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Review

Capillary electrophoresis of cardiovascular drugs

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Abstract

This review surveys the use of capillary electrophoresis for the analysis of cardiovascular drugs. Each section presents examples of separations according to the class of the cardiovascular agent. The classes presented are β-adrenergic antagonists (β-blockers), acetylcholinesterase inhibitors, angiotensin-converting enzyme inhibitors, diuretics, α-adrenergic antagonists, calcium channel blockers, cardiac glycosides, hypolipidemics (HmG–CoA reductase inhibitors and fibric acid), vasodilators and sodium channel blockers. Examples of the separation modes discussed include capillary electrophoresis, micellar electrokinetic chromatography using many additives (e.g. sodium dodecyl sulfate, cyclodextrins, bile salts, proteins, oligosaccharides) and isotachophoresis.

Keywords: Reviews; Pharmaceutical analysis; Enantiomers separation; Drugs; Beta-blockers; Calcium channel blockers; Enzyme inhibitors; Diuretics; Cardiac glycosides; Adrenergic antagonists; Fibric acid; Vasodilators; Sodium channel blockers

Contents

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1. Introduction

There are several cardiovascular diseases and multiple cardiovascular agents for their treatment [1]. The most common cardiovascular disease is hypertension. Hypertension increases the risk of stroke and other heart diseases. Cardiovascular drugs are used to control this disease, with no cure being currently available. Diuretics, β-adrenergic antagonists, angiotensin-converting enzyme inhibitors (ACE) and calcium channel blockers are the primary routes of treatment for hypertension. Angiotensin II antagonists are a new class of drugs which it is hoped will be as effective as ACE inhibitors in the treatment of hypertension with fewer side effects.

Angina is another form of cardiovascular disease for which several agents are available for treatment. This disease is typically caused by atherosclerosis, the narrowing of blood vessels by lipid deposit on the walls of the vessels which supply blood to the heart. The common drug classes used for treatment of angina include β-adrenergic antagonists, calcium channel blockers and nitroglycerin.

Congestive heart failure is frequently the outcome of heart attacks. The body physiologically responds to this condition in a manner which makes it harder for the heart to function, the outcome being greater and greater stress on the heart. This condition is treated with ACE inhibitors and hydralazine combined with isosorbide dinitrate.

Atherosclerosis is treated by lowering the low-density lipoprotein (LDL) cholesterol levels. Hypolipidemics, the HmG–CoA reductase inhibitors, are the agents used to accomplish the lowering of LDL. These agents are frequently used in combination with bile acid sequesterants.

Stroke is a disease for which no treatment exists once it has occurred. Thrombosis is another cardiovascular disease for which thrombolytic agents are used to thin the blood. The use of these agents minimizes the risk of clot formation in patients predisposed to stroke. Antihypertensives also reduce the risk of stroke.

Capillary electrophoresis (CE) has undergone rapid growth over the past 15 years because of the short run times and high resolution [more than two orders of magnitude when compared with high-performance liquid chromatography (HPLC)]. There are many general reviews on CE separations [2–6] and this technique is becoming popular for the analysis of pharmaceuticals, as evidenced by the substantial number of reviews for this area of analysis [7–19].

In this review, the CE analysis of drugs used in the treatment of various cardiovascular diseases will be presented according to their pharmacological class. No specific effort will be made to indicate the specific cardiovascular disease for which each drug is used.

2. β-Adrenergic antagonists

β-Adrenergic receptor antagonists block the binding of catecholamines to the β-receptor, therefore slowing the heart rate and decreasing myocardial contractility [20]. This effect is low when β-receptor stimulation is low, but is large when the sympathetic nervous system is activated, e.g., during exercise or stress. Peripheral resistance is increased as a result of β_2-receptor blockade.

There are many marketed β-adrenergic antagonists and they have provided a rich source of compounds for study by CE. Owing to the potential for each isomer to have different pharmacological activity, potency, toxicity and mode of action, drug regulatory agencies throughout the world require monitoring of enantiomeric purity of drug substances and products [21–23]. This requirement has driven the development of analytical techniques for the resolution and characterization of chiral molecules. In addition to the resolution of enantiomers, CE has been utilized in the analysis of drugs from biological fluids, both for pharmacokinetic determinations and for drugs of abuse. The discussion that follows for β-adrenergic blocking agents will demonstrate the utility of CE for such analyses.

Fanali [24] resolved propranolol enantiomers through the addition of cyclodextrins (CDs) to the background analyte. Separation was effected through the difference in formation constants for

each enantiomer with CD and the resultant difference in the effective electrophoretic mobility of the complexes. The effect of α-, β- and γCD, 2,6-di-OMe-β-CD (DMCD) and 2,3,6-tri-OMe-β-CD (TMCD) on migration time and resolution was studied. Phosphate buffer (100 mM) of pH 2.5, used as the background electrolyte, presented the propranolol as a cation for interaction with the CDs. The addition of αCD did not affect the migration time and provided no optical resolution, whereas all other CDs studied retarded the retention of propranolol. Increasing the amount of CD resulted in a larger increase in migration time. While the migration time of propranolol was increased by the CDs, resolution was only achieved using TMCD (maximum resolution 40 mM TMCD). Addition of 4 M urea and utilizing 30% methanol in 50 mM phosphate buffer (pH 2.5) provided optical resolution when using βCD. The urea enhanced the solubility of βCD and methanol was believed to affect the solvation of the complexes differentially; both additives were required to effect resolution. Resolution was not achieved when the urea–methanol background electrolyte was used with TMCD. Similar results were observed by Palmarsdottir and Edholm [25], who examined the use of αCD, βCD, DMCD and hydroxypropyl-βCD (HPCD) to separate propranolol enantiomers. The isomers could not be resolved using α- or βCD, whereas DMCD and HPCD were suitable chiral resolving agents using 50 mM phosphate buffer (pH 2.5).

A model was developed by Wren and Rowe [26] to describe the resolution of enantiomeric pairs by CE using CD additives. The model accounts for a freely soluble analyte and an analyte–chiral selector complex, each having its own electrophoretic mobility. The apparent mobility is the mean of the electrophoretic mobilities of the analyte and the complex, as described by

$$\overline{\mu_{app}} = \frac{\mu_1 + \mu_2 K_1 C}{1 + K_1 C} \quad (1)$$

where μ_{app} is the apparent mobility, μ_1 the mobility of the uncomplexed analyte, μ_2 the mobility of the complexed analyte, K_1 the formation constant for the complex and C the concentration of CD. Thus, the difference in the apparent mobilities of two chiral species can be described by

$$\Delta\mu = \frac{C(\mu_1 - \mu_2)(K_2 - K_1)}{1 + C(K_1 + K_2) + K_1 K_2 C^2} \quad (2)$$

where $\Delta\mu$ is the difference in the apparent mobilities of the two enantiomers, μ_1 the electrophoretic mobility of the free analyte in solution, μ_2 the electrophoretic mobility of the complex, K_1 and K_2 the formation constants for the complexes of the two enantiomers and C the concentration of cyclodextrin. The difference in apparent mobilities of the enantiomers reaches a maximum as the concentration of the chiral selector increases, but then decreases with further increases in concentration. In addition, the larger the difference between the formation constants, the greater is the apparent mobility difference for the enantiomers. Another aspect of the model is that the larger the formation constant difference, the lower is the concentration of chiral selector needed to achieve maximum resolution. The equation also suggests that the apparent mobility difference will be maximized when the electrophoretic mobility of the complex is in the opposite direction to the analyte. This led to a suggestion that positively charged ionizable chiral selectors would be valuable for enhancing resolution. The model was applied to the mobilities determined for propranolol enantiomers using βCD (with 4 M urea) or DMCD using 40 mM lithium phosphate buffer (pH 3.12). The experimental data were very well fitted by the model (Fig. 1), with maximum separations achieved at 5.5 mM for DMCD and slightly higher for βCD (ca. 7 mM), indicating a lower affinity of propranolol for βCD.

Wren and Rowe [27] expanded discussion of their model to incorporate the role of organic solvents on the migration difference. While the model was a good fit for the data, no improvement was observed for resolution of propranolol enantiomers when organic modifiers were incorporated into the background electrolyte. In a

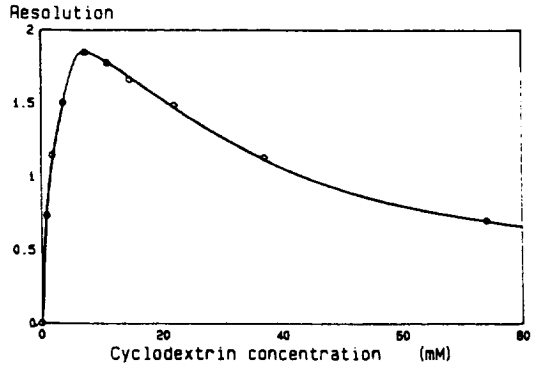

Fig. 1. Resolution between the propranolol enantiomers as a function of MBCD concentration. Run conditions: capillary 57 cm (50 cm to detector) × 75 μm I.D., 20 kV, 25°C, 40 m*M* lithium phosphate buffer (pH 3.12) containing 0–74 m*M* DMCD. (From Ref. [26]).

further study [28], they correlated the optimum level of CD additives to the log *P* values for propranolol, metoprolol and atenolol, but not oxprenolol. Wren et al. [29,30] provided further evidence for the validity of the equations using atenolol and practolol.

Guttman and Cooke [31] studied the effects of pH, applied electric field, temperature and concentration of HPCD on the resolution of propranolol isomers. Their results matched those presented by Wren and Rowe described above. Aumatell et al. [32] performed similar studies using HPCD or sodium sulfobutyl ether-βCD (SBECD) to separate labetalol, nadolol, oxprenolol, atenolol, alprenolol, propranolol, metoprolol, acebutolol and timolol isomers.

The enantiomers of carvedilol and pindolol were analyzed using micellar electrokinetic chromatography (MECC) with DMCD on uncoated capillaries by Soini et al. [33]. Hexadecyltrimethylammonium bromide (HTAB) was the micellar additive and methylhydroxyethylcellulose 4000 (MHEC) was added to improve reproducibility. Optimum conditions established for resolution of verapamil enantiomers (a calcium channel blocking agent, discussed in Section 6) were applied to the separation of carvedilol and pindolol enantiomers. The optimum separation conditions were 10 m*M* DMCD, 18 m*M* Tris buffer (pH 2.9), 0.1% MHEC and 0.03 m*M* HTAB, although only a partial resolution of pindolol was achieved. In addition, resolution of carvedilol enantiomers was achieved on polyacrylamide-coated capillaries with only a slight decrease in the degree of resolution compared with uncoated capillaries.

Gonzalez et al. [34] used pulsed-laser fluorescence detection for the CE analysis of acebutolol. Separation was optimized based on the effect of the background electrolyte pH on migration time, peak width, and detection for a mixture containing acebutolol, bendroflumethiazide and triamterene. A pH of 8.0 provided the best compromise for these parameters and was used throughout the remainder of the work presented. The detection limit for acebutolol was 88 fmol with an R.S.D. of 5.6%. Acebutolol was spiked into urine, along with the two other drugs, and analyzed by direct injection. Peak widths were only slightly poorer than those for standards and probably resulted from mismatch to the background electrolyte. This paper supports the use of CE for analysis of urine samples.

The effect of cyclodextrin type [βCD, DMCD, hydroxyethyl-β-cyclodextrin (HECD) and HPCD] on the resolution of propranolol, atenolol and betaxolol stereoisomers was studied by Peterson [35]. She demonstrated that propranolol could be resolved using HECD or HPCD, whereas atenolol was only partially resolved using DMCD. Betaxolol was not successfully stereoresolved using any of the CDs. These basic β-blockers were only resolved at low pH (pH 2.4 using Tris–phosphate buffers), where they were charged.

Lukkari et al. [36] demonstrated the utility of MECC in the determination of β-blockers in the analysis of urine samples for sports doping agents. A single separation system for acebutolol, nadolol, labetolol, timolol, atenolol, metoprolol, oxprenolol, pindolol, alprenolol and propranolol in urine samples was achieved using 80 m*M* sodium phosphate buffer (pH 7.0) with 10 m*M* N-cetyl-N,N,N-trimethylammonium bromide (CTAB). A simple sample clean-up was achieved using filtration through 0.5-μm filters. Linearity was observed from 25 to 250 μg ml^{-1} using 2,6-dimethylphenol as an internal standard

and measuring peak heights. The R.S.Ds varied from 2 to 8% ($n = 6$) using hydrodynamic injection. Limits of detection (LOD) were 10 μg ml^{-1} for all compounds except timolol (20 μg/ml). In similar work, Lukkari et al. [37] developed and validated a simultaneous assay for drug screening of plasma samples. The method was not sensitive enough for drug screening of human serum samples, although the authors suggested that a preconcentration step could be readily incorporated into the analysis. The poor reproducibility was attributed to the sample matrix and injection technique, again indicating the need for injection systems with greater precision.

Complexation with proteins was examined by Busch et al. [38] for chiral separation of pindolol, propranolol and atenolol by CE. They examined the proteins bovine serum albumin (BSA), ovomucoid (OM), orosomucoid and fungal cellulose for use in separating the enantiomers. They were successful in separating pindolol enantiomers using fungal cellulose. The other compounds were not resolved.

Valtcheva et al. [39] examined the chiral separation of propranolol, pindolol, labetalol, alprenolol and metoprolol using non-immobilized cellulase as an enantioselective protein. Capillaries were coated with non-cross-linked polyacrylamide. High resolution was achieved using cellobiohydrolase I (CBHI) at high concentration (40 mg ml^{-1}) in high ionic strength (0.4 M) phosphate buffer (pH 5.1) incorporating 2-propanol (30%) to improve peak shape of the most highly interacting isomer. At the experimental pH, the drugs were positively charged, thus migrating toward the cathodic detector and the protein negatively charged migrating away from the detector. Through its interaction with the protein, the isomer with the highest affinity for CBHI will be retained more than the other isomer, and thus resolution is effected. CBHI was also bound to an HPLC stationary phase, but no resolution was achieved, possibly owing to some physical change in the bound enzyme.

In an elegantly engineered CE system, Morita and Sawada [40] determined propranolol using direct injection of plasma samples and on-line sample pretreatment. In this system, an injection capillary filled with protein-coated octadecylsilane (ODS) gel was attached to a drain capillary via a T-connector and also to a separation capillary. All three capillaries were filled with 20 mM phosphate (pH 7) as the elution buffer. A sample was injected electrokinetically for 2.5–50 s at 15 kV. A field was then applied such that the gel-containing capillary would hold the drug molecule while allowing the plasma proteins to migrate through the drain capillary. This served the purpose of washing interfering plasma proteins to waste. The field was then switched to elute the injection capillary through the separation capillary. An elution buffer, 20 mM phosphate (pH 7) with 25% acetonitrile, was used electrokinetically for 15 s at 10 kv to elute the drug from the ODS gel. The separation was then performed using the original elution buffer. Recovery from plasma spiked with 150 μg ml^{-1} of propranolol was ca. 70% when column switching was not used. Dilution of the plasma 1:10 with phosphate buffer improved the recovery to 90% when column switching was not used, while dilution combined with the column-switching system resulted in 100% recovery of propranolol.

Lukkari et al. [41] examined the effect of buffer pH on the elution and separation of acebutolol, oxprenolol, nadolol, pindolol, timolol, alprenolol, atenolol, labetalol, sotalol, metoprolol and propranolol using MECC. CTAB (15 mM) was used as the micellar additive in 80 mM phosphate buffers with pH ranging from 6.0 to 7.8. The elution behavior as a function of pH for these compounds was modeled using molecular connectivity indices. While the model does not fit the data well, the greatest factors were substitution affecting the nitrogen atom common to all β-blockers and two factors describing the shape of the molecule. The same group [42] studied the elution of the same molecules by MECC using acetone, acetonitrile, ethanol, ethylene glycol, methanol and 2-propanol as additives. In all cases except with acetonitrile, labetalol migration did not change substantially as a function of organic modifier concentration, whereas the migration of all other agents in-

creased when the organic modifier concentration was increased. Other than for 2-propanol, no significant correlation was found using molecular connectivity indices for these solvent systems, Siren et al. [43] later described in more detail the use of migration indices for atenolol, alprenolol and propranolol, but this approach did not demonstrate good utility on a practical basis.

Quang and Khaledi [44] were unable to resolve propranolol enantiomers completely using 35 m*M* βCD with 150 m*M* tetrabutylammonium hydroxide (TBAH) added to the 50 m*M* phosphate buffer (pH 2.5). Use of 20 m*M* βCD with 100 m*M* tetramethylammonium hydroxide (TMAH) instead of TBAH afforded baseline resolution of the isomers. In later work [45], they examined the use of trimethyl-βCD (TMCD), DMCD and HPCD to resolve oxprenolol, alprenolol, pindolol, propranolol, nadolol, metoprolol, labetalol, acebutolol and atenolol. Only propranolol enantiomers were resolved using TMCD. DMCD provided good resolution of pindolol, alprenolol and propranolol, partial resolution of atenolol, oxprenolol and labetalol and no resolution of acebutolol. HPCD improved the resolution relative to DMCD for pindolol and labetalol, but decreased the resolution of nadolol and atenolol. Increasing the TMAH concentration from 70 to 150 m*M* provided improved the resolution of atenolol when using HPCD and oxprenolol when using DMCD, whereas acebutolol remained unresolved in both cases. It was hypothesized that *para* substitution of acebutolol interferes with its ability to complex with the CDs.

Schmitt and Engelhardt [46] examined the use of the charged cyclodextrins: carboxymethylated, carboxyethylated and succinylated βCD (CMCD, CECD and SCD, respectively) for the resolution of enantiomers. At pH >5, CMCD and CECD were deprotonated and, being negatively charged, migrated toward the anode, therefore functioning in a fashion similar to negatively charged micelles [e.g., sodium dodecyl sulfate (SDS)], providing the opportunity to resolve neutral isomers. At pH <4 these same chiral selectors were neutral and functioned in the same manner as non-ionizable CDs. It was shown that propranolol can be separated at either pH using CMDC. Similar results were obtained by Aturki and Fanali [47] using CMCD in different phosphate buffers (pH 2.5–6.2) to separate propranolol enantiomers. Since the resolution was greater below pH 4.5, CMCD clearly offered no enhancement for propranolol, but at pH 6.2 the migration was faster owing to increased electroosmotic flow (EOF).

The effect of temperature, effective capillary length and applied voltage on the retention of acebutolol, nadolol, timolol, atenolol, sotalol, oxprenolol, pindolol, alprenolol and propranolol using MECC was studied by Lukkari et al. [48]. CTAB was used as the micellar component of the background electrolyte. The migration times decreased with increasing temperature and applied voltage, while increasing capillary length obviously increased the migration time. A decrease in migration time with increasing temperature was attributed to the decrease in viscosity of the buffer and to the increased current observed. It was concluded that temperature control was crucial to obtain reproducible separations.

The utilization of chiral surfactants, (*R*)-, (*S*)-N-dodecoxycarbonylvaline (DCV) and (*S*)-dodecanoylvaline (DV), in MECC to resolve atenolol and pindolol isomers was discussed by Mazzeo et al. [49]. Enantioselectivity was controlled by pH for ionizable amines and partitioning was controlled through the optimization of surfactant concentration. They used (*R*)- and (*S*)-DCV owing to their lower background absorbance, which allowed more sensitive assays. Structurally, DCV has a carbamate whereas DV has an amide group adjacent to the chiral center. While it was expected that the two surfactants would have similar resolving properties, DCV was shown to have much greater ability than DV to resolve the stereoisomers studied. The retention order for benzoin isomers was reversed by switching from (*R*)-DCV to (*S*)-DCV, demonstrating the potential to reverse the order of migration of the enantiomers.

Penn et al. [50] derived, from the model presented by Wren and Rowe [26], the equation

$$\frac{\Delta\mu}{\mu_0 - \mu_\infty} = \frac{-\Delta K}{\bar{K}} \cdot \frac{\bar{K}C}{(1+\bar{K}C)^2} \qquad (3)$$

where $\bar{K}$ is the average binding constant for the stereoisomers, which is defined as $\sqrt{K_1 K_2}$, C the concentration of cyclodextrin and μ_0 and μ_∞ the mobilities of the free analyte and the analyte–cyclodextrin complex, respectively. Maximum differences in migration occurred at CD concentrations equal to the reciprocal of the average binding constant. This assumed that the diffusion coefficient is constant, although the authors went on to derive the equation

$$R_s = \frac{F\Delta K(\mu_0 - \mu_\infty)}{4\sqrt{2}\bar{K}}\sqrt{\frac{Vlze}{LkT\mu_\infty(\mu_\infty + \mu_{eo})}} \quad (4)$$

where V is the applied voltage, L the total length of the capillary, l the length of the capillary to the detector, z the charge of the analyte, e the electronic charge, k the Boltzmann constant, T the absolute temperature, μ_∞ the electroosmotic mobility and

$$F = \frac{\bar{K}C}{(1+\bar{K}C)(\beta+\bar{K}C)^{1/2}(\gamma+\bar{K}C)^{1/2}}$$

$$\beta = \frac{\mu_0}{\mu_\infty}$$

$$\gamma = \frac{\mu_0 + \mu_{eo}}{\mu_\infty + \mu_{eo}}$$

Differentiation with respect of $\bar{K}C$ provides a means of calculating maximum resolution. Determination of the electroosmotic flow (EOF), μ_0 and μ_∞ provides a simple means of predicting R_s and binding constants. This model was used to calculate the binding constants at pH 7.4 using βCD. No chiral resolution was achieved at pH 7.4, so additional experiments were performed at pH 3.0 using MBCD. The binding constants calculated under both CE conditions were comparable to those determined by HPLC at pH 7.4.

Aumatell and Wells [51] used MECC based on SDS or bile salts to examine the separation of atenolol, nadolol, oxprenolol, timolol, pindolol, alprenolol, metoprolol, propranolol, acebutolol and labetalol enantiomers. Resolution was examined using βCD, γCD, DMCD, TMCD, HPCD and sulfobutyl ether-β-cyclodextrin (SBECD) with SDS, but was unsuccessful except for nadolol. In the SDS system, nadolol enantiomers were only resolved using 20 mM borate buffer (pH 9)–1.45 mM SBECD–20 mM SDS with 5% (v/v) 1-propanol. Sodium taurodeoxycholate (STDC) (50 mM) with 60 mM HPCD provided resolution of metoprolol, timolol, pindolol and oxprenolol at 20 mM borate buffer (pH 9.5) when 5% (v/v) 1-propanol was added. In addition, nadolol enantiomers were separated using 20% (v/v) 1-propanol–30 mM HPCD–50 mM STDC in 50 mM borate buffer (pH 9.5).

The models of Wren and Rowe [26] were extended by Rawjee et al. [52] to incorporate terms for the effect of pH and drug ionization constants on selectivity for isomers using cyclodextrins:

$$\alpha_{R/S} = \frac{\mu_+^0 + (\mu_{HRCD^+}^0 K_{HRCD^+} C)}{\mu_+^0 + (\mu_{HSCD^+}^0 K_{HSCD^+} C)} \cdot \frac{1 + (K_{HSCD^+} C) + \dfrac{[OH]^-}{K_b}(1 + K_{SCD} C)}{1 + (K_{HRCD^+} C) + \dfrac{[OH]^-}{K_b}(1 + K_{RCD} C)} \quad (5)$$

where C is the cyclodextrin concentration, μ_0^+ is the mobility of the uncomplexed ionic analytes, $\mu_{HRCD^+}^0$ and $\mu_{HSCD^+}^0$ are the mobilities of the protonated enantiomer–CD complexes, K_{HRCD^+} and K_{HSCD^+} are the binding constants for the protonated enantiomer–CD complexes, K_{RCD} and K_{SCD} are the binding constants for the unprotonated enantiomer–CD complexes and K_b is the base dissociation constant. Using three sets of experiments, the various parameters can be calculated and the optimum conditions predicted for resolution of the analyte isomers. The equations predict three scenarios: only the non-dissociated enantiomers form complexes and resolution occurs only near the pK_a, only dissociated enantiomers complex and resolution occurs only below the pK_a with the best resolution occurring at pH < 2 units below the pK_a, or both the dissociated and undissociated species form complexes, giving rise to multiple ways to resolve the isomers. Propranolol was used to demonstrate the third case, where both undissociated and dissociated species form complexes.

The use of *n*-dodecyl-β-D-glucopyranoside 4,6-hydrogenphosphate (**1**), *n*-dodecyl-β-D-glucopyranoside 4-hydrogensulfate (**2**) or *n*-dodecyl-β-D-glucopyranoside 4,6-phenylphosphate (**3**) as micellar agents to resolve enantiomers was explored by Tickle et al. [53]. The critical micelle concentration (CMC) for these negatively charged surfactants was determined to be about 1 m*M*. Metoprolol enantiomers were resolved using 45 m*M* surfactant **1** in phosphate–borate buffer (pH 8.0) with 10% (v/v) methanol.

Gonzalez and Laserna [54] also worked on a rapid screening CE technique for banned drugs in sport. Included in the separation were oxprenolol, propranolol, atenolol, alprenolol, nadolol and acebutolol. Borate was used as the background electrolyte and LOD values of between $0.2 \cdot 10^{-6}$ and $4.6 \cdot 10^{-6}$ *M* were achieved. Injection reproducibility was between 3 and 7% by peak height for nadolol and atenolol, respectively. Electropherograms of atenolol spiked into urine and plasma were also presented.

Resolution of acebutolol, alprenolol, atenolol, bevantolol, celiprolol, metoprolol, pindolol, propranolol and sotalol using βCD, DMCD, TMCD and HPCD was studied by Bechet et al. [55]. Experiments performed with the CD additives showed that acebutolol, bevantolol and celiprolol were not resolved using any of the additives, atenolol, sotolol and pindolol enantiomers were well resolved using DMCD and propranolol was well resolved with either HPCD or TMCD. Studies were also conducted to establish optimum DMCD concentrations, which ranged between 5 and 20 m*M* for the various β-blockers studied. Resolution of alprenolol enantiomers using DMCD was enhanced with 30% (v/v) methanol.

Schmutz and Thormann [56] described the effect of pK_a, log *P* and protein binding on the separation of atenolol, pindolol, carteolol, timolol, metoprolol, propranolol, celiprolol, levobunolol and penbutolol by MECC using 75 m*M* SDS in phosphate–tetraborate buffer (pH 9.2). In this system, atenolol was resolved from all other β-blockers which were not resolved from each other despite significant differences in their log *P* values. This was attributed to the ionization state of these molecules, providing for interaction of the amine with the SDS. When 2-propanol was added (optimum at 5%, v/v), the migration time for these agents was correlated to log *P*. These agents were not well resolved from endogenous peaks when direct injection of spiked serum was examined. The effect of pK_a and protein binding was not explicitly explored for the β-blockers.

A simple way to determine the binding constants for analyte interactions with CDs was described by Vespalec et al. [57]. They determined the mean binding constants for practolol (50 l mol^{-1}) and atenolol (30 l mol^{-1}) from the CD concentration of maximum resolution using equations published by Wren and Rowe [26].

Quang and Khaledi [58] reversed the direction of the EOF through the incorporation of TMAH and TBAH into the background electrolyte. The magnitude of the reversed EOF was smaller than the electrophoretic mobility of the analyte, so the analytes were still detected at the anode. A mixture of thirteen drug molecules (including propranolol, metoprolol and nadolol) was analyzed using TBAH or TMAH; the optimum separation was achieved using 50 m*M* TBAH in phosphate buffer (pH 2.5). Resolution of various β-blocker stereoisomers was examined by incorporation of 20 m*M* βCD, TMCD, DMCD or HPCD into 50 m*M* TMAH phosphate buffer (pH 2.5).

Sample stacking for the determination of propranolol was described by Palmarsdottir and Edholm [59]. They examined field-enhanced preconcentration (stacking) and on-line coupling with column liquid chromatography for the concentration of plasma samples to increase the sensitivity without loss of separation performance. Propranolol isomers were analyzed with close to baseline resolution using a double stacking procedure, but the column switching technique was not applied to this compound.

Tanaka and Terabe [60] used affinity electrokinetic chromatography (EKC) to separate enantiomers of bunitrolol, pindolol, propranolol and arotinolol. Ovomucoid, conalbumin, BSA and α_1-acid glycoprotein (α_1-AGP) were the proteins used as chiral selectors. Substituting

protein concentration for CD concentration in Eq. 1, it was apparent that factors affecting the resolution for CD and protein-based chiral recognition were similar. K_1 and K_2 were estimated to range between 10^3 and 10^5 l mol^{-1} and the protein concentration of optimal resolution was predicted to be 10–1000 μM. According to this model, resolution would increase with increasing protein concentration, but unlike CDs, a maximum cannot be reached in practice since the maximum solubility of the protein will be below the level predicted for maximum resolution. Optimum resolution for bunitrolol, pindolol and arotinolol isomers was achieved by applying a partial separation zone technique using 500 μM ovomucoid in 50 mM phosphate buffer (pH 5) with the additives 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate (CHAPS), (10 mM), ethanol (8%) and 2-propanol (6%), respectively. Propranolol enantiomers were resolved by applying a partial separation zone technique using 500 μM BSA (pH 6.0) with no additive. In the partial separation zone technique, the capillary is filled with background electrolyte which does not contain the chiral selector, then partially filled with separation buffer containing the chiral selector and then a sample injected. The sample is resolved while migrating through the separation zone, passes into the background electrolyte and then passes to the detector. Since the chiral selector stays in the separation buffer and does not pass to the detector, the absorbance of the background chiral selector does not interfere with detection and quantification.

Schmitt and Engelhardt [61] examined the use of spermidine or spermine to reverse the EOF and the use of different CDs to optimize enantiomeric separations. While β-blockers were not determined in the presence of spermine and spermidine, these additives resulted in a very small EOF for the other drugs studied and did not enhance separation. DMCD (30 mM) in 20 mM phosphate buffer (pH 2.2) was used to resolve pindolol but not propranolol isomers. The use of 2% (w/v) CMCD in 20 mM citric acid (pH 2.5) provided good resolution of pindolol and propranolol (Fig. 2).

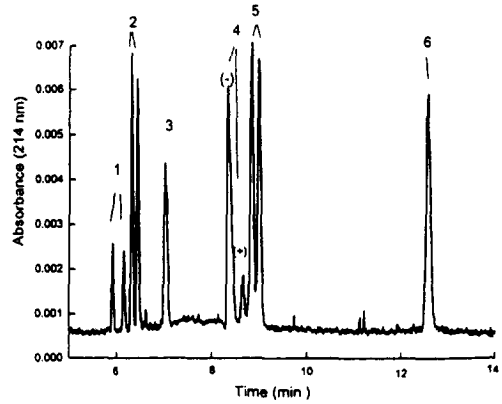

Fig. 2. Separation of basic amines using carboxymethyl-βCD. Run conditions: capillary 67 cm (60 cm to detector) × 75 μm I.D., 340 V cm^{-1}, 20°C, 20 mM citrate buffer (pH 2.5) containing 2% (w/v) of the CD. Recemic mixtures of: doxylamine (2), arterenol (1), ephedrine (4) spiked with (−)-ephedrine, pindolol (5), dimethindene (3) and propranolol (6). (From Ref. [61]).

Separation by MECC to resolve several cardiovascular drugs was further described by Bretnall and Clarke [62]. The effects of pH, concentration of SDS, different organic modifiers and concentration of organic modifier were examined in order to optimize the separation. The drugs studied were atenolol (the only β-blocker), nicardipine, nifedipine, diltiazem, verapamil and amlodipine. The optimum conditions with respect to resolution and migration time were 50 mM SDS in 100 mM borate buffer (pH 8.1) containing 15% (v/v) acetone.

3. The renin and angiotensin system: acetylcholinesterase inhibitors and angiotensin-converting enzyme antagonists

The renin and angiotensin system regulates hemodynamics and water–electrolyte balance. When the renin–angiotensin system is activated, the enzyme renin acts on angiotensinogen to release angiotensin I. Angiotensin I is then cleaved by angiotensin-converting enzyme (ACE) to yield angiotensin II, an active peptide. Angiotensin II is then hydrolyzed by aminopeptidase to yield angiotensin III (also active),

which is further cleaved to yield inactive peptides.

Angiotensin II stimulates the production of aldosterone in the adrenal cortex and raises blood pressure via constriction of the arterioles. It also stimulates the heart and sympathetic nervous system increasing blood pressure. The production of angiotensin II is rate limited by production of renin, predominantly produced in the kidney. The release of renin is controlled by anything that lowers renal perfusion (e.g., lower blood pressure), reduction of Na^+ concentration in the distal tubule and release of norepinephrine from sympathetic nerve terminals with activation of β-adrenergic receptors. There are two classes of renin–angiotensin inhibitors: the angiotensin II antagonists and the ACE inhibitors. The angiotensin II antagonists block receptors for the peptide whereas the ACE inhibitors slow the rate of formation of angiotensin.

3.1. Acetylcholinesterase inhibitors

Steuer et al. [63] compared separations using HPLC, supercritical fluid chromatography (SFC) and CE based on efficiency, performance, sensitivity, ability to modify selectivity, method development time, sample preparation and orthogonality. The method development time depends on column equilibration time, column efficiency and performance. Based on these factors, CE requires the shortest time, then SFC, followed by HPLC. Spirapril was determined by HPLC and CE with an order of magnitude greater sensitivity reported for the drug and its degradation products using HPLC when the same sample concentration was injected into both systems. It was shown that the sensitivity for CE could be increased by increasing the loading of the analyte, but CE did not fully match HPLC owing to loss of resolution. They also showed the utility of CE for the analysis of capsule dosage forms, but noted the need to match the extraction solvent to the background electrolyte in order to minimize injection artifacts, such as disturbances to the electric field or precipitation of co-extracted impurities or excipients. HPLC was considered to be less constrained by extraction solvents.

A CE method for the determination of enalapril was developed by Qin et al. [64]. In this study, 25–50 m*M* phosphate buffers or 80–100 m*M* borate buffers with pH ranging between 8.0 and 9.7, used as background electrolytes, were suitable for the analysis. The buffer system validated was 80 m*M* borate buffer (pH 9.7) (Fig. 3). Method linearity was demonstrated from 50 to 150% of the nominal analytical concentration (0.2 mg ml^{-1}). Analyses of samples using an external standard had a precision (R.S.D) of 0.62% ($n = 10$). The method was compared with HPLC for content uniformity determinations of tablets. CE had an R.S.D. of 1.1% and HPLC 3.2% with mean contents of 19.4 and 19.8 mg per tablet, respectively. These findings demonstrated the utility of CE for routine pharmaceutical analyses. Enalapril was also resolved from two degradation products, enalaprilat, formed by hydrolysis, and diketopiperazine, formed by cyclization of the hydrolysis product. Whereas an LOD of 0.2% was achieved for enalaprilat, diketopiperazine was difficult to determine

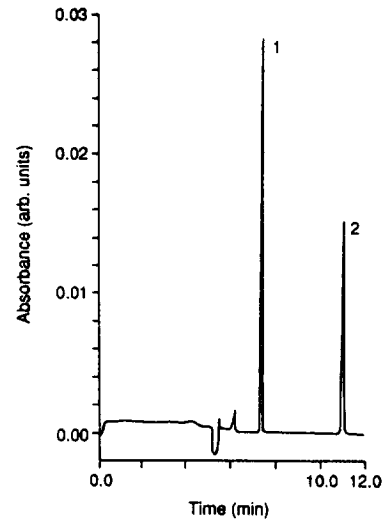

Fig. 3. Electropherogram of (1) enalapril and (2) enalaprilat recorded at room temperature. Conditions: capillary, fused silica [57 cm (50 cm to detector) × 75 μm I.D.]; electrolyte, 80 m*M* sodium borate buffer (pH 9.7); applied voltage, 16.1 kV. (From Ref. [64]).

owing to the possible co-elution with other neutral compounds. Enalapril also forms *cis* and *trans* rotamers in solution with an on-column interconversion being problematic for HPLC analyses owing to its effect on peak shape. MECC using 80 m*M* sodium borate buffer (pH 8.5) with 100 m*M* SDS allowed a good separation of these rotamers. The effect of temperature on the separation was determined from 20 to 50°C using 50 m*M* phosphate buffer (pH 8.25) with 40 m*M* SDS. The rotamers were separated at 20°C and coalesced into one peak at 50°C. At intermediate temperatures, on-column interconversion was observed.

Thomas and Ghodbane [65] developed a method for the quality control of enalapril-containing tablets. The initial separation developed demonstrated the same chromatographic behavior for the *cis–trans* rotamers described by Qin et al. [64], and did not result in separation of diketopiperazine from other neutral molecules. Incorporation of 20 m*M* SDS in 20 m*M* sodium borate buffer (pH 8.5) decreased the mobility of diketopiperazine, but resulted in a poor peak shape for enalapril. Adding 2% Brij-35 and 20 m*M* SDS to 20 m*M* sodium borate buffer (pH 8.5) resulted in a good peak shape for enalapril, decreased mobility for diketopiperazine and a method suitable for validation. Using these conditions, the method was validated for precision, accuracy, LOD, limit of quantification (LOQ) and linearity according to pharmaceutical industry standards used in the validation of HPLC analyses. Simvastatin was used as an internal standard in the quantification of the drug. Enalapril mixed with enalaprilat and diketopiperazine (5% each) was used to form five spiked samples ranging 50 to 150% of the normal assay concentration. Good linearity was achieved with only a small bias of 1.6% over three days. On a given day, the bias for an individual level sometimes exceeded the 2% acceptance limit and the precision (R.S.D. 6.2%) exceeded its 2% acceptance limit. The recoveries for enalaprilat had an acceptable bias of 0.5% and were more precise and reproducible than for diketopiperazine (2.2% bias). Linearity was good for enalaprilat, but inadequate for diketopiperazine. LOQs were determined to be 1 μg ml^{-1} and LODs 0.1 μg ml^{-1}. Ruggedness was also investigated by varying the SDS concentration, Brij-35 concentration, sodium borate concentration, pH, applied voltage and temperature. Overall the method did not demonstrate the precision necessary for a quality control method.

The determination of lisinopril and its *RSS* diastereomer by MECC was investigated by Qin et al. [66] using sodium cholate (SC), SDC and SDS. The *RSS* and *SSS* isomers of lisinopril were not separated in the absence of a surfactant. Limited resolution was achieved at 30°C using 50 m*M* SC in Tris–phosphate buffer (pH 9.55). Resolution was significantly enhanced when greater than 45% methanol was added to the buffer. As the pH increased from 8.25 to 9.55, the resolution increased. Optimum separation was achieved at 30°C using 50 m*M* SC in 25 m*M* Tris–phosphate buffer (pH 9.55) containing 55% methanol. The peak shape for the slower migrating isomer was degraded when the temperature was increased. The isomers were poorly resolved using αCD, βCD or γCD. Separation was found to be suitable when SDC was substituted for SC, but the separation was much better when SC was used under the same conditions. The use of 40 m*M* SDS in 25 m*M* phosphate buffer (pH 9) provided baseline separation of the diastereomers, which migrated in reverse order compared with the bile salt systems.

Lozano et al. [67] developed a quantitative CE analysis for fosinopril and compared the results with those obtained using HPLC. Fosinopril was resolved from fosinoprilat and two additional degradation products by both CE and HPLC. Shorter run times and better peak shapes were observed using CE. The response and peak shape were affected by the background electrolyte concentration and by the buffer concentration of the sample diluent. When 50 m*M* borate buffer (pH 8.3) was used as the background electrolyte at 22°C, good linearity was maintained from 1 to 400 μg ml^{-1}. The peak-area response for concentrations between 5 and 400 μg ml^{-1} ranged between 1 and 5% (R.S.D.) by CE versus 0.4% for HPLC. The precision of the CE method was not suitable for quality

control usage. The LOQ was estimated to be 0.3% (w/w) by CE, versus 0.1% (w/w) by HPLC. With a change in the sample concentration, it was projected that an LOQ of 0.1% (w/w) would be achievable, which would be consistent with industry standards for impurity profiling. It was concluded that CE had no significant advantages over HPLC for the determination of fosinopril. While the CE method provided superior selectivity and shorter run times, it did not match the sensitivity and precision of LC.

Lisinopril, hydrocholorthiazide (HCT) and chlorothiazide (CT) are used in several pharmaceutical combination products (e.g., Prinzide). A MECC separation was developed by Thomas et al. [68] for lisinopril, HCT and CT allowing for assay of combination products. The separation conditions were 20 m*M* sodium borate buffer (pH 9.5) containing 30 m*M* SDS. Validation experiments were performed on HCT and CT, but not lisinopril.

3.2. Angiotensin II-converting enzyme antagonists

Nguyen and Siegler [69,70] developed CE separations for SK&F 108566E and SB 203220E, angiotensin II receptor antagonists under development at SmithKline Beecham (Fig. 4). Both compounds degraded to form their *Z*-isomer when exposed to light. Separation as a function of pH was determined as part of optimization of resolution (Fig. 5). The optimized separation was

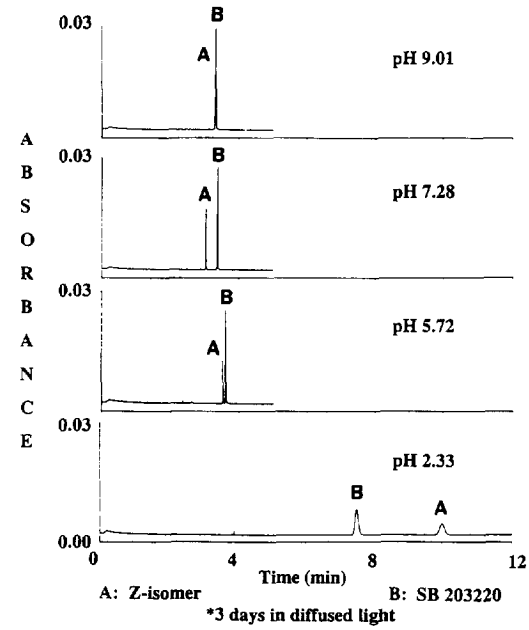

Fig. 5. Separation of SB 203220 (A) *Z*- and (B) *E*-isomers as a function of pH from pH 2.33 to 9.01 at a constant ionic strength of 30 m*M*. Separation voltage, 30 kV; column, fused silica, 57 cm (50 cm to detector) × 75 μm I.D.; detection at 230 nm; sample concentration, 0.05 mg ml^{-1}. Beckman P/ACE 2000. (From Ref. [70]).

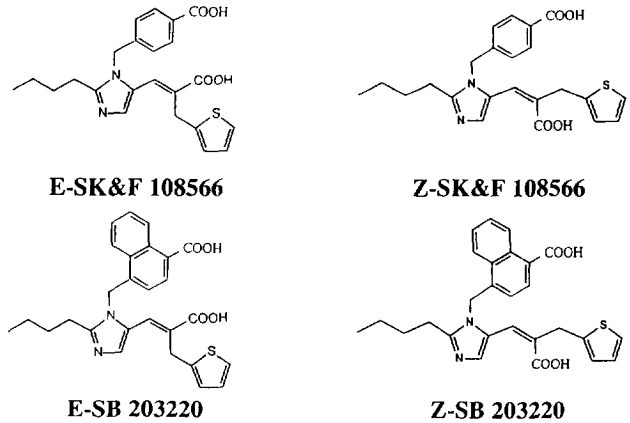

Fig. 4. Structures of SK&F 108566 and SB 203220 *E*- and *Z*-isomers.

performed in 10 m*M* phosphate buffer (pH 8.2) with detection at 230 nm and required only 4 min. The method was validated for analysis of intravenous (i.v.) and tablet dosage forms and the results were compared with those for a similar HPLC method (Fig. 6). Linearity was established from 20 to 160% and 20 to 200% for HPLC and CE, respectively. The LOD were 0.25 and 1% for HPLC and CE, respectively, and the LOQs were 0.8 and 1%, respectively. The precision ranged from 0.5 to 0.8% and from 1.8 to 3.5% for HPLC and CE, respectively. Recoveries for the i.v. dosage form ranged from 98 to 101% and from 92 to 106% for HPLC and CE, respectively, and those for the tablet dosage form ranged from 98 to 100% and from 94 to 104%, respectively. The validation experiments demonstrated that HPLC was still superior to CE based on injection reproducibility and detection limits, although CE provided faster method development, higher theoretical plate numbers and shorter analysis times.

Additional work by Siegler and Nguyen [71] utilized CE to determine pK_as for the *E*- and *Z*-isomers of SK&F 108566 and SB 203220. The apparent mobilities of the analytes were determined as a function of pH and corrected for EOF to give the electrophoretic mobilities. Data for the electrophoretic mobilities were fitted to a model providing the ability to obtain the electrophoretic mobility of each ionic species and the three pK_as for each species (Fig. 7). The pK_as determined using CE compared well with those obtained by titration. This work demonstrated the utility of CE for the determination of multiple pK_as for drug substances without the need for physically separating the degradation product from the drug substance. In addition, CE offered

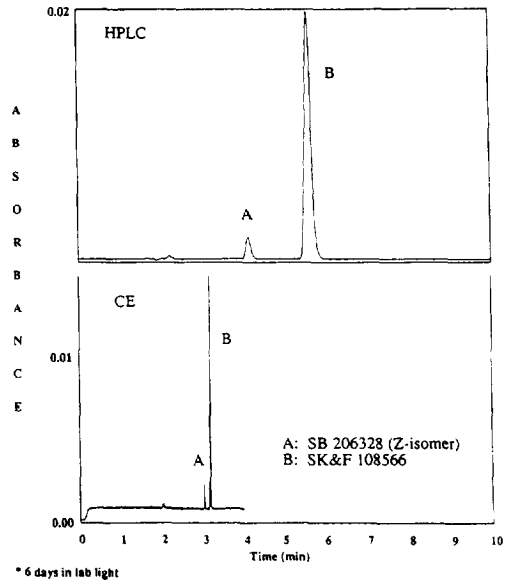

Fig. 6. Comparison of HPLC and CE for the separation of SK&F 108566 (A) *Z*- and (B) *E*-isomers. HPLC separation performed on a 5-μm Keystone Inertsil ODS-2 (CPG) column, using a mobile phase of acetonitrile–Na_2HPO_4 (50 m*M*)–TFA (18:82:0.1), with detection at 230 nm using a Shimadzu isocratic system. CE performed in pH 8.2 phosphate buffer at an ionic strength of 30 m*M*. Separation voltage, 30 kV; column, fused silica, 57 cm (50 cm to detector) × 75 μm I.D.; detection at 230 nm; sample concentration, 0.05 mg ml^{-1}. Beckman P/ACE 2000. (From Ref. [59]).

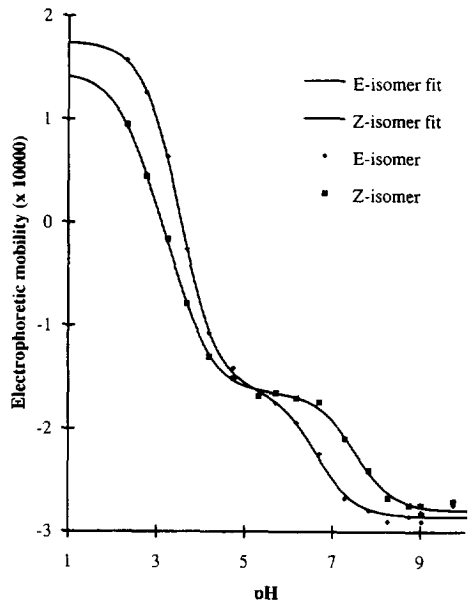

Fig. 7. Plot of fitted data for electrophoretic mobility of SK&F 108566 *E*- and *Z*-isomers as a function of pH from pH 2.33 to 9.75 at a constant ionic strength of 30 m*M*. Separation voltage, 30 kV; column, fused silica, 57 cm (50 cm to detector) × 75 μm I.D.; detection at 230 nm; sample concentration, 0.05 mg ml^{-1}. Beckman P/ACE 2000. (From Ref. [71]).

the ability to determine pK_as when only a very small amount of drug substance is available (e.g., 100 μg).

4. Diuretics

Diuretics alter the Na^+ balance and are the primary means of controlling hypertension. Thiazides and related agents are the most frequently used diuretics and it is not known how these agents effect their antihypertensive action. Hydrochlorothiazide is the best known diuretic. There are also loop diuretics which are less effective in patients with normal renal function, e.g., furosemide and bumethanide.

Prunonosa et al. [72] determined cicletanine enantiomers in human plasma and urine using MECC. SDS (110 m*M*) was used as the surfactant and 25 m*M* γCD as the chiral selector in 100 m*M* phosphate buffer (pH 8.6) with 10% acetonitrile. Internal standard was added to the plasma samples, double extracted using diethyl ether, evaporated under a stream of nitrogen and reconstituted in acetonitrile–water (1:9, v/v). The extraction procedure resulted in a fivefold concentration of the analytes. The assay linearity covered the range 10–500 ng ml^{-1} with an LOD of 10 ng ml^{-1}. Recoveries of spiked samples were determined by HPLC to range from 78.8 to 71.8% for 10 to 1000 ng ml^{-1} samples, respectively. The precisions were 20.8 and 7.4% at 10 ng ml^{-1} and 4.2 and 4.6% at 500 ng ml^{-1} for the *S*-(+)- and *R*-(−)-isomers, respectively. The distribution of cicletanine was followed using this method after a single oral dose containing racemic drug. Analyses showed that only the *R*-(−)-isomer was present in plasma. Glucuronidation and sulfation were the primary metabolic routes observed. The method described was also applied to the analysis of urine samples using diethyl ether and *n*-hexane (20:80, v/v) as the extraction solvent. Recoveries of 5.9 and 13.3% of the *S*-(+)- and 12.0 and 18.9% of the *R*-(−)-isomer were achieved from each of two patients, respectively, following hydrolysis with β-glucuronidase. The same group [73] compared results obtained using HPLC and MECC for the determination of total cicletanine in plasma. The separation conditions and the procedure for extraction of cicletanine from plasma were as described above. In this study, the linearity range was extended from 500 to 1000 ng ml^{-1} and the LOD increased from 10 to 20 ng ml^{-1}. Cicletanine plasma profiles for two subjects showed less than a 15% difference between the patients except for late time points. These results illustrated the suitability of CE for the performance of pharmacokinetic studies, although it appeared that the precision could be improved.

Using the MECC–γCD method described by Prunonosa et al. [72], Garay et al. [74] measured urine levels of cicletanine enantiomers after oral administration to rats of either (*R*)-(−)- or (*S*)-(+)-cicletanine. The total 24-h urinary excretion rates for the cicletanine sulfate metabolite were 3.8 versus 18.9% for the *R*-(−)- and *S*-(+)-enantiomers, respectively. Direct injection of the individual isomers of cicletanine sulfate showed the *S*-(+)-enantiomer to be 3–4 times more potent in stimulating sodium excretion. Their data also suggested that the *S*-(+)-enantiomer was predominantly excreted as the sulfate metabolite, whereas the *R*-(−)-enantiomer was predominantly excreted as the glucuronate metabolite. The utility of CE for performing studies on the metabolic fate of drugs after administration was thus demonstrated.

Evenson and Wiktorowicz [75] applied MECC based on 30 m*M* SDS in 30 m*M* borate buffer (pH 9.3) with 10% acetonitrile to the determination of furosemide, CT, tricholormethiazide (TCT) and HCT. The most hydrophilic molecules eluted first, which was consistent with the assumption that the more hydrophobic the drug, the more it would partition into the micelle and have its mobility retarded.

The separation of diuretics in blood and urine using CE in acetate (pH 3.5–5.5), phosphate (pH 6.0–8.5) or 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (pH 9.0–11.2) buffers was demonstrated by Jumppanen et al. [76]. Diuretics used in the treatment of cardiovascular disease included CT, furosemide, HCT, trichlorothiazide, triamterene, ethacrynic acid, benzthiazide, bumetanide, amiloride and bendroflumethiazide.

Diuretic drugs commonly abused by athletes were chosen for separation in this study. Urine was treated using solid-phase extraction to remove endogenous compounds, the sample eluted in methanol, evaporated to dryness and reconstituted in a methanol–aqueous buffer mix. Plasma and urine were treated with methanol to precipitate proteins prior to extraction and analysis using 0.06 *M* CAPS buffer (pH 10.6) at 20°C (Fig. 8). The effect of background electrolyte pH on the resolution of the diuretic analytes was studied. CT, benzthiazide, furosemide, bumetanide, HCT, trichlorothiazide and bendroflumethiazide were separated best using 0.06 *M* CAPS buffer (pH 10.6) at 20°C, while triamterene was separated best from other diuretics using 0.07 *M* acetate buffer (pH 4.5) containing betaine to limit the adsorption of endogenous compounds on the capillary wall.

Jumppanen et al. [77] developed equations to model the separation of thirteen diuretics for optimization of the separation using regression analysis. The diuretics studied were clopamide, chlorthalidone, probenecid, ethacrynic acid, bumetadine, bendroflumethiazide, acetazolamide, furosemide, TCT, benzthiazide, HCT, dichlorphenamide and CT. Equations were derived to describe performance parameters for total length of column, migration time of the last compound to elute and resolution between each successive pair. A general equation describing the quality of the separation was also developed for use in fitting the data and optimization. Separation at 20°C using 60 m*M* CAPS buffer (pH 10.6) with $[Cu(NH_3)_4]^{2+}$ varied between 0 and 140 μM to control the EOF for separations at 20°C. Using regression models, it was possible to predict the optimum electrophoretic conditions for resolution of the diuretics studied. It was also shown that diffusion coefficients could be deduced from mobility values if the charge of the analyte was known.

Pulsed-laser fluorescence detection was used by Gonzalez et al. [34] for CE analyses of triamterene and benzoflumethiazide. As discussed in Section 2 (β-adrenergic antagonists), separation was optimized based on the effect of background electrolyte pH on migration time, peak width and detection for a mixture containing acebutolol, bendroflumethiazide and triamterene. The best compromise for these parameters was pH 8.0 and this was used throughout the remainder of the work presented. The detection limit was 0.31 fmol (R.S.D. 5.8%) for triamterene and 18 fmol (4.1%) for bendroflumethiazide. Triamterene, benzoflumethiazide and acebutolol were spiked into urine and the sample was analyzed by direct injection. The peak widths were slightly poorer than those for standards and probably resulted from mismatch of the sample solvent with the background electrolyte. A patient was dosed with triamterene, the urine collected and the sample analyzed, with a result of 0.76 $\mu g\ ml^{-1}$ of triamterene.

Thomas et al. [68] validated drug substance assays for HCT and CT based on MECC using 20 m*M* sodium borate buffer (pH 9.5) containing 30 m*M* SDS. A major impurity, 4-amino-6-chloro-1,3-benzenedisulfonamide (ACBS), was resolved and determined. The precision of the method was optimized by careful control of several factors, such as minimizing the time the capillary ends were not immersed in buffer solution, minimizing the number of injections made using each anode and minimizing the number of injections made using each inlet vial. They used a 100 μm I.D. capillary to achieve an LOQ of 1 $\mu g\ ml^{-1}$ (0.1 and 0.2% of the assay concentration for HCT and CT, respectively). Analyses utilizing an internal standard gave consistent linear plots (50–150% of target) and accurate results with R.S.D.s less than 1%. The linearity for ACBs between 0.1 to 1.5% was good, excellent accuracy was demonstrated and a precision (R.S.D.) of less than 2% was achieved. Assays of HCT tablets on two days by two analysts on three different instruments gave excellent reproducibility for HCT (99.3–101.3% of the label claim for 25 different determinations on HCT drug substance) and ACBS (0.07–0.27% of the label claim for 25 different determinations on HCT drug substance). Assays of HCT tablets on two days by two analysts on three different instruments gave excellent reproducibility for CT (99.7–100.9% of the label claim for ten different determinations on CT drug substance). These

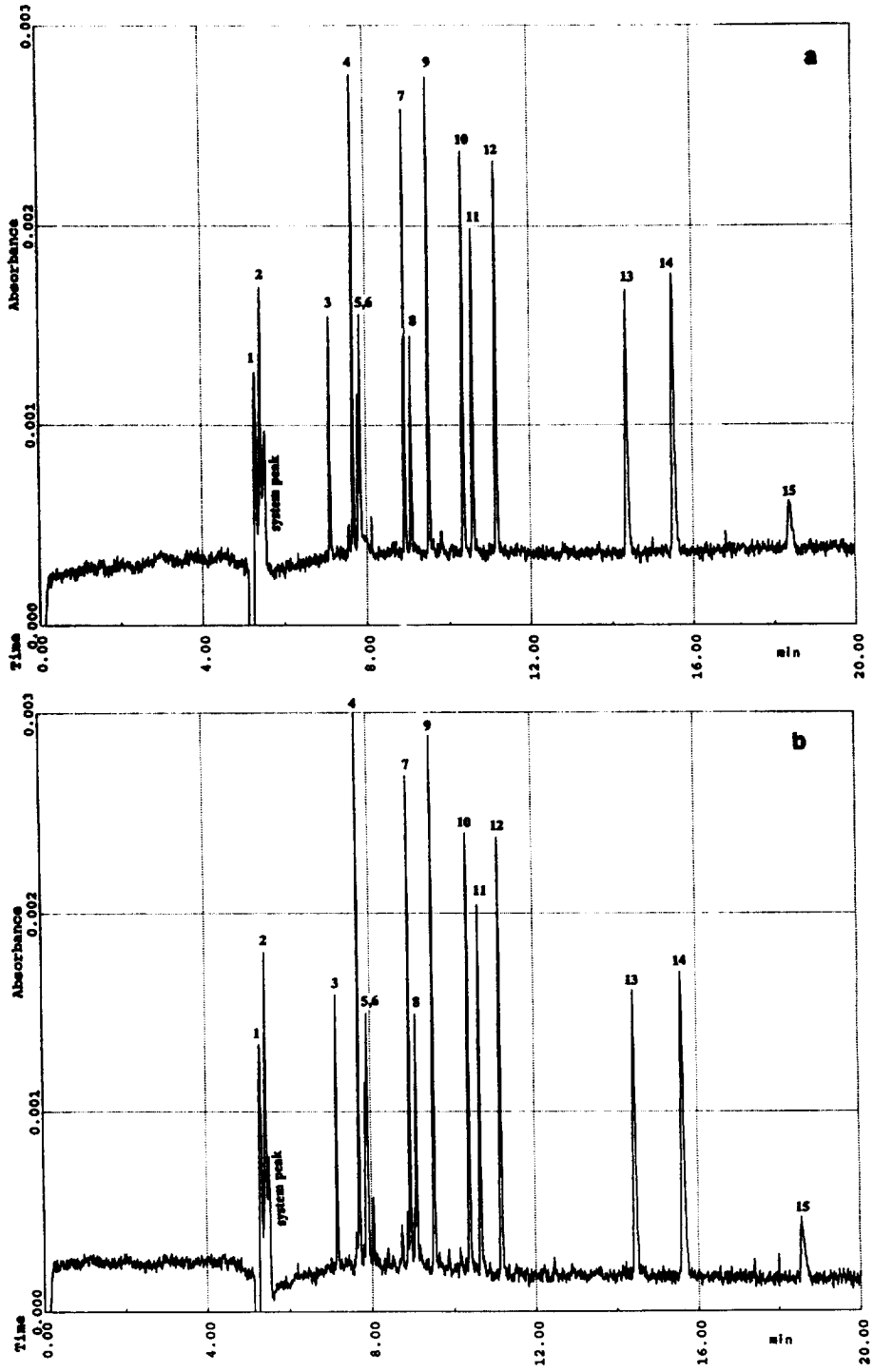

Fig. 8. Electropherograms of (a) spiked serum and (b) spiked urine. Running conditions: uncoated silica capillary, 67 cm (60 cm to the detector) × 50 μm I.D. × 360 μm O.D.; detection at 220 nm; 0.06 *M* CAPS (pH 10.6); 20°C; 25 kV; hydrostatic injection by pressure for 5 s. The spiked samples contained 10 ppm each of the following; 1 = metyrapone and caffeine; 2 = triamterene and amiloride; 3 = clopamide; 4 = chlorthalidone; 5 = ethacrynic acid; 6 = probenecid; 7 = bumetadine; 8 = bendroflumethiazide; 9 = furosemide; 10 = trichlormethiazide; 11 = benzthiazide; 12 = hydrochlorothiazide; 13 = dichlorphenamide; 14 = chlorothiazide; 15 = actazolamide. (From Ref. [76]).

results matched those achieved using HPLC and demonstrated the potential of CE for analyses of pharmaceutical products when all parameters are well controlled.

Quantification of diuretics in plasma for sports screening using CE was demonstrated by Gonzalez and Laserna [54]. The diuretics in the study included amiloride, triamterene, chlortalidone, HCT, bendroflumethiazide, bumetanide, xipamide, furosemide, etacrinic acid, acetazolamide and spironolactone. The background electrolyte was 0.11 *M* borate buffer (pH 8.0). LOD values of between $2.4 \cdot 10^{-7}$ and $1.3 \cdot 10^{-6}$ *M* were achieved with an injection reproducibility of 3.4 and 4.8% by peak height.

Soini et al. [78] used mixed polymer networks of poly(ethylene oxide) (PEO)–polydextran (PD) to determine drugs such as diltiazem. Initial separations of a drug mixture containing diltiazem and other components using 5% (w/w) PD in 32 m*M* 6-aminocaproic acid–18 m*M* adipic acid buffer (pH 4.5) containing 5% methanol resulted in high mobilities and limited resolution. Substitution of 0.02% (w/w) PEO for the PD in the buffer system resulted in low analyte mobility with better resolution. Incorporation of both 5% (w/w) PD and 0.02% (w/w) PEO in the buffer system provided enhanced resolution and reasonable migration times. Studies using different fractions of polydextrans indicated that mobility was primarily controlled by the viscosity of the dextran buffer solution. The effect of adding 2-propanol and acetonitrile was an increase in electroosmosis, whereas methanol had no effect. In addition, it was established that zone electrophoresis and not electrokinetic chromatography was the primary mode of separation using polydextrans. Although use of these additives in the analysis of urine samples was reported, diltiazem was not used as an example.

Quaglia et al. [79] studied the stability of furosemide in perfusion solutions using MECC. The background electrolyte was 20 m*M* phosphate buffer (pH 8) containing 30 m*M* SDS. Quantification utilized an internal standard to achieve a 1.58% R.S.D. for furosemide and spiked recoveries ranged from 91 to 112% in saline. Furosemide was stable for up to 6 h in the presence of aminophylline and methylprednisolone.

5. α-Adrenergic antagonists

α-Adrenergic antagonists have a significant affect on the cardiovascular system. Blockade of the α_1-receptors inhibits vasoconstriction induced by endogenous catecholamines with resulting vasodilation and a fall in blood pressure due to decreased peripheral vascular resistance. These changes are frequently accompanied by increased heart rate and fluid retention. The physiological response can be further enhanced if the agent also blocks α_2-receptors, in which case release of norepinephrine is increased and β_1-adrenergic receptor stimulation occurs.

Okafo et al. [80] successfully separated enantiomers of fenoldopam using MECC bile salts with cyclodextrin additives (20 m*M* βCD–50 m*M* taurodeoxycholate) in phosphate–borate buffer (pH 7.2). Fenoldopam was a drug examined for use in the treatment of congestive heart failure because of its ability to increase renal blood flow. Retention was primarily a function of the hydrophobicity of the molecules being analyzed with greater retention for the more hydrophobic molecules.

As discussed above under diuretics (Section 4), Soini et al. [78] used mixed polymer networks of poly(ethylene oxide)–polydextran to determine drugs. Prazosin, an α-adrenergic antagonist, was among the drugs studied. Initial separations of a drug mixture using 5% (w/w) PD in 32 m*W* 6-aminoicaproic acid–18 m*M* adipic acid buffer (pH 4.5) resulted in high mobilities and limited resolution, whereas replacement of PD with 0.02% (w/w) PEO resulted in low analyte mobilities with better resolution. Incorporation of both 5% (w/w) PD and 0.02% (w/w) PEO provided enhanced resolution an reasonable migration times. Although the use of these additives in the analysis of urine samples was reported, prazosin was not used as an example.

Tickle et al. [53] used long-chain alkyl glucopyranoside uncharged surfactants in the MECC

separation of several enantiomeric drugs. The α-adronergic antagonist fenoldopam was resolved using 30 m*M* *n*-dodecyl-β-D-glucopyranoside 6-hydrogensulfate in phosphate–borate buffer (pH 8). Peaks were broad for the enantiomers and this was hypothesized to be the outcome of fenoldopam's high capacity for hydrogen bonding.

6. Calcium channel blockers

Increased concentrations of cytosolic Ca^{2+} cause increased contraction of the myocardium and vascular smooth muscle. The entry of extracellular Ca^{2+} is important in the initiation of myocardial contraction, whereas release of Ca^{2+} from storage sites affects vascular smooth muscle activity. There is a high concentration of extracellular Ca^{2+}, so a gradient exists which is maintained by active Ca^{2-} transport by membrane pumps and intracellular storage. Many hormones and neurohormones increase Ca^{2+} transport through receptor-mediated channels while high K^+ levels and stimuli which depolarize the cells cause Ca^{2+} influx via voltage controlled channels. Blockade of these channels decreases the Ca^{2+} flux, decreasing contraction of the myocardium and vascular smooth muscle and resulting in a lessening of coronary vascular resistance. There are several types of calcium channels, but only the L channel, found predominantly in the heart, is sensitive to the calcium channel blockers. The approved calcium channel blockers all decrease vascular resistance and increase coronary blood flow, relieving the pain associated with angina and lowering blood pressure in hypertensive patients.

Nishi et al. [81] separated benzothiazapin analogs by MECC using bile salts. Diltiazem and several of its derivatives were determined in drug substance and tablet samples. Initial work with 50–100 m*M* SDS in 20 m*M* phosphate–borate buffer (pH 7–9) did not provide resolution. The effect of SC, SDC and sodium taurocholate (STC) on resolution was studied. Good separations were achieved for diltiazem and its derivatives using 50 m*M* STC in 20 m*M* phosphate–buffer (pH 9.0). Similar results were achieved using SC while separation of these compounds was not feasible using SDC.

The elution order was proportional to lipophilicity. The migration time increased with increasing bile salt concentration and organic solvent (up to 20% methanol) without changing the elution order for SC and STC. Validation for diltiazem tablets was performed on a method using 20 m*M* phosphate borate buffer (pH 8) with 100 m*M* SC. A LOD of 0.1% of the label claim was achieved for diltiazem, based on a signal-to-noise ratio of 3. Tablet samples were spiked with 0.3–0.5% of related substances and analyzed, demonstrating excellent resolution and detection at these levels. The precision for the standard solution by peak area was 2.2% ($n = 5$). Linearity was demonstrated from 40 to 120% with a zero intercept and excellent correlation. The average assay result ($n = 6$) was 101.5% with an R.S.D. of 1.7%.

Chiral bile salts were employed by Nishi et al. [82] to separate diltiazem enantiomers at neutral and alkaline pH. The bile salts studied included SC, SDC, STC and sodium taurodeoxycholate (STDC). The enantiomers and several related substances were only resolved using phosphate–borate buffer (pH 7) with 50 m*M* STDC (Fig. 9).

Soini et al. [33] separated the enantiomers of verapamil using MECC with TMCD on uncoated capillaries. HTAB was the micellar additive and MHEC was incorporated to improve reproducibility. Analysis of serum samples using 10 m*M* TMCD, 20 m*M* Tris buffer (pH 2.7) and 0.1% MHEC 1000 provided a good separation of the enantiomers but required a 40-min run time. Addition of 0.05 m*M* HTAB shortened the total analysis time to 30 min by minimizing protein adsorption on the capillary walls. Studies on the effect of increasing HTAB concentration showed an initial increase in resolution up to a concentration of 0.03 m*M* and a decrease in resolution thereafter. The optimum separation conditions were 10 m*M* TMCD, 20 m*M* Tris buffer (pH 2.7), 0.1% MHEC 1000 and 0.03 m*M* HTAB.

Protein binding of verapamil was studied via frontal analysis using polyacrylamide-treated

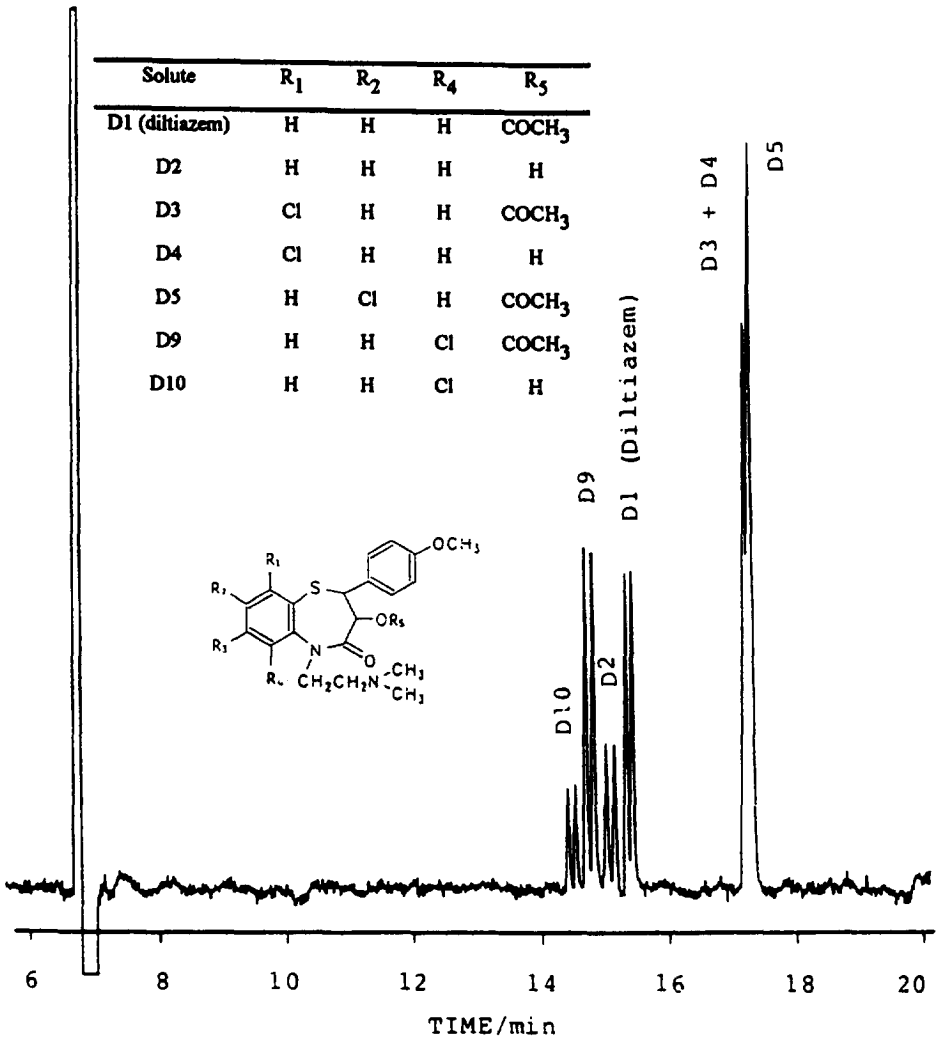

Fig. 9. Chiral separation of diltiazem hydrochloride and related compounds. Buffer, 0.05 *M* STDC in 0.02 *M* phosphate–borate buffer solution (pH 7.0). Capillary, 650 mm × 0.05 mm I.D. (effective length, 500 mm); applied voltage, 20 kV; detection at 210 nm; temperature, ambient. (From Ref. [82]).

capillaries with 0.17 *M* ionic strength phosphate buffer (pH 7.4) by Shibukawa et al. [83]. In frontal analysis, the drug–protein complex is analyzed on a CE system in which it does not migrate. As the voltage is applied, the drug rapidly equilibrates with the background electrolyte and is eluted away from the protein. This perturbs the equilibrium, causing more drug to be released from the protein until all drug is released. Owing to the constant desorption of the drug from the protein, the drug peak elutes as a zonal peak which has a plateau. By performing this analysis at several ratios of drug to protein, and measuring the plateau concentration of the zonal peak, information relating to the amount of unbound drug can be determined. Using this type of analysis Scatchard plots can be generated and drug–protein binding constants determined. Verapamil was determined to have a binding constant with α_1-AGP of $1.13 \cdot 10^6$ l mol^{-1}, which

was in good agreement with constants reported in the literature for equilibrium dialysis studies ($0.946 \cdot 10^6$ l mol^{-1}).

Dethy et al. [84] studied the separation of verapamil and norverapamil enantiomers in plasma using DMCD, TMCD, HPCD and γCD in phosphate buffers. Plasma samples were prepared using gallopamil as internal standard by extraction in hexane–2-propanol. After extraction, the sample was evaporated to dryness and the residue reconstituted in methanol–water. The sample preparation concentrated the analyte tenfold. Enantiomers of both drugs were poorly resolved in DMCD, HPCD and γCD. Using short capillaries, 60 m*M* TMCD in phosphate buffer (pH 2.5) provided separation in 10 min. Increasing the pH from 2.5 to 5.5 decreased the elution times and decreased the resolution. No interfering peaks were observed in blanks prepared from the plasma from six different volunteers and an LOQ of 2.5 ng for each enantiomer was achieved. Calibration graphs in plasma were prepared from 2.5 to 250 ng ml^{-1} and analysis gave good mean response factors, with R.S.D.s between 3 and 6%. Analyte recoveries at 2.5 ng ml^{-1} were 91–100% with R.S.D.s below 15%, well within current industry practice. The precision for actual clinical samples ranged from 6.1 to 10.6% and recoveries from 92.5 to 99.9%, in agreement with the validation results.

Maltooligosaccharides were used as chiral selectors by Soini et al. [85] in the determination of norverapamil and verapamil by CE. Verapamil was partially resolved and norverapamil enantiomers baseline resolved using 20% (w/w) Dextrin 10 in 25 m*M* Tris–phosphate buffer (pH 3.4) on coated polyacrylamide columns. In general, enantiomeric resolution was believed to arise from differential interaction with helical structures of the maltooligosaccharides.

Quang and Khaledi [58] discussed the ability to control enantioselectivity and migration order using βCD and modified CDs, such as TMCD, DMCD and HPCD. Verapamil was not separated using βCD or DMCD, whereas resolutions of 3.7 and 0.78 were achieved using TMCD and HPCD, respectively. The background electrolyte was 50 m*M* TMAH–phosphate buffer (pH 2.5) with 20 m*M* chiral selector.

A "partial zone separation technique" was used by Tanaka and Terabe [60] for the resolution of verapamil enantiomers with a protein pseudo-stationary phase for chiral recognition. In this system, BSA, ovomucoid, conalbumin or α_1-AGP were used as the chiral selector. The partial zone separation technique and the effect of increasing protein concentration on resolution were discussed in Section 2. Optimum resolution for verapamil used 500 μ*M* ovomucoid in 50 m*M* phosphate buffer (pH 5) containing 2-propanol (10%, v/v).

Bretnall and Clarke [62] studied several cardiovascular drugs using MECC with SDS. The calcium channel blockers studied included nicardipine, nifedipine, diltiazem, verapamil and amlodipine. Changes in pH had a small effect on migration time and little effect on resolution. Incorporation of acetone in the background electrolyte had significant effects on migration time and resolution. The optimum conditions were 50 m*M* SDS in 100 m*M* borate buffer (pH 8.1) containing 15% (v/v) acetone.

7. Cardiac glycosides

Digitalis and some cardiac glycosides increase the contractility of cardiac muscle and are used in the treatment of heart failure. These agents provide improved circulation in congestive heart failure patients and decreased ventricular rate in patients exhibiting atrial fibrulation. These agents effectively increase the force of myocardial contraction and effect their activity through a complex mechanism which will not be discussed here.

Gaus et al. [86] separated six primary cardiac glycosides using 37.5 m*M* SDS and 7 *M* urea in 22.5 m*M* borate buffer (pH 9.3) (Fig. 10). SDS or SC was used as a surfactant with αCD, βCD or γCD to enhance solubility and resolution. Cardiac glycosides, being neutral molecules, required the addition of a surfactant to attain separation. CDs increase the solubility of cardiac glycosides so they were also examined for their potential to enhance resolution when used in conjunction with SDS-based MECC. αCD had no effect on resolution, but βCD and γCD

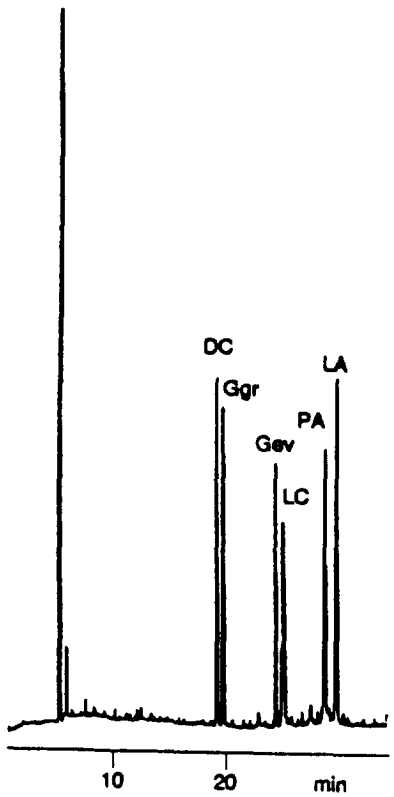

Fig. 10. Electropherogram of six primary cardiac glycosides. Buffer, 22.5 m*M* $Na_2B_4O_7$–37.5 m*M* SDS–7 *M* urea (pH 9.3); capillary length, 0.8 m (0.5 m to the detector); detection at 225 nm; voltage, 25 kV; current, 26 μA; 60-s hydrostatic injection. LA = lanatoside A; PA = purpureaglycoside A; LC = lanatoside C; DC = desacetyllanatoside C; Gev = glucoevatromonoside; Ggr = glucogitoroside. (From Ref. [86]).

enhanced resolution, with γCD providing optimum resolution. MECC using SC provided poor separation, but SC–SDS mixed micelles provided good separation. Crude extracts of *Digitalis lanata* were resolved using 25 m*M* SC and 25 m*M* SDS in 30 m*M* borate buffer (pH 9.3).

8. Hypolipidemics: HmG–CoA reductase inhibitors and fibric acids

Hyperlipoproteinemias are conditions in which the concentration of cholesterol or triglyceride carrying plasma lipoproteins exceed the "normal" limit. This can accelerate atherosclerosis. There are six classes of lipoproteins which differ in size and density of triglycerides and cholesteryl esters in the core and in the nature of the apoproteins on their surface. Each has its own tissue source and plays a defined role in the transport of lipids. HmG–CoA reductase inhibitors are one class of drugs used in the treatment of hyperlipoproteinemias (e.g., mevastatin, lovastatin, pravastatin and simvastatin). These drugs block cholesterol synthesis in the liver, causing a reduction of LDL in the plasma in compensation. Fibric acids (e.g., Clofibrate, Gemfibrozil, fenofibrate, ciprofibrate and bezafibrate) are another class which reduce plasma triglycerides by lowering the concentration of very low-density lipoproteins.

Noroski et al. [87] determined the enantiomer and diastereomer of BMS-180431-09 using MECC containing a CD. SDS was used as the surfactant in the presence of either βCD, γCD, HPCD, DMCD or triacetyl-βCD (TACD). βCD and HPCD provided limited resolution of the enantiomers in borate buffer systems. Resolution was not observed for γCD, TACD or DMCD under the same conditions. Changing the voltage from 10 to 20 kV resulted in a slight decrease in resolution, while increasing the length of the capillary from 72 to 122 cm increased the resolution slightly. Increasing the SDS concentration from 20 to 40 m*M* increased the resolution, although equivalent resolution was achieved in shorter run times in the absence of SDS. Increasing the borate concentration from 10 to 150 m*M* had a marked effect on resolution, presumably owing to complexation of borate anion with the vicinal hydroxyl groups of the analyte in a manner which enhanced the stereoselectivity of βCD. When HPCD was substituted for βCD, studies with increasing HPCD concentration from 0.5 to 2.0 g per 100 ml showed an increase in resolution up to 1.5 g per 100 ml, then a decrease on further addition of HPCD. The *cis* diastereomer was resolved from the parent drug in the absence of CD but not in free-zone CE, hence the diastereomers have different affinities for the SDS micelles. The assay was linear from 0.15 to 0.40 mg ml^{-1} for the parent drug and from 0.12 to 1.1% (w/w) of the parent drug for

the enantiomer. In addition, the LOD was estimated to be 0.06% (w/w). Recovery studies demonstrated a 93.8–110% recovery for the spiked enantiomer. The precision of the method for repeated injections of BMS-180431-09 solutions was between 1 and 2%. The results obtained using the optimized system were comparable to those of analyses by HPLC, although HPLC required the use of two separate analyses to determine the enantiomer and *cis* diastereoisomer.

9. Vasodilators

Vasodilators are useful in the treatment of hypertension, with hydralazine being the first drug marketed in the USA. It was found to have unacceptable side effects, but combination with sympatholytic agents and diuretics provided a successful treatment regimen. This agent causes arteriolar smooth muscle relaxation, although how this affect is achieved is unknown. Minoxidil is the most effective drug for the treatment of patients having severe hypertension and drug-resistant hypertension. Minoxidil is extensively metabolized and its N–O sulfate metabolite is responsible for its activity. This metabolite relaxes vascular smooth muscle but, again, the mechanism of action is understood.

Valka et al. [88] developed an assay for formulations containing papaverinium by capillary isotachophoresis (ITP) (Fig. 11). Papaverinium is an isoquinoline alkaloid derived from opium and is a potent vasodilator. In this early ITP work, 10 m*M* potassium acetate buffer (pH 4.5) was the leading electrolyte and 20 m*M* β-alanine the terminating electrolyte. Poly(vinyl alcohol) was used as an additive in the leading electrolyte. The tablets were disintegrated and suspended in 1 m*M* HCl to extract the drug. Direct injection was performed after filtration to remove undissolved excipients. Several formulations were assayed using the method, with papaverin tablets providing representative results of $103.0 \pm 1.7\%$ of the label claim versus $99.7 \pm 0.7\%$ by titration. No data were provided on detection limits in this early example of method validation. The method demonstrated high efficiency and selectivity relative to the titration method.

Minoxidil tablets were analyzed using ITP by Fanali et al. [89]. They mentioned studying several electrolyte systems, although 10 m*M* potassium acetate–acetic acid buffer (pH 5.1) as the leading buffer and 10 m*M* β-alanine as the terminating buffer provided the best separation. They also indicated that detection limits were improved by incorporating pyridinecarboxylic acid as a counter ion which, owing to its low mobility, allowed shortening of the analysis time. Tablets were ground, suspended in water to extract the drug and filtered to remove undissolved excipients prior to injection. Linearity studies for solutions containing $0.2 \cdot 10^{-9}$–$2.0 \cdot 10^{-9}$ mol gave a good correlation ($r = 0.9998$) with an R.S.D. of 0.5%. Two tablet formulations and one topical solution were assayed with results ranging from 99 to 103% of the label claim. No data were provided on detection limits, limits of quantification or accuracy with spiked samples.

10. Sodium channel blockers

Sodium channel blockers are one class of drugs useful in the treatment of arrhythmias. Arrhythmias manifest themselves as abnormal rate, regularity, site origin of the cardiac impulse or activation sequence of the atria and ventricles. Cardiac cell surface membranes have a characteristic potential as characterized by four phases [20]: phase 0 with inward movement of sodium ions through activated membrane channels, phase 1 with rapid polarization to a characteristic plateau potential and phase 2 having an outward movement of K^+ terminating the plateau with return to the normal action potential. In most cases cells remain at the normal action potential until activated, but some cells undergo spontaneous Phase 3 depolarization and exhibit self-excitation.

Gladdines et al. [90] used ITP to determine lidocaine and 25 other drugs in injectable solutions. The leading ion was 0.01 *M* potassium (pH 5.0), the counter ion was acetate with 0.05% Mowiol added and the terminating ion was 0.005

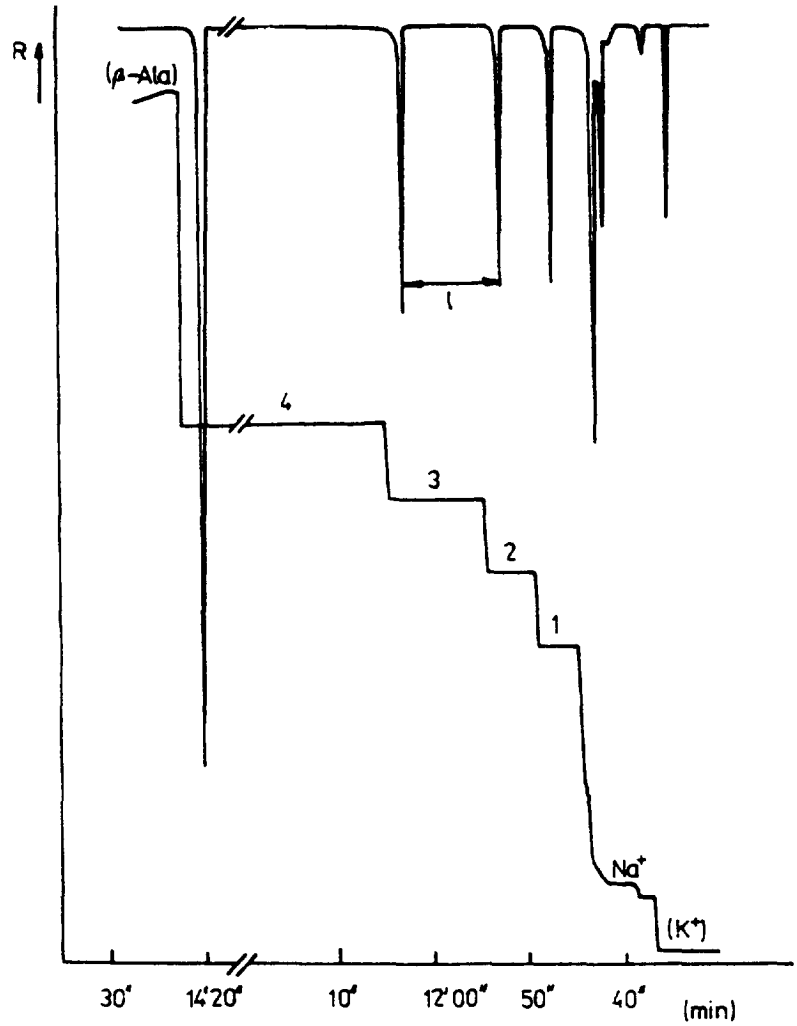

Fig. 11. Isotachopherogram of Spasmoveralgin tablets. The leading ion was K^+ (10^{-2} *M*), the counter ion acetate (pH 4.5) with PVA (0.5 g l^{-1}) as an additive and the terminating electrolyte β-alanine ($2 \cdot 10^{-2}$ *M*); capillary, 250 mm × 0.3 mm I.D.; separation current, 50 μA; conductivity detection. 1 = Ephedrine; 2 = codeine; 3 = papaverine, 4 = aminophenazone. (From Ref. [88]).

M H^+. An equation was derived for a dimensionless response factor which they then calculated using a single injection of the analyte standard. Reproducibility of the response factors determined ranged from 3.5 to 4.8%. Lidocaine intravenous injection solutions were assayed and there was no deviation between the label claim and the value determined by ITP, whereas for the 25 drugs determined, the deviation ranged from 0 to 2.0% for all but one drug, which deviated by 5.5%.

In a study not pertaining to cardiologic effects, Hernandez et al. [91] examined procaine and lidocaine for differential dopaminergic activity. CE was used to measure the amount of drug which diffused out of a probe in the absence of insertion into an animal and in dialyzate from animals. These studies were performed using 50 m*M* borate buffer (pH 8.3) with UV detection at 200 nm.

Belder and Schomburg [92] studied the separation of enantiomers of four tocainamide ana-

logs using γCD in SDS-based MECC. Using 30 m*M* γCD and 40 m*M* sodium sulfate buffer (pH 3), the isomers were not resolved. In order to decrease the EOF (decrease the migration time), 0.05% (w/w) poly(vinyl alcohol) (PVA) was added to the background electrolyte. Two analogs were baseline resolved from their enantiomers while the remaining analogs were only partially resolved. Substitution of hydroxyethylcellulose for PVA yielded similar resolution. They also applied a radial electrical field to control the EOF through changes in the zeta potential of the capillary. Comparison of electrical modification (no hydroxyethylcellulose or PVA) with chemical modification (with hydroxyethylcellulose and PVA) demonstrated that chemical modification offered better resolution of the isomers. In a continuation of this work, Belder and Schomburg [93] performed chiral separations using α- and βCD. In this study, α CD and βCD were found to be better chiral selectors for tochainide derivatives having less substituted phenyl rings, whereas γCD provided better resolution for the more highly substituted rings. Owing to its greater solubility, DMCD was found to offer better resolution than βCD or methyl-βCD in 40 m*M* phosphate buffer (pH 3.0) with 0.05% (w/w) PVA (Fig. 12). Resolution was also enhanced when the concentration of the CD was increased in the presence of PVA or hydroxyethylcellulose.

MECC using SDS was applied by Evenson and Wiktorowicz [75] to the determination of procainamide, N-acetylprocainamide, disopyramide and 5-chloropyramide. They observed that the most hydrophilic molecules eluted first, consistent with the assumption that the more hydrophobic the drug the more it would partition into the micelle, decreasing its mobility. The acetylated derivative of procainamide and the chloro derivative of disopyramide eluted with longer migration times than expected. The longer migration time for these derivatives was believed to arise from a greater dipole moment which decreased their mobility. The four cardioactive drugs were baseline resolved using 30 m*M* borate buffer (pH 9.3) with 30 m*M* SDS.

Chee and Wan [94] presented a CE separation for multiple classes of drugs. Lidocaine and procaine were among the drugs separated using 50 m*M* phosphate buffer (pH 2.35). The reproducibilities (R.S.D.s) for lidocaine and procaine measured by peak area were 2.15 and 2.80%, respectively. Urine and plasma samples were successfully analyzed using the same buffer system. The drug mixture was also analyzed using SDS-based MECC, which required longer run times and provided limited resolution.

CE was used to probe drug–protein binding for metabolites of procainamide to histone and hemoglobin proteins by Thomas et al. [95]. The migration time change for the protein was measured as a function of the ligand concentration and a Scatchard analysis was performed. The analysis provided the thermodynamic association constant and the number of binding sites for drug. Electropherograms were obtained in 60 m*M* borate buffer (pH 8.5). The analysis required the irreversible binding of the drug to the protein in order to prevent disruption of the equilibrium during the CE analysis. Nitrosoprocainamide was determined to have 2.3 binding sites per ferro hemoglobin molecule with an apparent binding constant of $1 \cdot 10^5$ using fluorescence immunoassay–electroconductivity (FIA–EC) versus 2.1 sites and $1 \cdot 10^5$ by CE. Procainamide hydroxylamine also exhibits irreversible binding with the protein, but its susceptibility to oxidation prevented the analysis. CE has particular applicability for these types of binding studies because of the small sample requirements.

11. Conclusion

During the last 15 years, CE has developed to the point where it has demonstrated utility as a separation technique for the determination of cardiovascular drugs. Separations with high efficiency, short run times and low operating costs due to small sample and buffer volumes make CE a particularly appealing analytical technique. Optimization parameters are similar to those for HPLC, but the ease of changing the background electrolytes and the short run times make method development a more time-efficient process. The excellent efficiency has been utilized in the

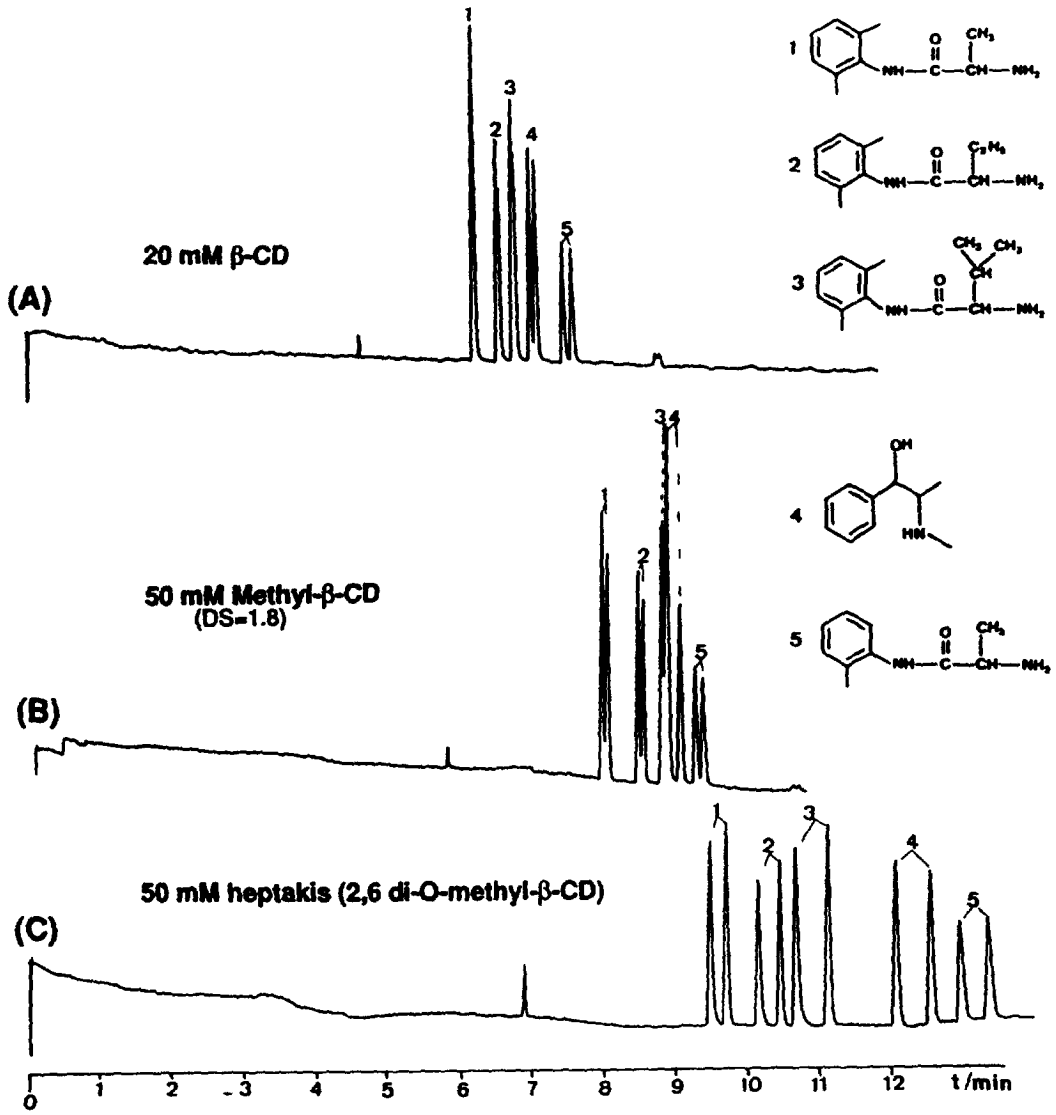

Fig. 12. Partially methylated βCD as selectors in chiral separations of tocainide, tocainide analogs and ephedrine (4). Capillary, 56.4 cm total length, 43.0 cm effective length × 50 μm I.D. × 0.36 mm O.D.; voltage, 35 kV, 30 μA; buffer, 40 mM sodium phosphate buffer (pH 3.0); (A) 50 mM αCD; (B) 20 mM βCD; (C) 50 mM γCD; 0.05% PVA; temperature, 20°C; sampling, vacuum ΔP 85 mbar, 3 s; detection, UV at 210 nm; samples, 0.02 mg ml^{-1} of each racemic compound dissolved in water. (From Ref. [93]).

separation of enantiomers using several chiral selectors as additives to the background electrolyte and in MECC. The separations of β-blockers and diuretics have many examples of such enantioselective separations using cyclodextrins, chiral surfactants, bile salts and oligosaccharides. Indeed, all of these properties make this technique applicable to all areas of pharmaceutical analysis.

The utility of this technique has been demonstrated in the pharmaceutical analyses of cardiovascular drugs with reference to separation of enantiomers, drug substance purity profiling, drug–protein binding constants and assay of

drugs in biological fluids, such as urine and plasma. There are published examples of validation to US Pharmacopeia (USP), Food and Drug Administration (FDA) and international drug development standards, although the results of these studies all point to the need for improved injection reproducibility. Improvement of injection reproducibility to the levels similar to HPLC, with R.S.D. less than 2%, remains as the major technical issue yet to be resolved by the manufacturing sector. Investigators have minimized this weakness through the use of internal standards, but in order to compete effectively with other existing techniques for quantification, this shortcoming must be overcome. Although there are some published examples of interlaboratory qualifications, more work remains to be done in proving that CE is rugged enough for routine analyses. There is also a need to improve the sensitivity for low-level monitoring of impurities in bulk drug substances, degradation products in bulk drug substances and products and for pharmacokinetic profiling of drugs in biological fluids.

CE has also demonstrated utility in the determination of physico-chemical properties such as pK_a and log P values. Owing to its high efficiency, CE is being used as an orthogonal technique to HPLC for specificity studies during method validation. Although no papers have been published on the application of capillary electrophoresis–mass spectrometry (CE–MS) to the structural analysis of cardiovascular drugs, several examples have been presented in the literature on other agents.

Looking to the future, we would envision the routine application of CE for therapeutic drug monitoring in biological fluids and in the quality control of bulk drug substances and drug products. There is also much potential for the use of CE in determining drug–receptor binding constants using mathematical modeling of the analyte–protein complexes. It is also expected that additional chiral selectors will be developed and utilized for the analysis of stereoisomers. There has been little published work on the incorporation of NMR chiral shift reagents into background electrolytes for this purpose.

12. Abbreviations

ACBS	4-Amino-6-chloro-1,3-benzenedisulfonamide
αCD	α-Cyclodextrin
ACE	Angiotensin-converting enzyme
α_1-AGP	α_1-Acid glycoprotein
βCD	β-cyclodextrin
BSA	Bovine serum albumin
CAPS	3-(Cyclohexylamino)-1-propanesulfonic acid
CBHI	Cellobiohydrolase I
CD	Cyclodextrin
CE	Capillary electrophoresis
CE–MS	Capillary electrophoresis–mass spectrometry
CECD	Carboxyethyl-β-cyclodextrin
CHAPS	3-[(3-Cholamidopropyl)dimethylamino]-1-propanesulfonate
CMC	Critical micelle concentration
CMCD	Carboxymethyl-β-cyclodextrin
CT	Chlorothiazide
CTAB	N-Cetyl-N,N,N-trimethylammonium bromide
DCV	N-Dodecoxycarbonylvaline
DMCD	2,6-di-OMe-β-cyclodextrin
DV	Dodecanoylvaline
EOF	Electroosmotic flow
EKC	Electrokinetic capillary electrophoresis
FDA	Food and Drug Administration
FIA–EC	Fluorescence immunoassay–electroconductivity
γCD	γ-Cyclodextrin
HCT	Hydrocholorthiazide
HECD	Hydroxyethyl-β-cyclodextrin
HPCD	Hydroxypropyl-β-cyclodextrin
HPLC	High-performance liquid chromatography
HTAB	Hexadecyltrimethylammonium bromide
ITP	Isotachophoresis
LDL	Low-density lipoprotein
LOD	Limit of detection
LOQ	Limit of quantification
MECC	Micellar electrokinetic chromatography
MHEC	Methylhydroxyethylcellulose

ODS	Octadecylsilane
OM	Ovomucoid
PD	Polydextran
PEO	Poly(ethylene oxide)
PVA	Poly(vinyl alcohol)
R.S.D.	Relative standard deviation
SBECD	Sodium sulfobutyl ether-β-cyclodextrin
SC	Sodium cholate
SCD	Succinyl-β-cyclodextrin
SDS	Sodium dodecyl sulfate or sodium lauryl sulfate
SFC	Supercritical fluid chromatography
STC	Sodium taurocholate
STDC	Sodium taurodeoxycholate
TACD	Triacetyl-β-cyclodextrin
TBAH	Tetrabutylammonium hydroxide
TCT	Trichloromethazine
TMAH	Tetramethylammonium hydroxide
TMCD	2,3,6-Tri-OMe-β-cyclodextrin
Tris	Tris(hydroxymethyl)aminomethane
USP	United States Pharmacopeia

References

[1] R.R. Ruffolo, Jr., Pharm. News, 2 (1995) 13–15.
[2] W.G. Kuhr and C.A. Monnig, Anal. Chem., 64 (1992) 389R–407R.
[3] S.G. Allenmark and S. Andersson, J. Chromatogr. A, 666 (1994) 167–179.
[4] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295–319.
[5] C.A. Monnig and R.T. Kennedy, Anal. Chem. 66 (1994) 280R–314R.
[6] M.M. Rogan, K.D. Altria and D.M. Goodall, Chirality, 6 (1994) 25–40.
[7] F.A. Chen, C. Liu, Y. Hsieh and J.C. Sternberg, Clin. Chem., 37 (1991) 14–19.
[8] R. Kuhn and S. Hoffstetter-Kuhn, Chromatographia, 34 (1992) 505–512.
[9] W. Thormann, S. Lienhard and P. Wernly, J. Chromatogr., 636 (1993) 137–148.
[10] K.D. Altria, J. Chromatogr., 646 (1993) 245–257.
[11] R.K. Gilpin and L.A. Pachla, Anal. Chem., 65 (1993) 117R–132R.
[12] Y. Xu, Anal. Chem., 65 (1993) 425R–433R.
[13] S.R. Rabel and J.F. Stobaugh, Pharm. Res., 10 (1993) 171–186.
[14] H. Nishi and S. Terabe, J. Pharm. Biomed. Anal., 11 (1993) 1277–1287.
[15] Z. Deyl, F. Tagliaro and I. Miksik, J. Chromatogr. B, 656 (1994) 3–27.
[16] N.W. Smith and M.B. Evans, J. Pharm. Biomed. Anal., 12 (1994) 579–611.
[17] J. Sadecka, J. Polonsky and H. Shintani, Pharmazie., 49 (1994) 631–641.
[18] H. Nishi and S. Terabe, J. Chromatogr. A, 694 (1995) 245–276.
[19] J.P. Landers, Clin. Chem., 41 91995) 495–509.
[20] J.G.G. Hardman, A. Gilman and L.L. Limbird, Goodman and Gilman's the Pharmacological Basic of Therapeutics, McGraw-Hill Health Professions Division, New York, 9th ed., 1995.
[21] Guideline for Submission of Supporting Documentation in Drug Applications for the Manufacture of Drug Substances, United States Food and Drug Administration, Washington, DC, 1987.
[22] Guideline for Submission of Supporting Documentation in Drug Applications for the Manufacture of and Control for Drug Substances, United States Food and Drug Administration, Washington, DC, 1987.
[23] International Conference of Harmonization: Draft Guidelines on Impurities in New Drug Substances, Fed. Regist., September 22 (1994) 59FR48740.
[24] S. Fanali, J. Chromatogr., 545 (1991) 437–444.
[25] S. Palmarsdottir, L.E. Edholm, J. Chromatogr. A, 666 (1994) 337–350.
[26] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235–241.
[27] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 609 (1992) 363–367.
[28] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 635 (1993) 113–118.
[29] S.A.C. Wren, J. Chromatogr., 636 (1993) 57–62.
[30] S.A.C. Wren, R.C. Rowe and R.S. Payne, Electrophoresis, 15 (1994) 774–778.
[31] A. Guttman and N. Cooke, J. Chromatogr. A, 680 (1994) 157–162.
[32] A. Aumatell, R.J. Wells, D.K.Y. Wong, J. Chromatogr. A, 686 (1994) 293–307.
[33] H. Soini, M.L. Riekkola and M.V. Novotny, J. Chromatogr., 608 (1992) 265–274.
[34] E. Gonzalez, R. Montes and J.J. Laserna, Anal. Chim. Acta 282 (1993) 687–693.
[35] T.E. Peterson, J. Chromatogr., 630 (1993) 353–361.
[36] P. Lukkari, H. Siren, M. Pantsar and M.L. Riekkola, J. Chromatogr., 632 (1993) 143–148.
[37] P. Lukkari, T. Nyman and M. Riekkola, J. Chromatogr. A, 674 (1994) 241–246.
[38] S. Busch, J.C. Kraak and H. Poppe, J. Chromatogr., 635 (1993) 119–126.
[39] L. Valtcheva, J. Mohammed, G. Pettersson and S. Hjerten, J. Chromatogr., 638 (1993) 263–267.
[40] I. Morita and J. Sawada, J. Chromatogr., 641 (1993) 375–381.
[41] P. Lukkari, H. Vuorela and M.L. Riekkola, J. Chromatogr. A, 652 (1993) 451–457.

[42] P. Lukkari, H. Vuorela and M.L. Riekkola, J. Chromatogr. A, 655 (1993) 317–324.
[43] H. Siren, J.H. Jumppanen, K. Manninen and M. Riekkola, Electrophoresis, 15 (1994) 779–784.
[44] C. Quang and M.G. Khaledi, Anal. Chem., 65 (1993) 3354–3358.
[45] C. Quang and M.G. Khaledi, J. High Resolut. Chromatogr., 17 (1994) 99–101.
[46] T. Schmitt and H. Engelhardt, Chromatographia, 37 (1993) 475–481.
[47] Z. Aturki and S. Fanali, J. Chromatogr. A, 680 (1994) 137–146.
[48] P. Lukkari, A. Ennelin, H. Siren and M.L. Riekkola, J. Liq. Chromatogr., 16 (1993) 2069–2079.
[49] J.R. Mazzeo, E.R. Grover, M.E. Swartz and J.S. Petersen, J. Chromatogr. A, 680 (1994) 125–135.
[50] S.G. Penn, G. Liu, E.T. Bergstrom, D.M. Goodall and J.S. Loran, J. Chromatogr. A, 680 (1994) 147–155.
[51] A. Aumatell and R.J. Wells, J. Chromatogr. A, 688 (1994) 329–337.
[52] Y.Y. Rawjee, R.L. Williams, L.A. Buckingham and G. Vigh, J. Chromatogr. A, 688 (1994) 273–282.
[53] E.C. Tickle, G.N. Okafo, P. Camilleri, R.F.D. Jones and A.J. Kirby, Anal. Chem., 66 (1994) 4121–4126.
[54] E. Gonzalez and J.J. Laserna, Electrophoresis, 15 (1994) 240–243.
[55] I. Bechet, P. Paques, M. Fillet, P. Hubert and J. Crommen, Electrophoresis, 15 (1994) 818–823.
[56] A. Schmutz and W. Thormann, Electrophoresis, 15 (1994) 1295–1303.
[57] R. Vespalec, S. Fanali and P. Bocek, Electrophoresis, 15 (1994) 1523–1525.
[58] C. Quang and M.G. Khaledi, J. Chromatogr. A, 692 (1995) 253–265.
[59] S. Palmarsdottir and L.E. Edholm, J. Chromatogr. A, 693 (1995) 131–143.
[60] Y. Tanaka and S. Terabe, J. Chromatogr. A, 694 (1995) 277–284.
[61] T. Schmitt and H. Engelhardt, J. Chromatogr. A, 697 (1995) 561–570.
[62] A.E. Bretnall and G.S. Clarke, J. Chromatogr. A, 700 (1995) 173–178.
[63] W. Steuer, I. Grant and F. Erni, J. Chromatogr., 507 (1990) 125–140.
[64] X. Qin, D.P. Ip and E.W. Tsai, J. Chromatogr., 626 (1992) 251–258.
[65] B.R. Thomas and S. Ghodbane, J. Liq. Chromatogr., 16 (1993) 1983–2006.
[66] X. Qin, D.T. Nguyen and D.P. Ip, J. Liq. Chromatogr., 16 (1993) 3713–3734.
[67] R. Lozano, F.V. Warren, Jr., S. Perlman and J.M. Joseph, J. Pharm. Biomed. Anal., 13 (1995) 139–148.
[68] B.R. Thomas, X.G. Fang, X. Chen, R.J. Tyrrell and S. Ghodbane, J. Chromatogr. B, 657 (1994) 383–394.
[69] N.T. Nguyen and R.W. Siegler, presented at the Fourth Annual Frederick Conference on Capillary Electrophoresis, 19–20 October 1993, Frederick, MD.
[70] N.T. Nguyen and R.W. Siegler, presented at the Sixth International Symposium on High Performance Capillary Electrophoresis, 31 January to 3 February, 1994, San Diego, CA.
[71] R.W. Siegler and N.T. Nguyen, presented at the American Association of Pharmaceutical Scientists Meeting, 6–10 October, 1994, San Diego, CA; in preparation.
[72] J. Prunonosa, R. Obach, A. Diez-Cascon and L. Gouesclou, J. Chromatogr., 574 (1992) 127–133.
[73] J. Prunonosa, R. Obach, A. Diez-Cascon and L. Gouesclou, J. Chromatogr., 581 (1992) 219–226.
[74] R.P. Garay, C. Rosati, K. Fanous, M. Allard, E. Morin, D. Lamiable and R. Vistelle, Eur. J. Pharmacol., 274 (1995) 175–180.
[75] M.A. Evenson and J.E. Wiktorowicz, Clin. Chem., 38 (1992) 1847–1852.
[76] J. Jumppanen, H. Siren and M.L. Riekkola, J. Chromatogr. A, 652 (1993) 441–450.
[77] J.H. Jumppanen, M.L. Reikkola and H. Haario, J. Microcol. Sep., 6 (1994) 595–604.
[78] H. Soini, M.L. Riekkola and M.V. Novotny, J. Chromatogr. A, 680 (1994) 623–634.
[79] M.G. Quaglia, E. Bossu, C. Desiderio and S. Fanali, Farmaco, 49 (1994) 403–406.
[80] G.N. Okafo, C. Bintz, S.E. Clarke and P. Camilleri, J. Chem. Soc., Chem. Commun., 17 (1992) 1189–1192.
[81] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 513 (1990) 279–295.
[82] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 515 (1990) 233–243.
[83] A. Shibukawa, Y. Yoshimoto, T. Ohara and T. Nakagawa, J. Pharm. Sci., 83 (1994) 616–619.
[84] J.M. Dethy, S. De-Broux, M. Lesne, J. Longstreth and P. Gilbert, J. Chromatogr. B, 654 (1994) 121–127.
[85] H. Soini, M. Stefansson, M.L. Riekkola and M.V. Novotny, Anal. Chem., 66 (1994) 3477–3484.
[86] H.J. Gauss, A. Treumann, W. Kreis and E. Bayer, J. Chromatogr., 635 (1993) 319–327.
[87] J.E. Noroski, D.J. Mayo and M. Moran, J. Pharm. Biomed. Anal., 13 (1995) 45–52.
[88] I. Valka, D. Walterova and V. Simanek, J. Chromatogr., 350 (1985) 336–338.
[89] S. Fanali, M. Cristalli and P. Catellani, J. Chromatogr., 405 (1987) 385–388.
[90] M.M. Gladdines, J.C. Reijenga, R.G. Trieling, M.J.S. Van Thiel and F.M. Everaerts, J. Chromatogr., 470 (1989) 105–112.
[91] L. Hernandez, N.A. Guzman, B.G. Hoebel, Psychopharmacology, 105 (1991) 264–268.
[92] D. Belder and G. Schomburg, J. High Resolut. Chromatogr., 15 (1992) 686–693.
[93] D. Belder and G. Schomburg, J. Chromatogr. A, 666 (1994) 351–365.
[94] G.L. Chee and T.S.M. Wan, J. Chromatogr. B, 612 (1993) 172–177.
[95] C.V. Thomas, A.C. Cater and J.J. Wheeler, J. Liq. Chromatogr., 16 (1993) 1903–1921.