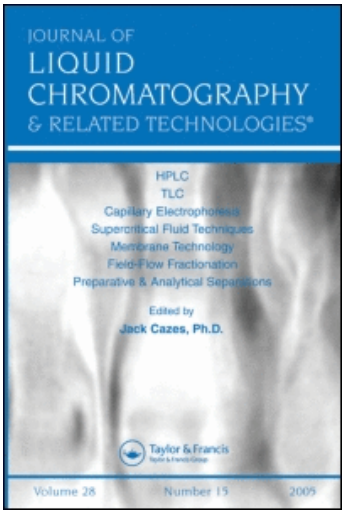

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Recent Progress in the Analysis of Pharmaceuticals by Capillary Electrophoresis

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Recent Progress in the Analysis of Pharmaceuticals by Capillary Electrophoresis

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Abstract: Capillary electrophoresis is a powerful analytical technique which is increasing in utility in the pharmaceutical industry. It is used as an alternative or complementary technique to HPLC due to its high efficiency, speed of analysis, reduction in solvent and sample consumption, and low operating cost compared to HPLC methodology. Various modes have been developed and used for pharmaceutical analysis including capillary zone electrophoresis, non-aqueous capillary electrophoresis, micellar electrokinetic capillary electrophoresis, capillary gel electrophoresis, capillary isotachophoresis, chiral capillary electrophoresis, and capillary electrochromatography. This review summarizes the principles and gives recent pharmaceutical analysis applications used for each mode of capillary electrophoresis. The review will also describe recent developments for enhancing concentration limits of detection.

Keywords: Pharmaceutical analysis, Capillary electrophoresis

INTRODUCTION

The first modern capillary electrophoresis (CE) technique developed was isotachophoresis in 1976[1] followed by capillary zone electrophoresis (CZE) in the early 1980's.[2,3] CE is an analytical technique that employs narrow-bore fused-silica capillaries to perform high-efficiency separation of analytes based on their differential mobilities in an electrical field.

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Additives can be included in the background electrolyte (BGE) to adjust the selectivity of the separation. High electric field strengths are used to separate molecules based on differences in charge and size. Samples are injected into the capillary by the hydrodynamic methods of applying positive pressure, vacuum or siphoning, or the electrokinetic method by applying voltage.[4]

The analysis of pharmaceuticals is predominantly performed by high performance liquid chromatography (HPLC) methods in industry. This is because there are many fully automated HPLC systems available, the technique is an established technology and the methods are highly sensitive, precise, selective, and accurate. CE has been used as an alternative or complementary technique to HPLC in the pharmaceutical industry due to the high efficiency, speed of analysis, reduction in solvent and sample consumption, lower operating cost compared to HPLC methodology,[4] and selectivity differences. Additionally, CE uses primarily aqueous buffers and the columns are inexpensive and have long lifetimes. CE has been successfully applied for the analysis of API, drug product formulations, pharmacokinetic profiling, bioavailability determinations, plasma protein binding studies, and drug activity level determinations.[5] The major disadvantages of using CE applications in the pharmaceutical industry are that the sensitivity of the technique with UV detection is limited due to the short optical path length of the capillary column and the technique is not as robust as HPLC in terms of handling pharmaceutical in-process samples. Samples which contain a variety of impurities and by-products such as in-process reaction samples are more difficult to analyze using CE due to the presence of catalysts and reaction by-products which may interfere with the analysis.

CE encompasses several separation modes to separate a variety of large and small molecules. These techniques include capillary zone electrophoresis (CZE), non-aqueous capillary electrophoresis (NACE), micellar electrokinetic capillary electrophoresis (MEKC), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), and chiral CE. The theory and practice of the different electromigration modes has been discussed in several texts.[6–10] CE also has been successfully hyphenated with mass spectrometric,[11–14] laser induced fluorescence (LIF),[15] and chemiluminescence (CL)[16] detectors to increase concentration sensitivity of the technique compared to conventional UV detection. Also, CE has been coupled with nuclear magnetic spectroscopy[17,18] for the elucidation of molecular structures.

A hybrid technique of CE is capillary electrochromatography (CEC). This technique combines the high efficiency of CE and the high selectivity of high performance liquid chromatography (HPLC).[19] CEC or pressurized capillary electrochromatography (pCEC) are analytical separation techniques that employ the separation principles of HPLC and the electroosmotically-driven mobile phase flow of CE. Open tubular, packed, or monolithic columns

have been used in CEC separations. A gradient elution pCEC system has been recently commercialized.[20] The theory and mechanism of CEC separations have been reviewed.[21–23]

This review will survey recently published CE applications for the analysis of pharmaceuticals. Numerous manuscripts have been published using the various CE modes and detectors. Each section of the text will provide an overview of the CE mode separation principles and give a comprehensive collection of CE pharmaceutical applications. The references given in the paper represent only a segment of the current literature available. Additional references can be found in the publications cited.

CAPILLARY ZONE ELECTROPHORESIS

Overview of CZE

CZE is a widely used mode of CE because it is applicable to separations of anions and cations in the same analysis, but CZE does not separate neutral compounds. The capillary is filled with a free solution of buffer of constant composition, and the source and destination vials are filled with the same buffer. A sample is injected into the capillary filled with buffer and voltage is applied and the solutes migrate through the capillary as zones. Solutes are separated as they move through the capillary due to differences in their rates of migration which are dependent on their electrophoretic mobilities. The separation mechanism is based on differences in charge-to-size ratio of the analytes. Electroosmotic flow (EOF) moves the solute through the capillary from the anode to the cathode. The EOF can be reversed or eliminated.[4]

The detector most commonly used in CE is UV detection due to its versatility, ease of operation, and availability in automated systems. A major disadvantage of CE with conventional UV detection is that the concentration limits of detection are inadequate for analysis requiring high sensitivity due to the short optical path length of the capillary column.[24] The path length of the UV cell in CE is the inner diameter of the capillary, typically 50–100 μm compared to HPLC path lengths which are approximately 100 times larger.[25] There have been several different approaches used to enhance UV detection sensitivity.[24–31] The approaches to enhance sensitivity of UV detection include the use of wider bore capillaries, detection at wavelengths <200 nm, indirect UV detection for non-UV absorbing analytes, use of precolumn or on-column sample concentration techniques, and derivatization of the analyte. Bubble cells or extended path length capillary column, Z-shaped flow cell, multireflection nanoliter cell,[30] and T-shaped post-column flow cell[31] also have been used to increase the sensitivity of the UV detector. Alternative detectors other than

UV have been used to increase method sensitivity. These detectors include mass spectrometric (MS), laser induced fluorescence (LIF), chemiluminescence (CL), and electrochemical (ECD) detectors.

CZE Pharmaceutical Applications

CZE with UV Detection

CZE has found many applications in the analysis of pharmaceuticals with UV detection. The analysis of antibiotics using CZE has been reviewed.[32] Many CZE techniques have been used to provide an alternative pharmaceutical assay method. Selected applications and detailed method descriptions are given in Table 1. Conclusions and advantages of selected applications given in Table 1 are discussed.

A simple and fast CZE method for assay determination of indinavir sulfate was developed by Aurora Prado et al.[33]. The method used a diazepam internal standard and was applied to the assay of capsule formulations. The 2.5 minute method was found to provide excellent recovery of 100.8% at three different concentration levels and provide advantages of low solvent consumption and long column lifetimes.

CZE with indirect UV detection also can be applied to compounds that lack a suitable UV chromophore. Fosfomycin, an antibiotic, a small molecule with no UV chromophore, was found to be readily detected by CZE with indirect UV.[34] The method was applied to microdialysis and plasma samples from clinical pharmacokinetic studies. The samples were analyzed with limited sample pretreatment prior to analysis and the method provided adequate sensitivity and accuracy.

The sample preconcentration technique of field amplified sample stacking (FASS) was applied by Ho et al.[35] for the assay determination of clozapine and the metabolites clozapine *N*-oxide and desmethylclozapine in plasma samples. FASS was used to improve the sensitivity and accuracy of the technique. A highly viscous buffer which contained 50% ethylene glycol was injected prior to a preinjection plug of water. Ethylene glycol acted as a trap to reduce the speed of the analytes and improve the sensitivity of detection. The FASS technique was effective in the determination of the parent compound and also provided a more sensitive method for detection of the metabolites.

A CZE method which separated eleven protease and reverse transcriptase inhibitors was developed by Gutleben et al.[36] The method enabled the simultaneous screening of protease and reverse transcriptase inhibitors with good selectivity and sensitivity in a single 9 minute run. The chromatogram of the separation is given in Figure 1. Serum samples were treated with

Table 1. (a) Applications and detailed method conditions for the CZE-UV analysis of pharmaceuticals; (b) Applications and detailed method conditions for the CZE-UV analysis of pharmaceuticals

Analyte	Sample matrix	Pharmaceutical method type	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
(a)							
Indinavir sulfate	Capsules	Assay	20 mmole/L phosphate (aq) pH 2.52	UV 214 nm	<1.5%	4.6 μg/mL	[33]
Fosfomycin	Plasma, microdialysis samples	Assay	25 mM benzoic acid (aq), 0.5 mM CTAB[c] (aq), pH 6.95 and pH 8.05	Indirect UV 254 nm	≤8%	0.6–2.0 μg/mL	[34]
Clozapine, Closapine N-oxide, Desmethylclozapine	Plasma	Assay	50:50 400 mM phosphate (aq), pH 3.0:ethylene glycol	UV 214 nm	1.4–10.7%	5–10 ng/mL	[35]
Indinavir, Saquinavir, Nelfinavir, Ritonavir, Amprenavir, Delavirdin, Nevirapin, Zalcitabin, Lamivudin, Abacavir, Didanosin	Serum	Assay	67:30:3 20 mM phosphoric acid (aq), pH 2.16: acetonitrile: ethanol with 0.001% hexadimethrin bromide	UV 200 nm	3.7–9.9%	0.5–1.0 μg/mL	[36]
LAS 35917	API	Impurity profile	60 mM borate (aq), pH 9.2	UV 220 nm	1.1%	1.0 μg/mL	[37]
Hydrochlorothiazide, Candesartan, Eprosartan, Irbesartan, Losartan, Telmisartan, Valsartan	Tablets	Assay	60 mM sodium phosphate (aq), pH 2.5	UV 214 nm	0.5–1.3%	n.r.[d]	[38]

(*continued*)

Table 1. Continued

Analyte	Sample matrix	Pharmaceutical method type	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
Naphazoline, Diphenhydramine, Phenylephrine	Nasal drop formulation	Assay	63 mM phosphate (aq), pH 3.72	UV 210 nm	1.1–1.5%	4.5–6.6 mg/L	[39]
Meloxicam	Tablets	Assay	95:5 100 mM borate (aq), pH 8.5: MeOH	UV 205 nm	1.3%	0.3 μg/mL	[40]
Cyclizine hydrochloride	Tablets, Suppositories	Assay	50 mM phosphate (aq), pH 2.3	UV 200 nm	0.4–3.3%	0.1 μg/mL	[41]
Rimantadine hydrochloride	Tablets	Assay	5 mM 4-methyl benzylamine (aq) in 20% ethanol	Indirect UV 210 nm	1.8%	n.r.	[42]
Raloxifene	Tablets, plasma	Assay	20 mM acetate (aq), pH 4.5	UV 286 nm	1.6–1.8%	6.1 ng/mL	[43]
Ursodeoxycholic acid	Tablets	Assay	5 mM sodium *p*-hydroxybenzoic acid (aq), pH 8.0	Indirect UV 250 nm	3.3–3.8%	3 μg/mL	[44]
(b)							
Fenofibric acid metabolite of Fenofibrate	Serum, plasma	Assay	Borate-carbonate (aq), 5 g/L polyethylene glycol	UV 280 nm	3.8%	0.5 mg/L	[45]
Sulfamethoxazole, Trimethoprim	Plasma	Assay	15 mM phosphate (aq), pH 6.2	UV 220 nm	0.3–6.4%	2.0–10.0 μg/mL	[46]
Amitriptyline, Nortriptyline	Plasma	Assay	1.4 M Tris[f], pH 4.50, 1.0 mM β-CD (aq), 50% ethylene glycol	UV 200 nm	0.2–3.3%	2.0 ng/mL	[47]
Cerivastatin	Plasma	Assay	50 mM phosphate (aq), pH 3.5	UV 254 nm	<0.2%	5 μg/mL	[48]

Ribavarin	Plasma, serum	Assay	100 mM borate (aq), pH 9.1, 5 mM spermine	UV 214 nm	2.4–3.4%	0.05 μg/mL	[49]
Fosmidomycin	Serum, urine	Assay	14 mM KH_2PO_4/ 56 mM K_2HPO_4 (aq), pH 10.8, 0.2 mM HTAB[e] (aq), 5% methanol (serum) or 20% methanol (urine)	UV 214 nm	3.1–6.4%	0.1–0.5 μg/mL	[50]
Tramadol and metabolites	Urine	Assay	65 mM borate (aq), 162.5 mM NaOH (aq), pH 10.65	UV 200 nm	3.1–6.7%	0.7–7.0 μM	[51]
Sulfate in aminoglycoside antibiotics	API	Assay	15 mM chromic acid, 40 mM Tris[f] (aq), pH 8.1, 0.2 mM CTAB[c]	Indirect UV 276 nm	0.6%	2 μg/mL	[52]

[a]BGE = background electrolyte.
[b]LOD = limit of detection.
[c]CTAB = cetyl trimethyl ammonium bromide.
[d]n.r. = not reported.
[e]HTAB = hexadecyltrimethylammonium bromide.
[f]Tris = tris(hydroxymethyl)methylamine.

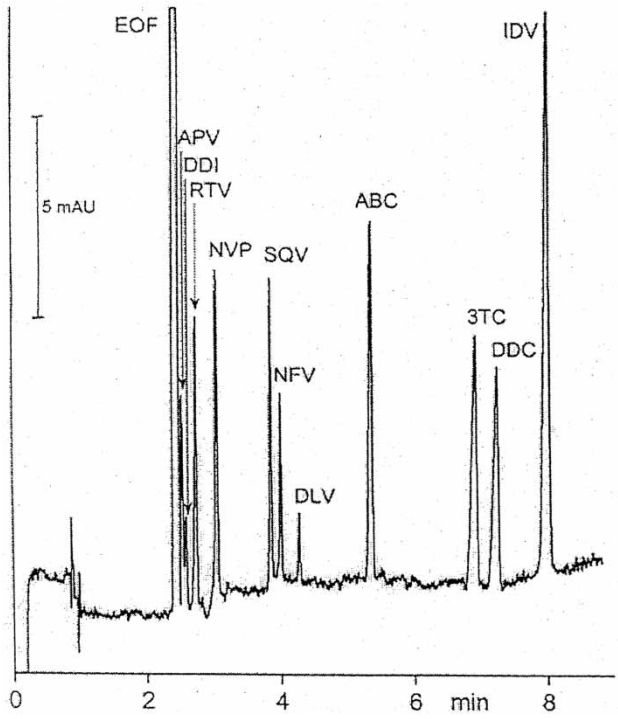

Figure 1. Counter-electroosmotic separation of a standard mixture of protease and reverse transcriptase inhibitors. Electrophoretic conditions: see Table 1. (Reprinted from J. Sep. Sci. **2003**, *26*, 1198–1202 with permission).

solid phase extraction (SPE) prior to injection. The CZE method utilized a bubble cell in the capillary column to enhance method sensitivity.

Toro et al.[37] developed a CZE impurity profile method for a new drug candidate LAS 35917. Impurities which were closely related in structure but previously could not be separated by conventional HPLC methodology were resolved by the CZE method. The CZE method was faster, detected impurities $>0.04\%$, and separated the known impurities and degradates. The method was found to be precise with 1.1% RSD and the robustness of the technique was found to be satisfactory by limiting the number of sample injections performed for each set of BGE vials and column preconditioning between injections.

CZE with MS, LIF, and CL Detection

The disadvantage of CE with conventional UV detection is that the concentration limit of detection is limited to the short optical path length of

the capillary column. Therefore, alternative detectors other than UV have been used to increase sensitivity of the technique. CZE hyphenated with MS, LIF, or CL detectors have been used to improve method sensitivity and also specificity. Selected applications which used CZE-MS, CZE-LIF, and CZE-CL are given in Table 2. Conclusions and advantages of selected applications given in Table 2 are discussed.

Zheng et al.[53] developed a CZE-ESI-MS method for determination of lamotrigine in human plasma. The BGE pH, concentration of ammonium acetate and nebulizer gas pressure were key parameters which influenced the separation and MS detection sensitivity. The CZE-ESI-MS method demonstrated higher chromatographic efficiency, specificity, sensitivity, and provided faster throughput than the previously used conventional HPLC-UV method.

An in-capillary derivatization technique was developed by Le Potier et al.[54] for the determination of insulin in plasma samples using CZE-LIF. The derivatization reagent to fluoresce insulin was 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate. The analysis was completed by introducing successive plugs of sample and derivatization reagent and applying voltage. The molar ratio of the sample and reagent and the length of plugs introduced into the capillary were found to be critical in the method optimization. It was found that the in-capillary derivatization technique was superior in terms of simplicity, ease of automation, and applicability to smaller sample sizes compared to pre-capillary derivatization techniques. An efficient derivatization method and separation of insulin and its two glycated forms were obtained using the in-capillary technique.

A CZE method which used chemiluminescence detection for the direct detection of $\propto$-amino acids, peptides, and proteins was developed by Tsukagoshi et al.[16]. The chemiluminescence system used a luminal-hydrogen peroxide–Cu(II)-catalyzed CL reaction. The CZE-CL method provides a direct detection method (no pre-derivatization or labeling) for detection of biomolecules. The method was applied to twenty α-amino acids, four peptides, and eleven proteins. The electropherogram of a mixture of glycine and peptides is shown in Figure 2. The method was found to be selective and sensitive for the biomolecules studied.

NON-AQUEOUS CAPILLARY ELECTROPHORESIS

Overview of NACE

NACE is the separation of analytes which uses a BGE composed primarily of organic solvents. The viscosity and dielectric constants of organic solvents affect both sample ion mobility and the level of electroosmotic flow. The use of a non-aqueous BGE allows additional selectivity options in methods

Table 2. Applications and detailed method conditions for the CZE-MS and CZE-LIF analysis of pharmaceuticals

Analyte	Sample matrix	Pharma-ceutical method type	Sample pre-treatment procedure	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
Lamotrigine	Plasma	Assay	Vortexing, filtration, centrifugation	20 mM NH_4OAc (aq), pH 5.0	ESI^+-MS	n.r.[c]	0.05 μg/mL	53
Insulin	API	Assay	None	10 mM sodium tetraborate (aq), pH 8.9	LIF: Excitation $\lambda = 325$ nm	4.6%	5.0×10^{-7} M	54
α-Amino acids, peptides, proteins	Biomolecules	Assay	None	10 mM phosphate (aq), pH 10.8, 5.0×10^{-5} M luminal, 5.0×10^{-6} M Cu(II), 5.0×10^{-5} M potassium sodium tartrate (aq) in the inlet; 10 mM phosphate (aq), pH 10.8, 0.4 M hydrogen peroxide in the outlet	CL	n.r.	0.6–1.1 fmol	16

Gabapentin	Plasma	Assay	Precolumn derivatization	50 mM sodium borate (aq), pH 9.5	LIF: Excitation $\lambda = 488$ nm; Emission $\lambda = 520$ nm	$\leq 10.8\%$	60 nM; 4.8 nM with stacking	55
Pranoprofen; Fenoprofen; Flurbiprofen; Ketoprofen; Ibuprofen	API	Assay	Precolumn derivatization	1.7 M acetic acid, 0.06 M ammonium acetate, 2.4 mM β-CD, 0.6 M urea (aq), pH 3.1	LIF: Excitation $\lambda = 488$ nm; Emission $\lambda = 520$ nm	n.r.	0.16–0.3 fmol	56
Zaleplon and its metabolites	Urine	Assay	Preconcentration using solid phase extraction	0.2 M boric acid (aq), 1.0 M Tris[e] 50 mM carboxymethyl-β-CD, pH 9.4	LIF: Excitation $\lambda = 325$ nm; Emission $\lambda = 450$ nm	n.r.	10–100 ng/mL	57
Ephedrine; Pseudoephedrine	Tablets	Assay	Extraction, centrifugation, precolumn derivatization	60 mM borate (aq), pH 9.9	LIF: Excitation $\lambda = 488$ nm; Emission $\lambda = 520$ nm	2.5–2.7%	0.16–0.17 μM;	58

[a]BGE = background electrolyte
[b]LOD = limit of detection
[c]n.r. = not reported
[e]Tris = tris(hydroxylmethyl)amino methane

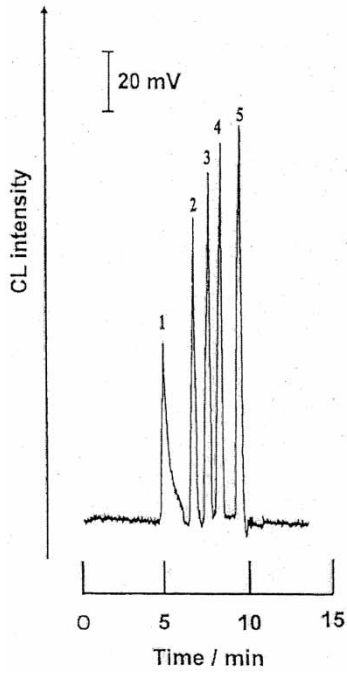

Figure 2. Electropherogram of a mixture of glycine and peptides: 1, glycine; 2, glycylglycine; 3, glycylglycylglycine; 4, glycylglycylglycylglycine; 5: glycylglycylglycylglycylglycine. Separation conditions: see Table 2. (Reprinted from Anal. Chem. **2004**, *76*, 4410–4415 with permission. Copyright (2004). American Chemical Society).

development, valuable for the separation of water-insoluble compounds and allows separations to be performed at subambient temperatures.[59] NACE has been found to be useful for analysis of highly hydrophobic and structurally similar compounds without the use of surfactant or complexing agent additives.[60] NACE is suitable for routine procedure for the rapid separation and characterization of basic compounds and is a viable alternative to HPLC.

NACE Pharmaceutical Applications

Selected NACE applications for pharmaceutical analysis are given in Table 3. Conclusions and advantages of selected applications given in Table 3 are discussed.

The application of non-aqueous capillary zone electrophoresis (NACZE) stacking and low temperature batch non-aqueous capillary zone electrophoresis

Table 3. (a) Applications and detailed method conditions for the NACE analysis of pharmaceuticals; (b) applications and detailed method conditions for the NACE analysis of pharmaceuticals

Analyte	Sample matrix	Pharmaceutical method type	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
(a)							
3,4-methylenedioxy-meth amphetamine	Tablets, urine	Assay	50 mM ammonium acetate in 90:10 methanol:water	Fluorescence, Excitation $\lambda = 285$ nm Emission $\lambda = 320$ nm	n.r.[c]	4.7×10^{-6} M (NACZE); 2.6×10^{-8} M (NACZE stacking) 5.0×10^{-9} M (LTB-NACZE[d] stacking)	[59]
Imipramine, Desipramine, Amitriptyline, Nortriptyline	Tablets, plasma	Assay	50 mM ammonium acetate, 1 M acetic acid in acetonitrile	UV 214 nm	1.2–14.1%	0.5 μg/mL (tablets); 20–30 ng/mL (plasma)	[60]
Protriptyline, Desipramine, Nortriptyline, Clenbuterol, Terbutaline, Imipramine, Amitriptyline, Clomipramine, Trimipramine	API	Assay	80 mM ammonium formate 80:20 methanol:acetonitrile	UV 195 nm UV-MS	n.r.	n.r.	[61]

(*continued*)

Table 3. Continued

Analyte	Sample matrix	Pharmaceutical method type	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
Paroxetine and metabolite, Tamoxifen and metabolite, Clomipramine	Urine	Assay	18 mM ammonium acetate/1.1% acetic acid (aq) in 80:20 methanol:acetonitrile	UV 209 nm	1.7–2.8%	3.0–7.1 μg/L	[62]
Ammonium cation	API	Assay	Methanol with 0.5% formic acid, 3 mM imidazole	Indirect UV 214 nm	1.1%	50 ppb	[63]
Lidocaine, Mepivacaine, Bupivacaine, Cinchocaine, Procaine, Prilocaine, Chloroprocaine, Cocaine, Ketamine	API	Assay	25 mM ammonium acetate in methanol	UV 200 nm	2.6%	n.r.	[64]

(b)							
Paroxetine and metabolites	Urine	Assay	9:1 methanol: acetonitrile with 25 mM ammonium acetate, 1% acetic acid	UV 209 nm	1.9–2.8%	9.6–23.1 μg/L	[65]
Atenolol, Betaxolol, Metoprolol, Sotalol	API	Assay	0.75 M formic acid, 31 mM potassium formate in 63:37 ethanol:acetonitrile containing 40 mM potassium camphorsulphonate	UV 230 nm	n.r.[c]	n.r.	[66]
Ephedrine, Pilocarpine, Papaverine, Berberine, Atropine, Matrine	API	Assay	50:50 methanol: acetonitrile containing 25 mM Tris and methane sulfonic acid (aq), pH 3.78	UV	n.r.	n.r.	[67]
Pb^{2+}, Zn^{2+}, Cd^{2+}	API	Assay	2% acetic acid, 2% acetonitrile in 1.5 mM 4-aminopyridine	Indirect UV 262 nm	4.5–5.1%	1.9–3.8 μM	[68]

[a]BGE = background electrolyte.
[b]LOD = limit of detection.
[c]n.r. = not reported.
[d]LTB-NACZE = low temperature batch nonaqueous capillary zone electrophoresis.

(LTB-NACZE) stacking with fluorescence detection were used to increase method sensitivity by Tsai et al.[59] in the analysis of 3,4-methylenedioxymethamphetamine in tablets and biological samples. A 160-fold improvement in detection sensitivity was obtained using the NACZE stacking technique. The sensitivity was further improved by 4-fold using the LTB-NACZE technique with a LOD of 5.0×10^{-9} M.

A NACE method for simultaneous determination of imipramine, amitriptyline and their metabolites in tablets and plasma was developed by Cantu et al.[60] The method optimization varied electrolyte concentration in acetonitrile to achieve the separation. The plasma samples used a liquid-liquid extraction procedure as a clean-up step prior to analysis. The CE method gave the advantage of no interferences from the incipient neutrals present in the formulation. The method was fully validated.

Peri-Okonny et al.[61] used NACE with conventional UV absorption and UV/MS detection for the separation and characterization of seven tricyclic antidepressants and two bronchodilator drugs. The method was developed by optimization of the BGE composition and apparent pH. A significant improvement in separation efficiency was achieved in the separation compared to HPLC. Also, it was demonstrated that the NACE method could be applied to a wide range of pharmaceuticals of varying solubilities.

A method for the analysis of paroxetine, tamoxifen, and their metabolites in biological samples was developed using NACE by Rodriguez Flores et al.[62] A mixture of non-aqueous solvents, acetonitrile and methanol, provided efficient separation of the pharmaceuticals. Varying the concentration of methanol in acetonitrile changed migration time and resolution due to changes in viscosity and dielectric constant. The concentration of the ammonium acetate electrolyte was also varied in the method optimization. Increased concentration of electrolyte was found to increase the migration time and current. The samples used a solid-phase extraction procedure as a clean-up and pre-concentration step prior to analysis. The separation using the optimized method conditions is shown in Figure 3.

NACE also can be used for quantitation of inorganic ions in pharmaceuticals. Gong et al.[63] developed a NACE method with indirect UV detection for the determination of ammonium cations in API samples. Methanol was used as the BGE in the analysis. The apparent pH of the BGE was optimized to achieve a close mobility match between the ammonium cation and the imidazole UV probe to obtain optimum peak symmetry. Resolution between ammonium and potassium cations was enhanced by addition of crown ether to the BGE. Addition of water, acetonitrile, and tetrahydrofuran to the methanol-based BGE allowed the method to be applied to pharmaceuticals that have poor solubility in methanol. The method was fully validated and applied to ammonium cation determinations in API and in-process samples.

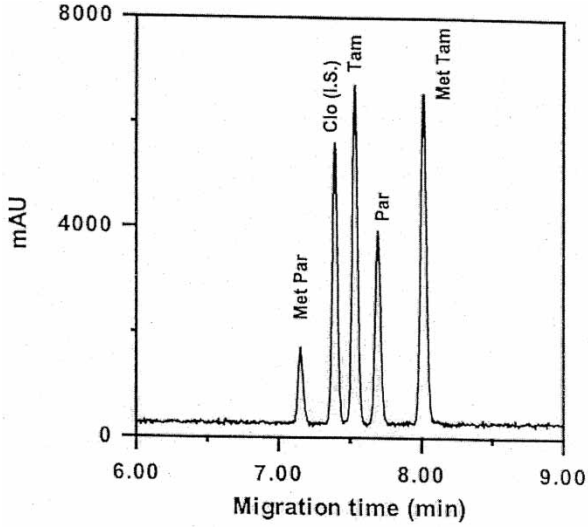

Figure 3. Electropherogram for a standard mixture of 2 mg/L of paroxetine (Par), tamoxifen (Tam), the metabolite of paroxetine (Met Par), the metabolite of tamoxifen (Met Tam) and the internal standard clomipramine (Clo). Operating conditions given in Table 3. (Reprinted from Anal. Chim. Acta **2004**, *512*, 287–295 with permission from Elsevier).

MICELLAR ELECTROKINETIC CAPILLARY ELECTROPHORESIS

Overview of MEKC

MEKC was developed by Terabe et al.[69–71]. MEKC is a mode of electrokinetic chromatography in which surfactants are added to the buffer solution at concentrations that form micelles. The separation principle of MEKC is based on partitioning of analytes between the micelle and the BGE. The retention times of the analytes are proportional to their hydrophobicity. Ionized solutes are retained due to electrophoretic migration, ionic interactions between the solutes and micelles, and solubilization of the solutes by the micelles. Other substances (such as cyclodextrins, organic solvents, metal ions) can be added to the BGE to adjust selectivity. MEKC has great utility in separating mixtures that contain both ionic and neutral species. The technique has become valuable in the separation of hydrophobic pharmaceuticals from polar metabolites and is useful for impurity profile determinations of pharmaceuticals.[72] Microemulsion electrokinetic chromatography (MEEKC) is a technique similar to MEKC. A microemulsion is used which has a core of oil droplets inside the micelles. The oil droplets

are stabilized and the surface tension between the oil and the water phase reduced by the use of a surfactant and cosurfactant. Strategies of MEEKC method optimization for analysis of pharmaceuticals have been reviewed.[73]

The main advantage of MEKC compared to CZE is that both neutral and charged analytes can be analyzed since CZE is not applicable to the analysis of neutral compounds. Also, compounds with low aqueous solubility can be analyzed by MEKC since samples can be dissolved in organic solvent prior to injection and surfactants used in the analysis often contribute to better solubility. Another advantage of MEKC compared to CZE is it may be possible to directly inject biological samples without pretreatment methods in some applications.[73]

MEKC Pharmaceutical Applications

Hillhorst et al. has reviewed MEKC use for impurity profile determination of pharmaceuticals.[72] The analysis of antibiotics[32] and nonsteroidal anti-inflammatory drugs in biological samples[74] using MEKC have been reviewed.

Applications and detailed method descriptions of selected MEKC methods of pharmaceutical analysis are given in Table 4. Conclusions and advantages of some selected applications given in Table 4 are discussed.

An impurity profile MEKC method was developed for gentamicin and its impurities.[75] The method was found to be faster and yield improved peak shape compared to the European Pharmacopeia HPLC method. NMR spectroscopy was used as the orthogonal method to validate the results obtained from the MEKC method.

A simple, fast, and selective MEKC method was developed for the simultaneous assay of ketorolac tromethamine and its known related impurities in API and tablets.[76] The method resolved the components in 6 minutes and quantified the level of impurities at the 0.1 wt-% level. The electropherogram of the separation is shown in Figure 4. The experimental parameters which influenced the separation were systematically investigated by means of an experimental design to achieve optimal separation and analysis time.

Alternative detectors other than UV have been used for MEKC. Cao et al.[77] achieved detection limits of 7.0×10^{-8} M in the determination of reserpine in biological samples using MEKC coupled with CL detection. The CL detector was coupled to a 25 μm i.d. capillary without an electric field coupler. Field-amplified sample stacking (FASS) was used to further increase method sensitivity and no sample pretreatment was required prior to analysis. MEKC coupled with laser-induced fluorescence detection was used to increase method sensitivity for the analysis of vigabatrin in biological samples.[78] A pre-column derivatization and a simple sample clean-up procedure were used prior to injection. The limit of detection of vigabatrin in plasma was 0.13 μM.

Table 4. (a) MEKC applications and detailed method conditions for the analysis of pharmaceuticals; (b) MEKC applications and detailed method conditions for the analysis of pharmaceuticals

Analyte	Sample matrix	Pharmaceutical method type	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
(a)							
Gentamicin	API	Impurity profile	100 mM borate, 20 mM Dchol, 15 mM β-CD (aq), pH 10.0	UV 340 nm	n.r.[c]	n.r.	[75]
Ketorolac tromethamine	API, tablets	Assay, Impurity profile	13 mM boric acid and phosphoric acid (aq), pH 9.1; 73 mM SDS[d]	UV 323 nm	0.2–5.4%	0.6–1.0 μg/mL	[76]
Reserpine	Urine	Assay	20 mM/L H_3BO_3/ $Na_2B_4O_7$ (aq), pH 9.0, 2.0 mM/L SDS (aq)	CL: 1.1 V	4.3%	7.0×10^{-8} M	[77]
Vigabatrin	Plasma	Assay	50 mM sodium borate (aq), pH 9.5, 10 mM SDS	LIF, excitation $\lambda = 544$ nm; emission $\lambda = 589$ nm	11.2–14.3%	0.13 μM	[78]
Hydrochlorothiazide, Candesartan, Eprosartan, Irbesartan, Losartan, Telmisartan, Valsartan	Tablets	Assay	55 mM sodium phosphate (aq), pH 6.5, 15 mM SDS	UV 214 nm	0.5–1.0%	n.r.[c]	[38]

(*continued*)

Table 4. Continued

Analyte	Sample matrix	Pharmaceutical method type	BGE[a]	Detection	Precision (% RSD)	LOD[b]	Reference
Acetaminophen, Phenylephrine, Chlorpheniramine	Capsules	Assay	40 mM phosphate (aq), pH 6.20, 0.5 mM SDS	UV 200 nm	0.9–5.5%	n.r.	[79]
Tramadol, its phenolic and glucuronide metabolite diastereomers	API	Assay	25 mM borate (aq), 70 mM SDS (aq), pH 10.45	UV 200 nm	n.r.	8.5–15 μM	[51]
Vitamin B6, Vitamin B12, Dexamethasone phosphate, Piroxicam (b)	Tablets	Assay	20 mM borate (aq), pH 7.5, 20 mM SDS, 50 mg/L acetonitrile	UV 240 nm	n.r.	0.02–0.56 mg/L	[80]
Benzylpenicillin, Procaine, Benzathine, Clemizole	API	Assay	3.1 g/L phosphate, 7.6 g/L borate (aq), pH 8.7, 14.4 g/L SDS[d]	UV 214 nm	1.0–1.5%	0.0004–0.02 mg/mL	[81]
Cefozopran	Serum	Assay	25 mM borate, 0.1 N sodium hydroxide, pH 10.0, 50 mM SDS (aq)	UV 244 nm	2.4–7.7%	0.5 mg/L	[82]

Prednisolone, Naphazoline, Phenylephrine	Nasal drops, aerosol	Assay	5 mM phosphate, 5 mM borate, pH 8.2; 40 mM SDS (aq)	UV 205 nm	≤2.5%	0.03–0.22 mg/L	[83]
Fenofibrate	Capsules, tablets	Assay	100 mM/L borate, pH 8.0; 2% SDS (aq)	UV 280 nm	4.9%	0.02 mg/mL	[45]
Benzodiazepines	API	Assay	Borate (aq), pH 9.2; 2% dextran sulfate, 20 mM SDS (aq)	UV 254 nm	n.r.	0.2 μg/mL	[84]
Piribedil	Tablets	Assay	50 mM borate (aq), pH 8.0, 50 mM SDS (aq)	UV 205 nm	0.4–2.2%	1 μg/mL	[85]
Vigabatrin, Glutamate, L-Aspartate, γ-Aminobutyric acid	Brain extracellular fluid	Assay	75 mM borate, 60 mM SDS, 5 mM hydroxylpropyl-β-CD (aq), pH 9.2	LIF, excitation $\lambda = 442$ nm; emission $\lambda = 490$ nm	2.0–10.3%	0.4×10^{-9}–3×10^{-9} moles/L	[86]

[a]BGE = background electrolyte.
[b]LOD = limit of detection.
[c]n.r. = not reported.
[d]SDS = sodium dodecyl sulfate.

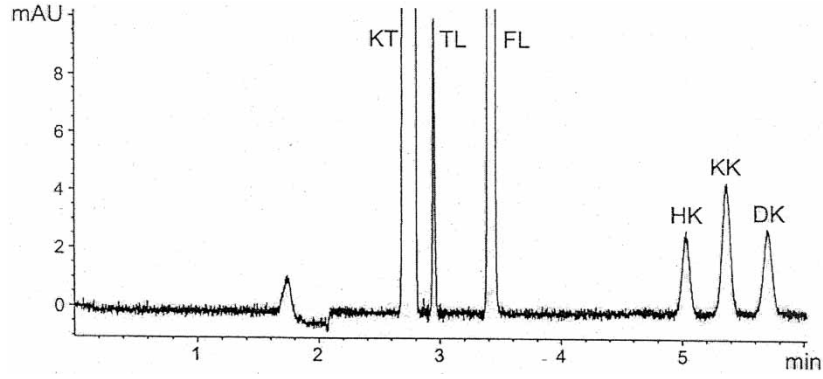

Figure 4. Electropherogram of ketorolac (KT) and related substances. TL = tolmetin sodium salt dihydrate internal standard. FL = flufenamic acid internal standard. HK = (±)-(7-hydroxy-6,7-dihydro-5H-pyrrolizin-3-yl)-phenyl-methanone. KK = 5-benzoyl-2,3-dihydro-pyrrolizin-1-one. DK = (6,7-dihydro-5H-pyrrolizin-3-yl)-phenyl-methanone. (Reprinted from J. Chromatogr. A **2004**, *1032*, 253–263 with permission from Elsevier).

CAPILLARY GEL ELECTROPHORESIS

Overview of CGE[87]

CGE is the adaptation of traditional gel electrophoresis using capillary columns filled with polymers in solution to create a molecular sieve. This allows analytes having similar charge-to-mass ratios to be resolved by size. The gel usually is a polyacrylamide/bisacrylamide crosslinked polymer or a linear, noncrosslinked polyacrylamide polymer. Dextran, polyethyleneoxide, and agarose gels are also used. Ionic solutes that have different sizes, but similar charge-to-size ratios, such as oligonucleotides, DNA restriction fragments, and SDS-proteins can be separated by CGE. Selectivity can be changed in CGE through the use of modifiers which interact with solutes. Resolution and efficiency are comparable to that obtained in CZE.

CGE Pharmaceutical Applications

Characterization of a protein pharmaceutical, polyethylene glycolylated interferon alpha (PEGylated IFN), was completed by Na et al.[88] using SDS-CGE with a hydrophilic polymer network. The method was found to be able to resolve PEGylated IFN's of different sizes, provide a faster reaction monitoring method, and required less sample than the previously used SDS-polyacrylamide gel electrophoresis (SDS-PAGE) method.

Antisense oligonucleotide therapeutic targets have been characterized by CGE. Palm and Varga[89] developed an automated, rapid method using on-column electroextraction-injection and separation of antisense oligonucleotides from human plasma. Plasma samples were diluted and injected without further pre-treatment steps. The method used dextran polymer with buffer containing boric acid, urea, and tris(hydroxymethyl) aminomethane at pH 8.0. Yu et al.[90] used CGE to determine levels of antisense oligonucleotides in biological samples. The analysis provided pharmacokinetic, tissue distribution and metabolism data for pharmaceutical clinical trials. On-line coupling of CGE-ESI-MS for analysis of oligonucleotides was completed by von Brocke et al.[91]. The CGE-ESI-MS coupling parameters of sheath liquid-flow rate and composition of the separation capillary alignment in the interface were optimized for the oligonucleotides analyzed.

CAPILLARY ISOTACHOPHORESIS

Overview of CITP

CITP is a moving boundary technique in which the sample is sandwiched between a leading and terminating trailing buffer.[4] The leading buffer must have a higher electrophoresis mobility than any of the solutes, and the terminating buffer must have a lower electrophoretic mobility than any of the solutes. An electric field, in the constant current mode, is applied, and the solutes separate into zones based on differences in their electrophoretic mobilities. Solutes having mobilities intermediate to those of the leading and termination electrolytes stack into sharp, focused zones. The output of CITP is different from the other modes in that the zone widths are proportional to the amount of ions in the sample. A limitation of the technique is that anions and cations are not separated in the same run[4] and method sensitivity is less than CZE with conventional UV detection. Conductometric detection is the standard mode of detection and high sample loading capacity is possible. Transient CITP has been used as a sample preconcentration technique to enhance the sensitivity of CZE;[92] the analyte detectability can be extended to the nanomolar range. Methodological, instrumental aspects, and general applications of CITP have been reviewed previously.[93]

CITP Pharmaceutical Applications

Several recent papers have been published which have used CITP with conductometric detection for the analysis of pharmaceutical API and drug product formulations. Microchip capillary electrophoresis (MCE) in

the CITP mode also has been utilized. Method descriptions are given for the applications.

The assay determination of tolfenamic, flufenamic, mefenamic, and niflumic acid in drug product formulations[94] was completed by CITP with conductometric detection. The method used 10 mM HCl/20 mM imidazole (aq), pH 7.1 as the leading electrolyte and 10 mM 5,5′-diethylbarbituric acid (aq), pH 7.5 as the terminating electrolyte with a 20 minute analysis time. The method was linear between 10–100 mg/L and the precision of the method was satisfactory, yielding 1.0–1.6% RSD.

CITP with conductometric detection was used to determine levels of amproxol and bromhexine in drug product formulations.[95] The method used 5 mM picolinic acid and 5 mM potassium picolinate (aq), pH 5.2 as the leading electrolyte and 10 mM formic acid (aq) as the terminating electrolyte with an 8 minute analysis time. The method was linear between 10–200 mg/L concentrations and the precision was acceptable with 1.2–1.6% RSD's.

Determination of naproxen in the presence of its metabolite 6-*O*-desmethylnaproxen in serum was completed by CITP with the conductometric method by Cakrt et al.[96]. The leading electrolyte contained 10 mM HCl, β-alanine (aq), pH 4.0 with 0.1% methylhydroxypropylcellulose. The terminating electrolyte contained 10 mM 2-(N-morpholino)ethane sulfonic acid-tris(hydroxymethyl)aminomethane (aq), pH 6.9 and 20 v/v% ethanol. Naproxen was determined in the sample supernatant after deproteination with ethanol and the results matched well with those obtained by fluorescence spectrometry. The limit of detection for naproxen and its metabolite was 0.2 mg/L.

A method which used CITP with conductometric detection to determine bopindolol in pharmaceutical tablets was developed by Urbanek et al.[97]. The electrolytes used were 5 mM potassium picolinate/5 mM picolinic acid (aq), pH 5.4 as the leading electrolyte and 10 mM formic acid (aq) as the terminating electrolyte. The method analysis time was 12 minutes. The method was linear between 10–100 mg/L and the precision was acceptable at 0.9% RSD.

A CITP method with conductometric detection was developed by Meissner et al.[98] for determination of alkylsulfonates in API. Alkylsulfonates from methanesulfonic acid to decanesulfonic acid were separated by CITP using a 10 mM HCl/ε-aminocaproic acid (aq), pH 4.8/0.1% methylhydroxyethylcellulose leading electrolyte and 20 mM caproic acid (aq)/0.05% methylhydroxyethylcellulose (aq) as the terminating electrolyte. The method was used to determine salt levels in API alkylsulfonate samples and also used for trace analysis of alkylsulfonates with a limit of detection <1 mg/L.

Analysis of the β-blockers oxprenolol, atenolol, timolol, propranolol, metoprolol, and acebutolol in biological samples was completed by a combination of CZE and transient ITP.[99] Transient ITP was used as a CZE

preconcentration technique. The electrolyte system used was 10 mM sodium morpholinoethane sulfonate (aq), pH 5.5 with 0.1% methylhydroxyethylcellulose as the leading electrolyte and 30 mM ortho phosphoric acid (aq), pH 2.0 (aq) as both the terminating electrolyte and BGE. The method also was applied to a poly(methyl methacrylate) microchip with integrated platinum electrodes for conductivity detection. The microchip system used the same leading electrolyte, but 5 mM glutamic acid (aq), pH 3.4, as the terminating electrolyte with a 20 minute analysis time. The method was rugged with up to 200 analyses of β-blockers performed on the same microchip.

Olvecka et al.[100] developed a MCE method which allowed the direct determination of valproate in serum by zone electrophoresis-ITP. A poly(methyl methacrylate) column-coupling chip with integrated conductivity detection was used for the analysis. Dilution of the serum sample and filtration were found to be the only required sample pretreatment steps prior to analysis. The method yielded a limit of quantitation at 1–2 μM/L and yielded recoveries between 90–94%.

CHIRAL CAPILLARY ELECTROPHORESIS

Overview of Chiral CE

Pharmaceuticals containing asymmetric carbons that exist as enantiomers are challenging to analyze as the stereoisomers are physically and chemically identical. CE is a powerful analytical tool for chiral separation of pharmaceuticals[101] due to its high resolving power, low consumption of sample and solvents, and flexibility with regard to using and changing chiral selectors. Addition of a chiral selector to the BGE is the most popular technique for CE chiral separations. CE chiral selectors have been reviewed.[102] General aspects of enantiomer migration order in chiral CE have been reviewed.[103] Approaches for coupling CE and electrospray ionization-mass spectrometry also have been reviewed by Shamsi.[104]

Chiral CE Pharmaceutical Applications

Selected chiral CE pharmaceutical analysis applications and detailed method descriptions are given in Table 5. Conclusions and advantages of some of the applications for selected chiral selectors given in the table are discussed. MCE in the chiral CE mode also has been utilized for enantioseparation and the applications are given in the text.

Table 5. Chiral CE applications and detailed method conditions for enantiomeric separation of pharmaceuticals; (b) Chiral CE applications and detailed method conditions for enantiomeric separation of pharmaceuticals; (c) Chiral CE applications and detailed method conditions for enantiomeric separation of pharmaceuticals

Analyte	Sample matrix	Capillary column	BGEoa and chiral selector	Detection	Precision (% RSD)	LOD[b]	Reference
(a)							
Itraconazole, Hydroxyitraconazole	Plasma, serum	Uncoated fused silica	0.95% w/v sulfated-β-CD, 66% v/v 40 mM phosphate (aq) pH 2.0, 34% v/v methanol, 4.0% w/v PEG[c] 4000	UV 214 nm	9.3%	5 μg/mL	[108]
Fenoprofen, Flurbiprofen, Ibuprofen, Ketoprofen	API	PVA coated silica	4 mM HS-β-CD[e], 18 mM PMMA-β-CD[f] in 100 mM phosphate/triethanolamine, pH 2.5	UV 214 nm	n.r.[g]	n.r.	[111]
Deprenyl metabolites	Urine	Uncoated fused silica	2 mM CM-β-CD[h], 0.5% HPMC[i], 4 mM DM-β-CD[j] in 20 mM Tris[k]-phosphate (aq), pH 2.7	UV 200 nm	0.7–3.0%	0.2–0.5 μM	[112]
Salbutamol	Urine	Uncoated fused silica	NACE: 10 mM ammonium formate, 15 mM HDAS-β-CD[l] in methanol acidified with 0.75 M formic acid	UV 230 nm	2.6–7.7%	125 ng/mL	[113]
Levetiracetam	API	Uncoated fused silica	MEEKC: 1.0% w/v SDS, 0.5% v/v ethyl acetate, 1.2% v/v 1-butanol, 1.5% s-β-CD[m] in 100 mM phosphate	UV 215 nm	n.r.	n.r.	[114]

Ketoprofen, Dansyl-aminobutyric acid, Dansyl-norleucine, Dansyl-valine, Dansyl-methionine	API or raw materials	HDB[n] dynamically coated fused silica	2 mM vancomycin, 50 mM Tris-phosphate (aq), pH 6.2, 0.001% HDB	UV 214 nm	n.r.	n.r.	[116]
Aromatic amino acids	API or raw materials	Uncoated fused silica	5 mM (+)-18C6H$_4$,or (−)-18C6H$_4$, 20 mM Tris-citric acid (aq), pH 2.50	UV 200 and 210 nm	n.r.	n.r.	[117]
Propranolol	Tablets, injectables	Uncoated fused silica	ACE: 67 mM phosphate buffer, pH 7.4, 100 μM human serum albumin injected prior to sample solutions	UV 220 nm	≤13%	4 mg/L	[118]
(b)							
Talinolol	Plasma	PVA[d] coated fused silica	3 mM HDAS-β-CD[l] in 100 mM phosphate (aq), pH 3.5	UV 200 nm	1.9%	14.6 ng/mL	[119]
Baclofen	API	PEO[p] dynamically coated fused silica	3% w/v HS-β-CD[e] in 25 mM phosphate (aq), pH 2.5	UV 196 nm, 200 nm	3%	0.13–0.18 μg/mL	[120]
DT4 anti-HIV nucleosides	API	PEO[p] dynamically coated fused silica	4% w/v HS-β-CD[e], 25 mM phosphate (aq) pH 2.5 with TEA	UV 200 nm	2.2%	1.5–1.9 mg/L	[121]
Citalopram	Tablet	Uncoated fused silica	0.04% β-CD sulfate, 25 mM citrate (aq), pH 5.5	UV 205 nm	1.7 – 4.5%	0.15 μg/mL	[122]
Ibuprofen, ketoprofen, fluribiprofen	API	Uncoated fused silica or HDB[n] dynamically coated fused silica	Normal phase separation: 75 mM TM-β-CD[o], 100 mM MES (aq), pH 6.0; Reverse phase separation: 30 mM TM-β-CD[o], 1.0% CM-β-CD[h], 0.01% HDB[n], 100 mM phosphate (aq), pH 3.0	UV 200 nm	<2%	0.5% minor detected	[123]

(*continued*)

Table 5. Continued

Analyte	Sample matrix	Capillary column	BGEoa and chiral selector	Detection	Precision (% RSD)	LOD[b]	Reference
Ibuprofen, Ketoprofen, Fluribiprofen	Serum	Uncoated fused silica	0.05 M TM- β-CD[o] in 0.02 M triethanolamine-phosphate buffer (aq), pH 5.0	UV 253 nm	0.7–14.4%	0.5–0.25 μg/mL	[124]
Melagatran, Ximelgatran	API	Uncoated fused silica	30 mM DM-β-CD[j] in 80:20 200 mM NaH_2PO_4 (pH 1.8): MeOH	UV 200 nm	6.6–6.9%	0.013–0.015 w/w%	[125]
(c)							
E-6006 thienylpyrazolylethanamine antidepressant	API	Uncoated fused silica capillary	10 mM HS-β-CD[e], 25 mM sodium phosphate (aq), pH 3	UV 225 nm	$\leq$3.3%	0.7 μg/mL	[126]
Salbutamol	Tablets, syrup, oral solution	Uncoated fused silica	13.1 mg/mL CM-β-CD[h] in 25 mM acetate (aq), pH 5	UV 230 nm	$<$6.5%	4 μg/mL	[127]
Organic disulfates	API	Uncoated fused silica	0.5 mM QA-β-CD[q], 10 mM glycine (aq), pH 2.4	UV 200 nm	$<$0.5%	0.3 ng/μL	[128]
Omeprazole	Tablets	Uncoated fused silica capillary	3% HS-β-CD[e], 20 mM phosphate (aq), pH 4	UV 202 nm	1.7%	1317 ng/mL	[129]
Citalopram, Desmethylcitalopram	Plasma	PVA dynamically coated fused silica	1% HS-β-CD[e], 25 mM phosphate (aq), pH 2.5, 12% acetonitrile, 0.1% PVA	UV 200 nm	$<$14.5%	1.4–3.4 ng/mL	[130]
3,5-dinitrobenzoyl leucine	API or raw material	Uncoated fused silica	NACE: 4 mM *O*-(*tert*-butyl carbamoyl)quinine,100 mM octanoic acid, 22 mM ammonium hydroxide, in 60:40 ethanol:methanol	On-line FTIR	n.r.	n.r.	[131]

[a]BGE = background electrolyte.
[b]LOD = limit of detection.
[c]PEG = polyethylene glycol.
[d]PVA = polyvinyl alcohol.
[e]HS-β-CD = highly sulfated β-cyclodextrin.
[f]PMMA-β-CD = permethyl-6-monoamino-6-monodeoxy-β-cyclodextrin.
[g]n.r. = not reported.
[h]CM-β-CD = carboxymethyl-β-cyclodextrin.
[i]HPMC = hydroxypropylmethylcellulose.
[j]DM-β-CD = Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin.
[k]Tris = tris(hydroxylmethyl)amino methane.
[l]HDAS-β-CD = Heptakis-(2,3-di-O-acetyl-6-O-sulfo)-β-cyclodextrin.
[m]s-β-CD = sulfated sodium salt β-cyclodextrin.
[n]HDB = Hexadimethrine bromide.
[o]TM-β-CD = Heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin.
[p]PEO = polyethylene oxide.
[q]QA-β-CD = quaternary ammonium-β-cyclodextrin.

Chiral Selector used for Enantioseparation

Cyclodextrins

Cyclodextrins (CDs) are chiral selectors most commonly used in CE chiral separations.[105] CDs are oligosaccharides that are made up of several D(+)-glucopyranose units. The advantages of CDs are that they have low UV absorbance and solubility in water.[106] CDs typically used in CE chiral separations are those containing six ($\propto$-CD), seven (β-CD), and eight (γ-CD) glucopyranose units. The outside of CD is hydrophilic which allows dissolution in the aqueous BGE. The cavity of the CD is lipophilic and is responsible for inclusion complexation. Enantiomeric separation is based on the formation of inclusion complexes between the CD and chiral analyte. The type and concentration of CD chosen are critical for achieving enantiomeric separation.

The most useful CD for chiral CE analysis of pharmaceuticals has been β-CD due to its size and properties.[105] The solubility of β-CD in water is 16 mM.[107] Thus, the low solubility of β-CD in aqueous solution limits method optimization by increasing the concentration of the CD in the BGE. However, CDs have been derivatized which give higher solubilities in water and provide diverse enantioselectivity.[106] The CD derivatives commonly used are either charged or uncharged CDs. Single isomer charged CDs also have been useful chiral selectors. One of the most effective charged chiral reagents has been highly sulfated β-CD (HS-β-CD) which is negatively charged. It contains 7–11 sulfate groups per CD molecule and is used frequently as the chiral selector in pharmaceutical chiral CE separations.[108]

Dual CD systems have been used in the analysis of chiral pharmaceuticals[105] to improve enantiomeric resolution. Mixtures of CDs are generally used when several related analytes and enantiomers are to be separated and analyzed simultaneously. Dual CD systems, consisting of a charged and a neutral CD, have been shown to improve enantioseparations compared with the use of a single CD in solution.[109] A combination of a charged and a neutral CD is especially effective in chiral separation of neutral and hydrophobic analytes.[110]

Abushoffa et al.[111] developed CE enantiomeric separation methods for nonsteroidal anti-inflammatory drugs using a dual CD system with a mixture of charged CDs. The combination of the single-isomer cationic CD, permethyl-6-monoamino-6-monodeoxy-β-CD (PMMA-β-CD) and the single isomer polyanionic CD, heptakis-6-sulfato-β-CD (HS-β-CD) were used for the enantioseparations of profens at pH 2.5. The two CD derivatives were found to have an opposite effect on the analyte mobility. The opposite affinity patterns were found to be favorable for enhancement of selectivity and resolution. Affinity constants for the two CD derivatives with the enantiomers were determined using a linear regression approach. The binding

constants made it possible to predict conditions that would provide high selectivity and resolution using developed mathematical models.

Improvements in selectivity and enantioseparation using a dual CD system was also found by Tabi et al.[112] for the simultaneous enantiomeric separation of deprenyl and its eight metabolites. Use of carboxymethyl-β-CD (CM-β-CD) with heptakis-(2,6-di-*O*-methyl)-β-CD (DM-β-CD) in the BGE allowed simultaneous separation of all of the enantiomers, the electropherogram is given in Figure 5.

The use of NACE for chiral separation of pharmaceuticals is a promising alternative for chiral selectors that have limited solubility or stability in aqueous solution and do not exhibit desirable enantioselectivity in water-based background electrolytes due to strong hydrophobic interaction with the analyte. NACE was used for determination of salbutamol enantiomers in biological samples using heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD) chiral selector and methanol containing buffers.[113] The biological samples used a solid phase extraction clean-up step prior to analysis. The method could be directly applied to CE-MS since the BGE contains volatile salts and the CD used migrates in a direction opposite to the detector.

Microemulsion electrokinetic chromatography (MEEKC) has been used for chiral CE separations. A CD-MEEKC chiral method was developed for the separation of *S*- and *R*-levetiracetam.[114] Variations in the concentration, surfactant, oil, pH, and BGE were varied in the method development. A dual sodium dodecylsulfate (SDS) randomly sulfated β-CD pseudostationary phase with aqueous phosphate buffer was found to provide the separation of enantiomers. CD-MEEKC provided another option for enantioseparation of the hydrophobic compounds. The CE-based method for chiral analysis also provided a lower cost method and more rapid chiral analysis than the previously used HPLC method.

Fast chiral separations using a variety of basic and acidic pharmaceuticals were developed using MCE with microfluidic quartz chips, a linear imaging UV-detector and HS-β-CD chiral selector,[115] Baseline separation of the enantiomers of 19 pharmaceuticals was achieved in <1 minute. The results demonstrate that MCE has a high potential for chiral high throughput screening.

Macrocylic Antibiotics

Macrocyclic antibiotics are glycopeptides or cyclic peptides which have been used as chiral selectors. These selectors include vancomycin, ristocetin A, and teicoplanin. The enantiorecognition mechanism is based on a combination of inclusion complexation and affinity interaction.[102] Vancomycin is an advantageous macrocyclic antibiotic due to solubility in aqueous buffer, high enantiomeric selectivity, and commercial availability.[116]

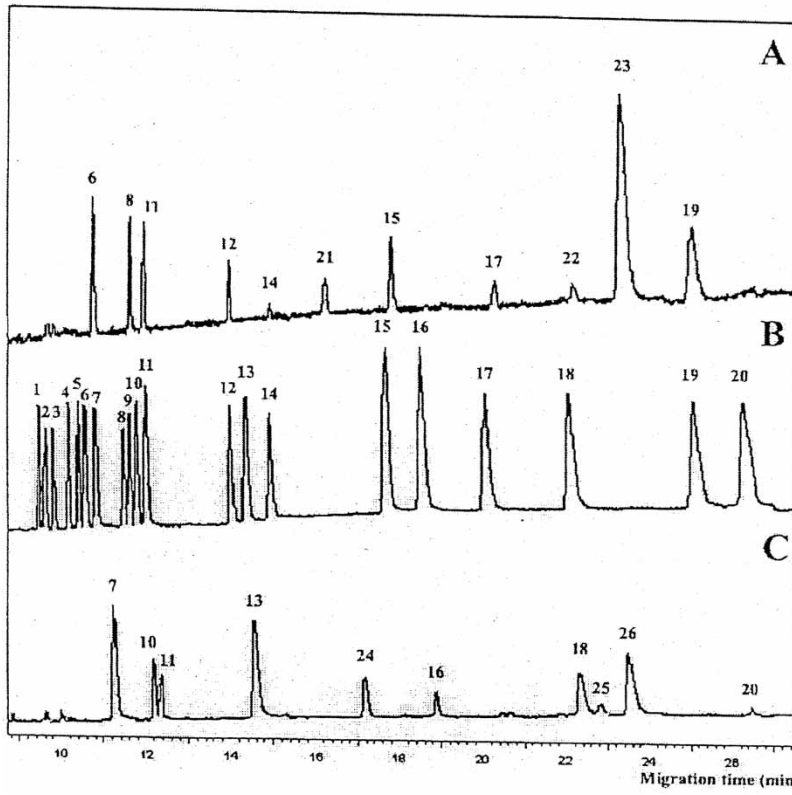

Figure 5. Electropherograms of (B) blank rat urine sample spiked with standards, (A) the urine sample of rats treated with *R*-(−)deprenyl, or (C) *S*-(+)-deprenyl. Separation conditions given in Table 5. Peaks: 1, 1*S*,2*R*-(+)-norephedrine; 2, 1*R*,2*S*-(−)-norephedrine; 3, 1*R*,2*R*-(−)-pseudoephedrine; 4, 1*S*,2*R*-(+)-ephedrine; 5, 1*R*,2*S*-(−)-ephedrine; 6, *R*-(−)-amphetamine; 7, *S*-(+)-amphetamine; 8, *R*-(−)-methamphetamine; 9, 1*S*,2*S*-(+)-pseudoephedrine; 10, *S*-(+)-methamphetamine; 11, *S*-(+)-*para*-fluorodesmethyldeprenyl (internal standard); 12, *R*-(−)-deprenyl; 13, *S*-(+)-deprenyl; 14, *R*-(−)-desmethyldeprenyl; 15, *R*-(−)-*para*-hydroxymethamphetamine; 16, *S*-(+)-*para*-hydroxymethamphetamine; 17, 1*R, NR*-(+)-deprenyl-*N*-oxide; 18, 1*S, NS*-(−)-deprenyl-*N*-oxide; 19, 1*R, NS*-(−)-deprenyl-*N*-oxide; 20, 1*S, NR*-(+)-deprenyl-*N*-oxide; 21–26, unknown peaks. (Reprinted from Electrophoresis **2003**, *24*, 2665–2673 with permission).

The disadvantage of macrocyclic antibiotics is that they exhibit significant UV-absorption. This disadvantage is reduced by using a partial filling technique and the countermigration mode.[102] The countermigration mode is a technique where the EOF is suppressed or controlled so that the chiral selector is not carried to the detection window.[102]

Vancomycin was used as a chiral selector by Kang et al.[116] for the separation of the dansyl-∝-amino-*n*-butyric acid enantiomers. A dynamic coating technique, co-electroosmotic flow technique, and the partial-filling technique were employed in the separation. Absorption of vancomycin onto the capillary was minimized by using hexadimethrine bromide in the BGE which formed a dynamic coating on the capillary wall. The absorption of vancomycin was then minimized via electrostatic repulsion between the coating and vancomycin. The co-electroosmotic flow electrophoresis technique created a positively charged capillary wall and reversed EOF which migrated in the same direction as the negatively charged analyte. This increased the analyte's electrophoretic mobility to significantly shorten analysis time. The partial filling technique was used to improve detector sensitivity since absorption interferences from vancomycin were not detected.

Crown Ethers

Crown ethers are also used as selectors in chiral CE. They are macrocyclic polyethers which form stable and selective complexes with primary amines. Inclusion complexation of the protonated amine groups in the cavity of the crown ether and interactions between the carboxylic acid groups and enantiomers occur.[105] An advantage of crown ethers is that they do not interfere with UV-detection.

Optically active (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18C6H$_4$) was used as a chiral selector in the enantioseparations of amino acids and chiral primary amines by Lee et al.[117]. This technique was successful in reversing enantiomer migration order by using (+)-18C6H$_4$ and (−)-18C6H$_4$, two chiral selectors with the opposite chiral recognition ability. It was found that the electromigration order of nine aromatic amino acids was reversed when (−)-18C6H$_4$ was substituted with (+)-18C6H$_4$ in the BGE.

Human Serum Albumin

Affinity capillary electrophoresis (ACE) can also be applied in chiral CE separations. Macromolecules, such as proteins, are employed as chiral selectors. The macromolecules are charged and possess electrophoretic mobility with the charge of the proteins being pH dependent. Examples of such selectors include bovine and human serum albumin (HSA), α_1-acid glycoprotein (AGP), ovomucoid, cellulose, avidin, transferring, pepsin, cellobiohydrolase I, and lysozyme.[105] The mechanism of the chiral recognition is based on the hydrophobic as well as electrostatic interactions.[105]

A simple, fast method for resolution of propanolol enantiomers was developed using affinity chromatography (ACE) with a HSA chiral selector.[118] Enantioresolution of *R*- and *S*-propanolol was achieved in

<5 min. Different affinity of the enantiomers for the HSA chiral selector were found to provide good separation of the enantiomers. The method has the advantages of low cost and high speed of analysis.

CAPILLARY ELECTROCHROMATOGRAPHY

Overview of CEC

CEC is a hybrid separation method that couples the advantages of high column efficiency of CE and selectivity of HPLC.[21] CEC separations are carried out using wall-coated open-tubular (WCOT) capillaries or capillary columns packed with stationary phase. A voltage is applied across the capillary that transports solutes along the capillary toward the detector and a dual separation mechanism occurs by differential partitioning and electrophoretic migration to provide enhanced selectivity.[22] The plug like flow of the EOF reduces flow-related band broadening, so high separation efficiencies can be achieved. Other advantages of CEC include the ability to analyze both neutral and charged analytes, and the sample and solvent requirements are lower than HPLC.[23] Hyphenation of CEC with MS has been recently reviewed.[132,133] Pressure-assisted CEC (pCEC) is a separation mode of CEC where the mobile phase is moved through the column by application of an electric field and pressure at the inlet side of the capillary.[134]

Research in column technology is at the forefront of development in CEC as there is a limited selection of commercial columns currently available. Therefore, the research in column technology is rapidly expanding. CEC column technology reviews have been recently published with papers discussing developments in open-tubular,[135] bare silica,[136] monolithic,[137,138] sol-gel,[139] polymer brush,[140] imprinted stationary phase[141] columns, and microchips.[142,143]

CEC Pharmaceutical Applications

CEC is in early development and currently it is not used for routine analysis in the pharmaceutical industry. Most CEC columns are fabricated in-house, which has precluded routine use of this technique. Also, as with CE, the sensitivity of the technique using on-column UV detection is limited by the path length through the column. CEC method development is also more complicated than CE. High EOF occurs at pH values >7 where silanols are ionized on conventional reverse phase stationary phase packings. However, at pH >7, basic analytes interact with the ionized silanols on the stationary phase which leads to peak tailing.[23] This is a drawback in the analysis of pharmaceuticals since many drugs contain basic nitrogen groups. Despite its

drawbacks, CEC has been used successfully for the analysis of pharmaceuticals in research applications. Selected applications and method descriptions in the analysis of pharmaceuticals are given.

An impurity profile method for ketorolac and its known impurities was developed by Orlandini et al.[144]. A LiChrospher RP18 capillary column, prepared in-house, was rugged with 300 analyses performed. Ammonium formate (aq), pH 3.5: acetonitrile mobile phases provided a suitable EOF for the separation. The peaks were baseline resolved and the analysis completed in 9 minutes. The method was validated and applied to the analysis of tablets.

Ohyama[145] developed a CEC method which allowed simultaneous separation of four barbiturates and three benzodiazepines with a 3-(1,8-naphthalimido)propyl-modified silyl silica gel stationary phase and 45% methanol in 1.0 mM citrate (aq) pH 5.0 mobile phase and at 20 kV. The column used for the separation was prepared in-house and the analysis time was 9 min.

The separation of indapamide, methatone, celiprolol, atenolol, isoprenaline, and timolol pharmaceuticals was achieved using a mixed mode of hydrophilic interaction and strong cation exchange by pCEC.[146] A SCX poly(2-sulfoethyl aspartamide)-silica capillary column fabricated in-house was used with 70:30 acetonitrile:5 mM phosphate buffer (aq), pH 4.5 mobile phases. The peak shapes were improved using the SCX column compared to reversed phase C_{18} columns for basic compounds. The same stationary phase was also applied in a gradient pCEC method[147] for a peptide separation. The SCX strong cation exchanger column and 75:25 acetonitrile:15 mM phosphate (aq), pH 2.5 mobile phases were used in the separation. The retention mechanism of the peptides was also due to a mixed mode of hydrophilic interaction, strong cation exchange, and EOF.

Bicker et al.[148] performed enantioseparations of ephedrine using CEC capillary columns packed in-house with a low molecular weight chiral cation exchanger based on a penicillamine-sulfonic acid derivative as the chiral selector. The non-aqueous mobile phases of 80:20 acetonitrile: methanol containing 50 mM formic acid and 25 mM (*RS*)-2-amino-1-butanol were used for the separation. The method was found to be applicable to other pharmaceuticals such as β-blockers, calcium channel blockers, and local anesthetics. The precision of the peak areas was 6.5–6.8% and the method could detect the minor enantiomers at levels $\leq$0.1% in the method validations.

Applications of chiral CEC analysis of pharmaceuticals has been recently reviewed.[149] It was concluded that CEC enantioseparations are currently in exploratory stages for chiral separations with the emphasis on developments in column fabrication. A fast screening strategy was used for CEC reversed phase enantioseparations.[150] Chiralcel OD-H and Chiralpak AD-H polysaccharide chiral stationary phases were packed into capillary

columns and 30:70 5 mM phosphate (aq), pH 11.5:acetonitrile with 0.15 v/v% hexylamine (aq) mobile phases were used for the enantiomeric separations. The screening strategy was applied to 29 pharmaceuticals and enantioseparation was observed for 25 of them. The strategy gave satisfactory results for both basic and bifunctional compounds, but it was not applicable to acidic compounds.

CONCLUSIONS

CE provides a useful complementary technique to conventional HPLC in the analysis of pharmaceuticals. CE methodology has been successfully utilized for routine use of selected applications in the pharmaceutical industry. It is a versatile method due to the number of modes which can be used with a single instrument and capillary column. Many pharmaceutical analytical laboratories have increased the use of CE methodology due to the advantages of rapid method development, reduced operating cost, and ease of operation.

Review of the current literature has demonstrated that CE has provided a suitable alternative technique in the pharmaceutical industry for the assay and impurity profile of API and drug product, pharmacokinetic profiling, and determination of bioavailability and drug activities in biological samples, however, the technique has not been shown to be sufficiently robust to directly analyze complex in-process samples which contain significant by-products and impurities. The CE disadvantage of insufficient concentration limits using conventional UV detection due to the short optical path length of the capillary column has been surmounted by a variety of approaches including sample pre-concentration, on-column sample stacking techniques, using a bubble cell or extended pathlength capillary, and alternative modes of detection.

CEC is currently in the stages of early development. Routine use of CEC and pCEC methods in the pharmaceutical industry will require significant improvements in column ruggedness and reproducibility. New stationary phases will need to be developed to minimize irreversible adsorption of analytes while at the same time provide significant EOF. The technique is not yet sufficiently robust in terms of column preparation, column stability, reproducibility, and EOF stability, but it is showing promise for the future.

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