined as follows:

1) pH of buffer was set at 2.5 because at 3.0 the small gain in resolution was largely offset by the long migration time. pH 2.0 damaged resolution seriously.

2) Concentration of HP-β-CD could be set either to 15 mM or to 20 mM because no difference in resolution and migration time between the two concentrations was found. Concentration of 25 mM only lengthened migration time with no gain in resolution.

3) Resolution kept inert to the change of buffer cation from sodium to trimethanolamine or tetramethylammonium ions. The over-prolonged migration time precluded the use of such ions in this work.

4) Addition of acetonitrile seriously damaged resolution. Methanol prolonged migration time. Neither of the two organic modifiers was helpful to separation.

5) Applied voltage was maintained at its nominal value (20 kV).

The above settings corresponded exactly to the values used in the nominal procedure. There was no need to proceed with further searches for optimum conditions. This nominal procedure was consequently applied to determine the optical purity of chlorpheniramine.

Determination of Optical Purity of Dexchlorpheniramine

Because the two components (dextro- and levo-) of enantiomers have almost identical detector responses, the internal normalization method can be used readily for their quantitation.[18] With this method, the content of one isomer is found from the ratio of its area to the sum of the two isomers. One dexchlorpheniramine chemical (as bulk substance) and two brands of dexchlorpheniramine commercial tablets were tested in this work. All of them were found to contain levorotatory isomer. Chromatogram obtained from one brand of dexchlorpheniramine tablets is shown in Figure 4. Migration times (n=7) were 10.28 min and 10.38 min for the (-)- and (+)-isomers, respectively, with RSD being

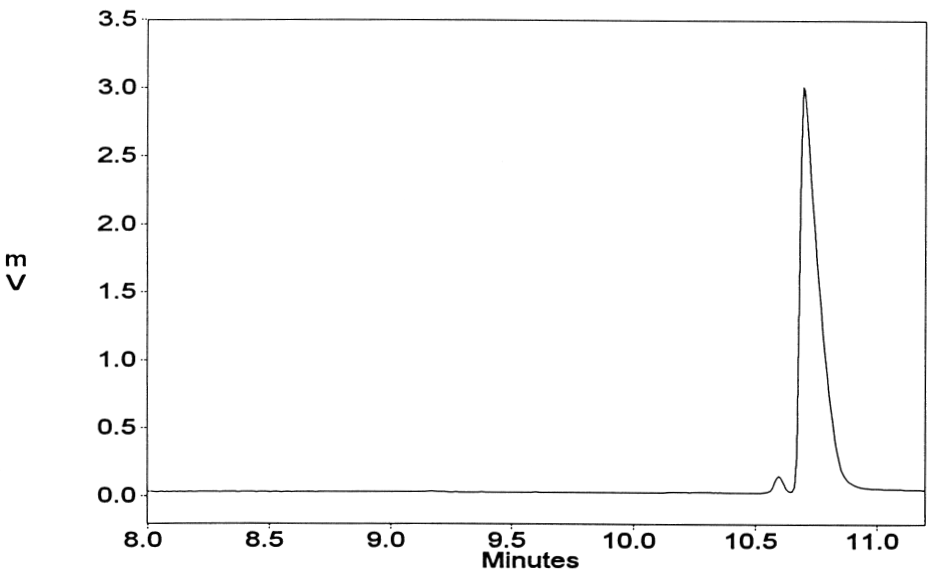

Figure 4. Electropherogram of commercial dexchlorpheniramine tablets from one pharmaceutical company obtained with optimized conditions. Conditions as described in Figure 2.

between 0.72% and 2.24% for the three tested samples. Repeatabilities of peak area (n=7) were between 1.44% and 3.53% RSD for the (-)-isomer.

To make quantitation more accurate, Altria proposed the correction of peak area relative to migration time.[19] RSD of peak area after correction were between 1.01% and 2.56%. The impurity percentages of (-)-chlorpheniramine, calculated with the internal normalization and corrected peak area, were found to be 1.82% (RSD 0.87%, n=7), 1.19% (RSD 0.74%, n=7), and 1.92% (RSD 1.04%, n=7) for the bulk substance and two commercial tablets, respectively.

CONCLUSIONS

With the aid of the Plackett-Burman design, this work developed a quick and simple procedure for determination of dexchlorpheniramine in bulk substances and commercial preparations. This procedure is suitable as a routine method for assay of dexchlorpheniramine.

The utility of the Plackett-Burman design as a screening tool for optimization has been shown in this work. The design allowed the test of qualitative factors that were discrete in nature, and the results showed that organic cations and organic modifiers were unsuccessful in the separation of chlorpheniramine enantiomers under the tested conditions.

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REFERENCES

1. Reynolds, J.E.F. *Martindale*, *the Extra Pharmacopoeia*, *31st Ed.*; Royal Pharmaceutical Society: London, 1996; 437.
2. Otsuka, K.; Terabe, S. J. Liq. Chromatogr. **1993**, *16*, 945-953.
3. Koppenhoefer, B.; Epperlein, U.; Christian, B.; Lin, B.; Ji, Y.; Chen, Y. J. Chromatogr. A **1996**, *735*, 333-343.
4. Bechet, I.; Paques, P.; Fillet, M.; Hubert, P.; Crommen, J. Electrophoresis **1994**, *15*, 818-823.
5. Quang, C.; Khaledi, M.G. J. Chromatogr. A **1995**, *692*, 253-265.
6. Rogan, M.M.; Altria, K.D.; Goodall, D.M. Chromatographia **1994**, *38*, 723-729.

7. Boonkerd, S.; Detaevernier, M.R.; Vander Heyden, Y.; Vindevogel, J.; Michotte, Y. J. Chromatogr. A **1996**, *736*, 281-289.
8. Plackett, R.L.; Burman, J.B. Biometrika **1946**, *33*, 305-325.
9. Massart, D.L.; Vandeginste, B.G.M.; Deming, S.N.; Michotte, Y.; Kaufman, L. *Chemometrics*: *A Textbook*; Elsevier: Amsterdam, 1988; 101-106.
10. Montgomery, D.C. *Design and Analysis of Experiments*, 4th Ed.; John Wiley: New York, 1997; 416-420.
11. Mulholland, M.; Waterhouse, J. Chromatographia **1988**, *25*, 769-774.
12. Vander Heyden, Y.; Questier, F.; Massart, D.L. J. Pharm. Biomed. Anal. **1988**, *17*, 153-168.
13. Fabre, H. J. Pharm. Biomed. Anal. **1996**, *14*, 1125-1132.
14. Vindevogel, J.; Sandra, P. Anal. Chem. **1991**, *63*, 1530-1536.
15. Bempong, D.K.; Honigberg, I.L. J. Pharm. Biomed. Anal. **1996**, *15*, 233-239.
16. Albert, A.; Serjeant, E.P. *The Determination of Ionization Constants*, 3rd Ed.; Chapman and Hall: New York, 1984; 167.
17. Wren, S.A.C. J. Chromatogr. **1993**, *636*, 57-62.
18. Altria, K.D.; Rogan, M.M. *Introduction to Quantitative Applications of Capillary Electrophoresis in Pharmaceutical Analysis*; Beckman Instruments: Fullerton, CA, 1994.
19. Altria, K.D. Chromatographia **1993**, *35*, 177-182.

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