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Chiral Separation of Alkylamine Antihistamines in Pharmaceuticals by Capillary Isotachophoresis with Charged Cyclodextrin

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Cyclodextrin-mediated capillary isotachophoresis (ITP) in cationic regime of the separation was developed for the separation and quantitation of alkylamine antihistamine dimethindene (DIM) and pheniramine (PHM) enantiomers in various pharmaceutical preparations (capsules, oral drops, gel, granulated powder). Several electrolyte systems of different compositions and pH were examined. The optimized chiral ITP electrolyte system was consisted of 10 mmol/L potassium acetate adjusted to pH 4.8 with acetic acid, containing 4 mmol/L negatively charged CE-β–CD (chiral selector) as the leading electrolyte with electroosmotic flow (EOF) suppressing additive, 0.2% (w/v) methylhydroxyethylcellulose (m-HEC), and 5 mmol/L β-alanine as the terminating electrolyte. The proposed electrophoretic method was successfully validated. It was convenient for the sensitive, simple, rapid, and highly reproducible assay of these antihistamine enantiomers. The calibration graphs relating the ITP zone length to the concentration of DIM and PHM enantiomers were rectilinear ($r = 0.999$) in the range 40.0–200.0 mg/L of each enantiomer. The relative standard deviations (RSD) were 0.75% for DIM(1), 0.63% for DIM(2), 1.05% for PHM(1), and 0.83% for PHM(2) ($n = 6$) when determining 100 mg/L DIM and PHM, respectively, standard solutions. According to the validation procedure based on the standard addition technique the recoveries were 97.66–98.34%. Good quantitation was obtained in short analysis time (a single analysis took about 12 min). The minimal sample pretreatment and low running costs make the proposed ITP method a good alternative to commonly used analytical methods (CZE, HPLC). The obtained results suggest that the proposed method is suitable for routine assay of dimethindene and pheniramine enantiomers in various pharmaceuticals.

Keywords capillary electrophoresis; cyclodextrins; dimethindene; enantioseparation; pharmaceutical analysis; pheniramine

INTRODUCTION

Dimethindene (DIM), *N*,*N*-dimethyl{2-[3-[1-(2-pyridyl) ethyl]-1*H*-inden-2-yl]ethyl}amine, and pheniramine (PHM), *N*,*N*-dimethyl-[3-phenyl-3-(2-pyridyl)propyl]amine (Figure 1), are potent H_1-receptor antagonists used as antihistaminics for the symptomatic relief of hypersensitivity reaction. They are usually administered in the form of granulated powders (PHM), capsules, oral drops, and gel (DIM). Capillary isotachophoresis (ITP) has recently emerged, as a promising analytical technique that consumes an extremely small amount of sample and that is capable of rapid, high-resolution separation, and reproducible quantitation of analytes.

Cyclodextrins are cyclic glucopyranoses that have a shape of a truncated cone with an open cavity, relatively hydrophobic and an outside hydrophilic due to the presence of hydroxyl groups (positions 2, 3, and 6 of glucopyranose). The hydroxyl groups present on the rim of the CDs can be easily modified by chemical reactions in order to obtain CD derivatives with a different degree of substitution. The modified CDs can exhibit very different properties than the native ones, which can be easily used for improving the selectivity of the enantiomers separation. A wide number of CD derivatives are currently used in CE for chiral analysis and among them we can mention the uncharged methylated-, hydroxyethylated-, hydroxypropylated-, acetylated-CDs, and the charged ones such as methylamino-, sulphobutylether-, carboxymethylated-, sulphated-, phosphated-CDs, etc. (Fanali, 2000).

Charged CD derivatives have been used as chiral selectors in CE for the first time by Terabe (1989). A high efficacy of this type of CDs in CE enantioseparations can be explained at least by two phenomena: (i) an effect of opposite analyte-selector migration and (ii) a higher stability of inclusion CD associates due to additional electrostatic interaction between the ionic substituents on the CD ring and the ionic guest compound (Chankvetadze et al., 1994; Mikuš et al., 2002).

Chiral CE separations of DIM and PHM (or its halogen-substituted analogues) have been accomplished using native and derivatized cyclodextrins (CDs) (Chankvetadze, Endresz,

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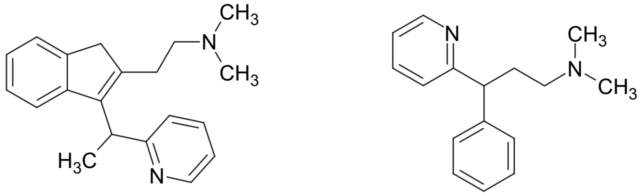

Dimethindene Pheniramine

FIGURE 1. Chemical structures of the drugs used.

& Blaschke, 1998; Chankvetadze, Pintore, et al., 2000; Chankvetadze, Schulte, Bergenthal, & Blaschke, 2000; Heuermann & Blaschke, 1993, 1994; Jin & Li, 1998; Matsunaga, Tanimoto, & Haginaka, 2002; Rudolf & Blaschke, 1999; Schuster, Gotte, Bernhardt, & Buschauer, 2001; Van Eeckhaut, Detaevernier, Crommen, & Michotte, 2004a, 2004b; Van Eeckhaut, Detaevernier, & Michotte, 2002; Wu, Huang, Chen, & Wu, 1999) and heparin (Jin & Stalcup, 1998) as chiral selectors. Liquid chromatography has also been reported for enantiomeric separations of these drugs (Hermansson & Grahn, 1995; Lai & Ng, 2004; Stephani & Cesare, 1998). Given the simplicity and robustness of cyclodextrin-based chiral separations (Schneiderman & Stalcup, 2000) it was decided to focus on this chiral selectivity mechanism. CDs and their derivatives differ significantly in their selectivities in CE separations of various groups of compounds. These differences provide a frame for a wide variety of the electrolyte systems suitable to the CE enantioseparations of ionogenic and nonionic pharmaceuticals (Fanali, 1997).

ITP is an electrophoretic separation technique in a discontinuous buffer system in which the analytes migrate according to their electrophoretic mobilities, forming a chain of adjacent zones moving with equal velocity between two solutions, leading (LE) and terminating (TE) electrolyte, bracketing the mobility range of the analytes. The major advantage of this method is that analytes cannot cross out of their zone and the net effect of this is that the zone compresses and the concentration of the analyte is increased (Kemp, 1998). ITP is a simple, quick, and low-cost method and therefore well suited for main drug determination. To the best of our knowledge, DIM and PHM enantiomers have not yet been determined by ITP so far. The aim of this work was to examine several chiral selectors for DIM and PHM, and to develop simple, sensitive, and selective capillary isotachophoresis (ITP) method for determination of DIM and PHM enantiomers in commercial pharmaceutical formulations, suitable for a routine use.

EXPERIMENTAL

Samples and Reagents

Chemicals used for the preparations of the electrolyte solutions were obtained from Merck (Darmstadt, Germany) and Aldrich (Steinheim, Germany). Methylhydroxyethylcellulose 30,000 (m-HEC) was obtained from Serva (Heidelberg, Germany). The electrolyte solutions were filtered before use through disposable membrane filters (1.2 μm pore size) purchased from Sigma (St. Louis, MO). Racemic DIM (maleate salt) was obtained as working standard from USP (USP Convention, Rockville, MA.) and racemic PHM (maleate salt) from ICN Biomedicals (Eschwege, Germany). The analyzed pharmaceuticals Fenistil® cps (containing 4 mg of DIM per capsule, Fenistil gtt (1 mg/mL of DIM), Fenistil gel (1 mg/g of DIM) (all are products of Novartis, Nyon, Switzerland) and granulated powder Fervex® (25 mg of PHM per powder) (UPSA, Agen, France) were obtained commercially. Native CDs were purchased from Aldrich (Steinheim, Germany). CE-β-CD (DS 3, CE purity) and HP-β-CD (DS 3, CE purity) are commercial product of Cyclolab (Budapest, Hungary).

Instrumentation and ITP Conditions

ACS Isotachophoretic Analyser (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in a single-column configuration of the separation unit. The analyzer was equipped with a 30 μL sampling valve, a 160 × 0.8 mm (i.d.) separation capillary made of fluorinated ethylene-propylene (FEP) copolymer and an on-column conductivity detector. Quantitative data were obtained by off-line processing of the stored isotachopherograms providing the length of the isotachophoretic zones by using the ITP software package supplied by the Villa-Labeco company. Prior to use, the capillary was given no particular treatment to suppress EOF. Dynamic coating of the capillary wall by means of 0.2% (w/v) m-HEC in leading electrolyte solution served for this purpose. The experiments were performed in constant current mode, in the cationic regime of the separation with direct injection of the samples. The driving current was 250 μA.

Sample and Standard Solution Preparations

Solid pharmaceuticals were prepared for analysis in the following way: ten capsules/granulated powders were finely powdered in the agate mortar. A portion of the powder equivalent to 5 mg of DIM or 10 mg of PHM was weighed accurately, transferred to a 10 mL volumetric flask and suspended in 5 mL of demineralized water and 0.5 mL of 1 mol/L acetic acid. The flasks were placed in ultrasonic water bath for 10 min before completion to the mark with the water. The mixtures were centrifuged (5000 rpm) for 10 min and appropriately diluted with demineralized water. The filtered samples [a 1.2 μm pore size (Sigma)] were injected into the capillary. Pharmaceuticals present in their liquid or gel dosage forms were weighed accurately and then appropriately diluted with slightly acid demineralized water and directly injected into the ITP equipment. The concentrations of the each drug in the injected pharmaceutical samples were in all cases 100 mg/L.

Pure standard stock solutions of DIM and PHM, respectively, were prepared in diluted acetic acid at 1000 mg/L concentration and they were stored at –8°C in the freezer. Working solutions were made by appropriate dilution of the stock solutions with demineralized water so that the concentrations of each drug in the injected model samples were 10–100 mg/L.

The water was demineralized by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona.). All chemicals used were of analytical grade or were additionally purified by the usual methods. Acetic acid was used for the preparation of the electrolyte solutions after isothermal distillation.

RESULTS AND DISCUSSION

Method Optimization

DIM and PHM represent molecules those migration velocities are influenced by a protonation of two basic groups so that a cationic regime of separation is convenient for their analysis. The principal operating parameters optimized in the present ITP separation was electrolyte systems composition, including type and concentration of CD, leading cation, counterion, and terminating cation and pH of the leading electrolyte. The ITP operational system was optimized with respect to the quality of separation [relative position of PRO (RSH), interferences], sharpness of zones, time of analysis and to achieve a sufficient enantioresolution of DIM and PHM.

Electrolyte systems with K^+ as the leading ion and acetate as the counter ion were tested in the pH range from 4.0 to 5.5. β-alanine was used as the terminating ion. Native α- (concentration range used 1–50 mmol/L) and β-CDs (1–10 mmol/L), neutral CD derivative HP-β-CD (1–50 mmol/L), and charged CD derivative CE-β–CD (1–6 mmol/L) were used in our experiments aimed at finding a chiral selector suitable for the separation of DIM and PHM enantiomers.

Charged CE-β-CD was found to be more effective than native CDs (no enantioseparation) and neutral HP-β-CD (partial enantioseparation). Sufficient ionization of both, analyte and chiral selector, was achieved at pH 4.8 resulting in a baseline enantioseparation of DIM and PHM with a low concentration of the oppositely migrating CE-β-CD and a short analysis time. Optimized separating conditions consisted of 10 mmol/L potassium acetate adjusted to pH 4.8 with acetic acid, containing 4 mmol/L negatively charged CE-β-CD (chiral selector) as the leading electrolyte, and 5 mmol/L β-alanine as the terminating electrolyte. An electroosmotic flow (EOF) was eliminated by a dynamic coating of the inner surface of the capillary. For this purpose served a polymeric additive, methylhydroxyethylcellulose (m-HEC), present in the leading electrolyte at a 0.2% (w/v) concentration. The experiments were performed in constant current mode and the driving current applied was 250 μA. One ITP run took about 12 min.

Validation

After optimization of the separation conditions, some analytical characteristics of the developed ITP method were investigated using standard solutions, as given in the Experimental section. The validation parameters included sensitivity, linearity, precision (run-to-run, day-to-day), accuracy/recovery, and robustness. The linearity was tested from 40.0 to 200.0 mg/L of each enantiomer, with a coefficient of determination (r^2) of 0.999. The equation for calibration curve is: $y = a + bx$, where y is the zone length in sec and x is the concentration (mg/L). Calibration data are given in Table 1. Relatively low values of the intercept a are positive sign of correctly passed migration and analytical stability of the ITP zone and the high values of the correlation coefficient confirms good rectilinearity of the calibration graphs.

The method was validated by evaluation of run-to-run and day-to-day precision. The relative standard deviations (RSD) of six replicate analyses of 100 mg/L DIM and PHM, respectively, standard samples were 0.75% for DIM(1), 0.63% for DIM(2), 1.05% for PHM(1), and 0.83% for PHM(2), indicating good run-to-run precision of the ITP method. Day-to-day reproducibility of the calibration curves was characterized by the RSD of the slope and intercept. The RSD values did not exceed 2.84%. The determined detection limit and the quantitation limit of each DIM and PHM enantiomer were 10 and 21 mg/L, respectively. The sensitivity of this proposed method was sufficient for the assay of DIM and PHM enantiomers in all dosage forms. The method accuracy was examined by adding a known amount of DIM or PHM reference substance to the real sample (capsules, oral drops, gel, granulated powder). The data from the recovery test, given in Table 2, clearly indicated an acceptable accuracy of the proposed ITP method.

TABLE 1
ITP Results for RSH[a] Reproducibility and Calibration of Dimethindene and Pheniramine Enantiomers

Parameter	DIM(1)	DIM(2)	PHM(1)	PHM(2)
RSH	0.81	0.85	0.84	0.88
R.S.D. (%) ($n = 5$)	1.01	0.82	0.91	1.07
Range (mg/L)	40.0–200.0	40.0–200.0	40.0–200.0	40.0–200.0
Intercept (a)	–0.085	–0.071	0.162	0.154
Sa[b]	0.045	0.038	0.082	0.077
Slope (b)	0.118	0.122	0.121	0.146
Sb[b]	0.039	0.057	0.079	0.069
r[2c]	0.9994	0.9995	0.9993	0.9994

[a] Relative step heights, relative position of the analyte zone toward zones of leading and terminating ions measured via changes of conductivity.

[b] Standard deviation values of intercept (Sa) and slope (Sb).

[c] Coefficient of determination.

TABLE 2
ITP Recoveries of Dimethindene and Pheniramine Added to Pharmaceuticals

Parameter	Fenistil (DIM) Capsules	Fenistil (DIM) Oral Drops	Fenistil (DIM) Gel	Fervex (PHM) Granulated Powder
Added (mg/L)	50	50	50	50
Found (mg/L) ± RSD (%)	48.83 ± 1.57	48.94 ± 1.89	49.06 ± 1.92	49.17 ± 1.64
Recovery (%)	97.66	97.88	98.12	98.34

An indication of acceptable ruggedness of the ITP method was based on RSD values of qualitative and quantitative migration parameters of DIM and PHM enantiomers obtained from the analyses of pharmaceutical samples in different laboratories by different workers over a period of one week. The RSD did not exceed 3%. The deliberate changes of experimental parameters included the pH of leading electrolyte (4.6–5.0) and the driving current (200 and 250 μA).

Application

The validated method was applied for the determination of DIM and PHM enantiomers in commercial pharmaceutical formulations including capsules, oral drops, gel, and granulated powder. Results from the determinations are given in Table 3. The contents of DIM and PHM, obtained by the proposed method, were in a good agreement with those declared by the manufacturers. The absolute differences between determined and declared values did not exceed 1.13% with RSD less than 1.63%. The chiral method clearly confirmed racemic composition of the active compound in the preparations. The isotachopherograms from the chiral analysis of the pharmaceutical samples (commercial oral drops, capsules, and gel Fenistil, and granulated powder Fervex) are shown in Figure 2. No detection interferences from the samples solvents, impurities and dosage form excipients were occurred separating DIM and PHM enantiomers in the samples.

CONCLUSION

This work outlined the significant potential of ITP employing charged chiral selector, CE-β–CD, for providing higly effective chiral separations of DIM and PHM. It was demonstrated that CE-β–CD could be effectively applied in ITP enantioseparations of antihistamines when present in (at least partially) ionized form. On the other hand, native and neutral forms of CD have not been effective for this purpose. The advantages of the proposed method are short analysis time, low consumption of electrolytes, chiral selectors, and samples. The major advantage of this method is low operating costs. The typical volume of aqueous buffer used per day is in the order of 10–100 mL. This compares favorably with HPLC, where liters of waste organic solvent are produced per day. Moreover, the direct injection of samples without their pretreatment is feasible. Performance parameters including precision (repeatability, intermediate precision, reproducibility) and accuracy are comparable or better than those obtained by alternative methods (CZE, HPLC) (Chankvetadze et al., 1998; Hermansson & Grahn, 1995; Jin & Li, 1998; Lai & Nai, 2004; Matsunaga et al., 2002). It is concluded that the proposed

TABLE 3
Enantioselective Determination of Dimethindene and Pheniramine in Pharmaceuticals[a]

Parameter	Fenistil (DIM) Capsules	Fenistil (DIM) Oral Drops	Fenistil (DIM) Gel	Fervex (PHM) Granulated Powder
Average content[b]	per 1 capsule	per 1 mL	per 1 g	per 1 powder
declared (mg)	4	1	1	25
determined ± RSD (%)	98.87 ± 1.53	99.68 ± 0.91	100.89 ± 1.04	99.72 ± 1.63
Enantiomeric ratio, (1) / (2)	49.12 / 50.88	49.24 / 50.76	48.97 / 51.03	50.24 / 49.76

[a] For the optimized separating conditions see Section 3 (other working conditions and sample preparation procedures, Section 2). Concentration of DIM and PHM, respectively, in the injected samples was ~100 mg/L.

[b] Average content of DIM and PHM, respectively, in pharmaceuticals was calculated (from five consecutive runs) as a sum of enantiomers (1) and (2) using their zone lengths.

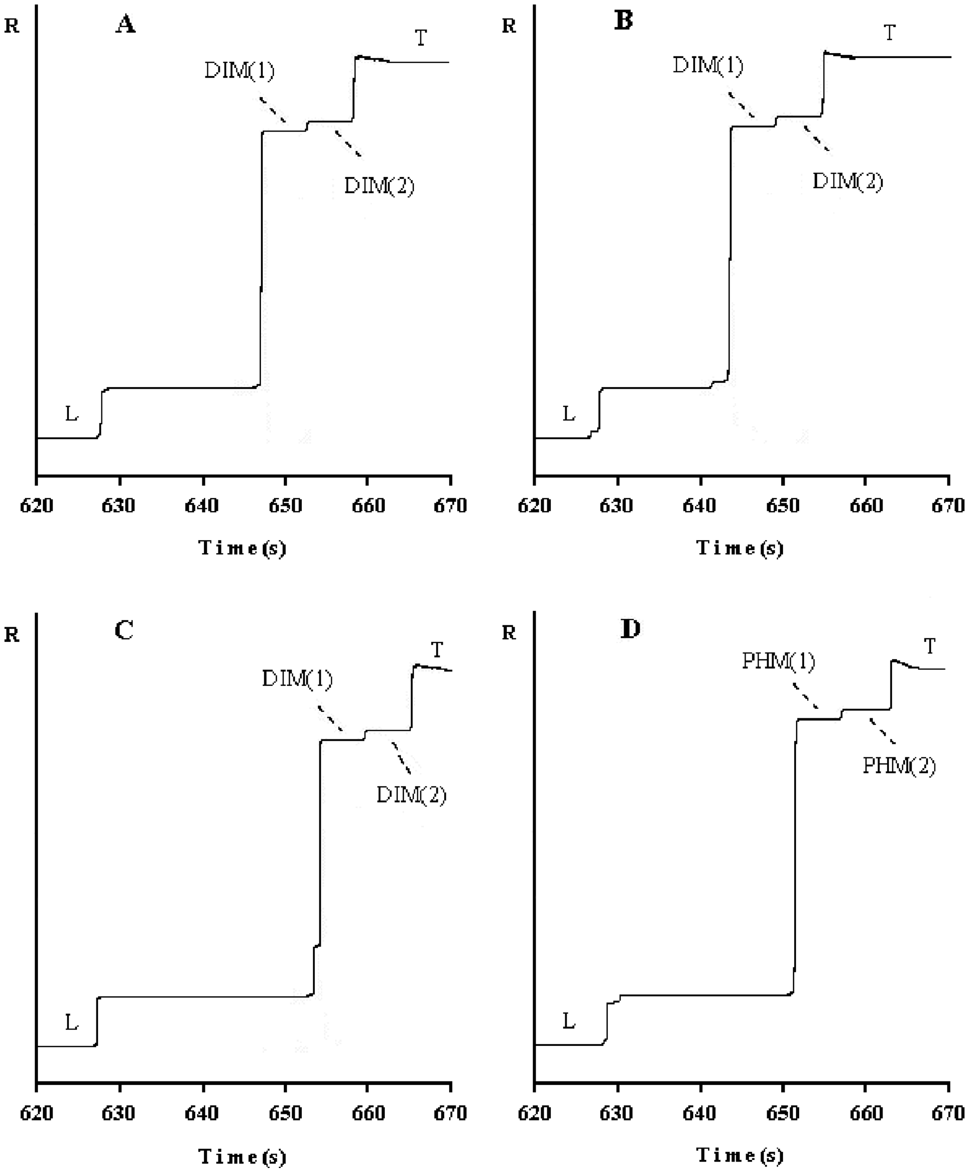

FIGURE 2. Isotachopherograms from the separations of DIM enantiomers in: (A) Oral drops fenistil, (B) Capsules fenistil, and (C) Gel fenistil; and from the separations of PHM enantiomers in (D) Granulated powder fervex. The separations were carried out in electrolyte system containing 4 mmol/L CE-β–CD (chiral selector). In all cases, the concentration of DIM and PHM, respectively, was 100 mg/L. DIM(1), DIM(2) = dimethindene enantiomers; PHM(1), PHM(2) = pheniramine enantiomers; L = leading ion; T = terminating ion; R = increasing resistance. For the samples preparation and other conditions see the experimental section.

method, with CE-β-CD as a chiral selector, is suitable for highly effective (reliable, simple, fast, and economic) enantioselective screening of pharmaceuticals containing DIM or PHM as an active ingredient. Moreover, the proposed ITP method can serve also to on-line preseparation and preconcentration of the drugs in complex (biological) samples as this technique is easily combinable with CZE method serving for the final separation and sensitive detection (for on-line coupled ITP-CZE methods, see, e.g., refs. Danková, Kaniansky, Fanali, & Ivanyi, 1999).

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REFERENCES

Chankvetadze, B., Burjanadze, N., Pintore, G., Bergenthal, D., Bergander, K., Mühlenbrock, C., Breitkreuz, J., & Blaschke, G. (2000b). Separation of brompheniramine enantiomers by capillary electrophoresis and study of chiral recognition mechanisms of cyclodextrins using NMR-spectroscopy, UV spectrometry, electrospray ionization mass spectrometry and X-ray crystallography. *J. Chromatogr. A, 875,* 471–484.

Chankvetadze, B., Endresz, G., & Blaschke, G. (1994). About some aspects of the use of charged cyclodextrins for capillary electrophoresis enantioseparation. *Electrophoresis, 15,* 804–807.

Chankvetadze, B., Pintore, G., Burjanadze, N., Bergenthal, D., Bergander, K., Breitkreuz, J., Mühlenbrock, C., & Blaschke, G. (2000a). Mechanistic study of opposite migration order of dimethindene enantiomers in capillary electrophoresis in the presence of native β-cyclodextrin and heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin. *J. Chromatogr. A, 875,* 455–469.

Chankvetadze, B., Schulte, G., Bergenthal, D., & Blaschke, G. (1998). Comparative capillary electrophoresis and NMR studies of enantioseparation of dimethindene with cyclodextrins. *J. Chromatogr. A, 798,* 315–323.

Danková, M., Kaniansky, D., Fanali, S., & Ivanyi, F. (1999). Capillary zone electrophoresis separations of enantiomers present in complex ionic matrices with on-line isotachophoretic sample pretreatment. *J. Chromatogr. A, 838,* 31–43.

Fanali, S. (1997). Controlling enantioselectivity in chiral capillary electrophoresis with inclusion–complexation. *J. Chromatogr. A, 792,* 227–267.

Fanali, S. (2000). Enantioselective determination by capillary electrophoresis with cyclodextrins as chiral selectors. *J. Chromatogr. A, 875,* 89–122.

Hermansson, J., & Grahn, A. (1995). Optimization of the separation of enantiomers of basic drugs retention mechanisms and dynamic modification of the chiral bonding properties on a α_1-acid glycoprotein column. *J. Chromatogr. A, 694,* 57–69.

Heuermann, M., & Blaschke, G. (1993). Application of cyclodextrin buffers in capillary electrophoresis to enantioselective determination of the H1-antihistamine dimethindene in human urine. *Pharmazie, 48,* 74–75.

Heuermann, M., & Blaschke, G. (1994). Simultaneous enantioselective determination and quantification of dimethindene and its metabolite *N*-demethyl-dimethindene in human urine using cyclodextrins as chiral additives in capillary electrophoresis. *J. Pharmaceut. Biomed. Anal., 12,* 753–760.

Jin, L. J., & Li, S. F. Y. (1998). Comparison of chiral recognition capabilities of cyclodextrins for the separation of basic drugs in capillary zone electrophoresis. *J. Chromatogr. B, 708,* 257–266.

Jin, Y. K., & Stalcup, A. M. (1998). Application of heparin to chiral separations of antihistamines by capillary electrophoresis. *Electrophoresis, 19,* 2119–2123.

Kemp, G. (1998). Capillary electrophoresis: A versatile family of analytical techniques. *Biotechnol. Appl. Biochem., 27,* 9–17.

Lai, X. H., & Ng, S. C. (2004). Preparation and chiral recognition of a novel chiral stationary phase for high-performance liquid chromatography, based on mono (6^A-*N*-allylamino-6^A-deoxy)-perfunctionalized β-cyclodextrin and covalently bonded silica gel. *J. Chromatogr. A, 1031,* 135–142.

Matsunaga, H., Tanimoto, T., & Haginaka, J. (2002). Separation of basic drug enantiomers by capillary electrophoresis using methylated glucuronyl glucosyl beta-cyclodextrin as a chiral selector. *J. Sep. Sci., 25,* 1175–1182.

Mikuš, P., Kaniansky, D., Šebesta, R., & Sališová, M. (2002). Cyclodextrins and their complexes—Structure and interactions. *Chem. Listy, 96,* 693–697.

Rudolf, J., & Blaschke, G. (1999). Stereoselectivity of the Phase-II-metabolism of the H-1 antihistaminic drug dimethindene investigated by capillary electrophoresis. *Enantiomer, 4,* 317–323.

Schneiderman, E., & Stalcup, A. M. (2000). Cyclodextrins: A versatile tool in separation science. *J. Chromatogr. B, 745,* 83–102.

Schuster, A., Gotte, C., Bernhardt, G., & Buschauer, A. (2001). Chiral separation of pheniramine-like 3-phenyl-3-heteroarylpropylamines by CE and HPLC methods. *Chirality, 13,* 285–293.

Stephani, R., & Cesare, V. (1998). Enantiomeric enrichment of non-racemic antihistamines by achiral high-performance liquid chromatography. *J. Chromatogr. A, 813,* 79–84.

Terabe, S. (1989). Electrokinetic chromatography—An interface between electrophoresis and chromatography. *Trends Anal. Chem., 8,* 129–134.

Van Eeckhaut, A., Detaevernier, M. R., Crommen, J., & Michotte, Y. (2004a). Differential effects of organic modifiers in the enantioseparation of dimetindene maleate with carboxymethly-beta-cyclodextrin in capillary electrophoresis. *J. Sep. Sci., 27,* 21–27.

Van Eeckhaut, A., Detaevernier, M. R., Crommen, J., & Michotte, Y. (2004b). Influence of methanol on the enantioresolution of antihistamines with carboxymethyl-beta-cyclodextrin in capillary electrophoresis. *Electrophoresis, 25,* 2838–2847.

Van Eeckhaut, A., Detaevernier, M. R., & Michotte, Y. (2002). Development of a validated capillary electrophoresis method for enantiomeric purity testing of dexchlorpheniramine maleate. *J. Chromatogr. A, 958,* 291–297.

Wu, H. L., Huang, C. H., Chen, S. H., & Wu, S. M. (1999). Chiral quantitation of pheniramine, chlorpheniramine, and brompheniramine maleates by capillary zone electrophoresis. *J. Chromatogr. Sci., 37,* 24–30.