Capillary Electrophoresis as a Routine Analytical Tool in Pharmaceutical Analysis

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Co-authored by separation scientists at three pharmaceutical companies, this instalment of “CE Currents” describes how these companies have successfully adopted capillary electrophoresis for routine analysis. The column discusses using capillary electrophoresis to support aspects of early drug discovery testing, to analyse protein-based pharmaceuticals, and to support drug development and routine quality control of marketed pharmaceuticals.

This “CE Currents” column examines the use of capillary electrophoresis (CE) in three important areas of pharmaceutical analysis. The first section deals with using CE to support aspects of early drug discovery testing, and it uses examples from Cardiome Pharma Corp. (Vancouver, British Columbia, Canada). The second section concentrates on using CE to analyse protein-based pharmaceuticals, a fast-growing area, and it incorporates several examples from Genentech Inc. (South San Francisco, California, USA) and other biotechnology companies. The last section provides details about using CE for supporting drug development and routine quality control of marketed pharmaceuticals, and it focuses on methods used within GlaxoSmithKline R&D (Ware, UK).

**Discovery Phase Implementation at Cardiome Pharma**

CE is routinely used early in the discovery process at Cardiome (1). Analysts can assess newly synthesized compounds varying in basicity and hydrophobicity for purity by CE before the release of the compound for pharmacological testing. Compounds are also analysed by high performance liquid chromatography (HPLC). Although a comparison of data from both techniques reveals good agreement in chemical purities, CE’s advantage over HPLC is clearly illustrated by its minimal method development time. Analysing one compound library required the development of several chromatographic conditions; however, a single CE method using a low-pH buffer sufficed for analysing the same collection of compounds. CE has also been used in Cardiome’s laboratory for reaction monitoring, enantiomeric excess determinations and drug stability studies.

One area in which the potential of CE has been explored minimally is bioanalytical chemistry. A few reviews have been published about using CE for the determination of drugs in biological matrices (2), for forensic applications (3) and for therapeutic drug monitoring (4). HPLC is used conventionally to analyse drugs in biological samples, but it often involves long method development times. For example, extensive sample preparation is usually necessary to obtain a chromatogram free of interference from the matrix. Moreover, plasma and tissue samples may contain structurally related metabolites, which would require additional method optimization to attain adequate resolution.

At Cardiome, scientists use pharmacokinetic profiling with CE to measure drug levels in biological tissues. In their hands, CE provides an excellent alternative to HPLC because of its greatly reduced method development time. The high efficiency of CE permits rapid separation of closely related compounds and analyses of drugs in plasma, blood, urine, bile and brain tissue with very little development time and no interference from the matrix.

The simple CE method used at Cardiome can quantify a broad range of compounds and metabolites in various biological matrices for pharmacokinetic screening, bioavailability studies, tissue level determinations, plasma protein binding and metabolism studies. The extraction procedure includes the precipitation of proteins from the biological matrix with trichloroacetic acid (alternatively, acetoniitrile can be used), the addition of sodium hydroxide to attain an alkaline pH, and liquid–liquid extraction with ethyl ether. A capillary zone electrophoresis (CZE) method using 100 mM sodium phosphate buffer (pH 2.5) is used to analyse all matrices and compounds. The extracts are reconstituted in 10 mM sodium phosphate buffer (pH 2.5). Any injection volume variability caused by differences in sample viscosity is corrected by using an internal standard. Analysts perform ultraviolet (UV) or photodiode-array detection, commonly at 200 nm or at a compound’s maximum absorption wavelength.

Higher limits of quantification are often a limitation to the use of CE in plasma determinations. Acceptable limits can be achieved, however, with the use of sample pretreatment (5). Cardiome scientists use standard curves in plasma with concentrations ranging from 75 ng/mL to 10 µg/mL for pharmacokinetic studies. Typically, they achieve 75–200 ng/mL limits of quantification, with precision and accuracy of 15% or lower. The limits of detection can be as low as 25 ng/mL.

Table 1 presents the results of a CE assay of drug analytes in rat plasma. Triplicate quality control samples were prepared with each batch to assess the performance of the method at the high and low ends of the standard curve. The assay showed...
accuracy of less than 6% and 8% for the high- and low-quality control results, respectively, and method precision was 1% and 7% for the high- and low-quality control results, respectively. Figure 1 shows electropherograms of low calibration standards. By using this method, Cardiome analysts can derive pharmacokinetic profiles of drug compounds after intravenous and oral administration to rats. The greater efficiency of CE enables the resolution of drug metabolites in plasma after oral administration of drugs without additional method optimization. Electropherograms of rat plasma extracts after oral administration of drug revealed the presence of several components with migration times (15–18 min) similar to that of the parent drug (16.5 min) (Figure 1). These peaks had UV spectra (photodiode-array detection) that were similar to those of the parent drug, and these results suggest that they are drug metabolites.

CE has been used at Cardiome to assess the extent of glucuronidation as a metabolic pathway for drugs. Because drug glucuronides are more hydrophilic than a parent drug, they normally require a different extraction procedure and analysis conditions. An attractive alternative to using multiple extraction and analysis methods would entail the cleavage of the glucuronides by incubation with β-glucuronidase to release the parent drug. This process would allow analysts to use a single method for extraction and CE analysis. Scientists at Cardiome compared rat bile extracts after oral drug administration, and they detected small amounts of unchanged drug in the bile after oral administration. After treatment with β-glucuronidase, the levels of parent drug increased dramatically, which indicated that glucuronidation is a major metabolic pathway for the elimination of this compound. Subsequent liquid chromatography–tandem mass spectrometry analysis revealed an additional peak with a migration time very similar to that of the parent drug. The peak was determined to be a hydroxylated metabolite excreted as a glucuronide.

Enantiomer separation can be achieved easily by CE with cyclodextrins in the separation buffer. Chiral analysis of plasma extracts can reveal stereoselectivity in the disposition of drugs. Two publications address the use of chiral CE in bioanalysis (1, 6). Electropherograms of plasma extracts were analysed by CE using highly sulfated γ-cyclodextrin (1). The corrected peak area ratio of the two enantiomers in the standard was 1.04. In rat plasma after oral drug administration, this ratio was calculated to be 1.35, which indicates preferential clearance of enantiomer 2 (1). The analysis of drugs in other tissues can also be performed using the CE method. Figure 2 shows representative electropherograms of brain tissue extracts in which the matrix caused no interference. An endogenous peak with an early migration time appeared, but it did not interfere with the analytical window.

Kim and co-workers (7) determined drug levels in urine using CE. They compared rat urine after drug infusion with a spiked standard. They obtained an electropherogram free of interference for the blank urine extract, and it allowed quantification.

CE has become the method of choice for routine plasma sample analysis at Cardiome Pharma. The ability to use the same method for a wide variety of compound structures and matrices is a

### Table 1: Standard Curve Parameters and Quality Control Data of the CE Method for Drug Analysis in Rat Plasma.*

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Deviation (%)</th>
<th>Relative Standard Deviation (%)</th>
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<tr>
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<tr>
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<td>–1</td>
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<td>Average</td>
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* Weighting of $1/x^2$ was used. The standard curve range was 75–10000 ng/mL. The slope was 0.2475 and the y intercept was 0.0019. The $r^2$ value was 0.9999.

**Figure 1:** CE analysis of rat plasma extracts. Shown are (a) standard at 300 ng/mL, (b) standard at 100 ng/mL, (c) standard at 75 ng/mL and (d) blank plasma. Capillary: 60 cm × 75 µm fused silica, 50 cm to detector; buffer: 100 mM sodium phosphate (pH 2.5); separation voltage: 25 kV; cartridge temperature: 20 °C; injection: 1 psi for 10 s; detection: UV absorbance at 200 nm. Peaks: 1 = drug, 2 = internal standard.
major advantage of the CE technique. Method development time is greatly reduced, and operational costs are minimal. In addition, CE's versatility also facilitates method adaptation for applications such as enantiospecific analysis and drug tissue level determination.

CE Methods Used in the Development and Routine Lot Release Testing of Protein Pharmaceuticals

The use of CE in the analysis of recombinant protein pharmaceuticals during product development and routine lot release testing has become a reality. Scientists representing several biotechnology companies presented examples of CE methods used for analysing protein pharmaceuticals at a symposium titled "CE in the Biotechnology Industry: Practical Applications for the Analysis of Peptides and Proteins." This symposium was sponsored by the California Separation Science Society, and the proceedings of the symposium were published in a supplement to the February 2001 issue of Chromatographia (8). These applications, including validated assays requiring regulatory approval, have been implemented in the routine analysis of Synagis (palivizumab), Enbrel (etanercept), human growth hormone from several manufacturers, Infenogen (interferon alfacon-1), and several therapeutic monoclonal antibodies produced by Genentech. This section summarizes some of the presentations to demonstrate the various applications for CE methods. The examples show the unique capabilities of CE and demonstrate that the results obtained by CE can be correlated to conventional methods.

Research from Amgen: Interferon alfacon-1 is a highly potent drug that is formulated at concentrations of approximately 30 mg/mL. At these low concentrations, interferon alfacon-1, as other proteins, is susceptible to non-specific adsorption to surfaces. Therefore, analysts add protein excipients to the formulation to prevent non-specific adsorption of the product to surfaces. Generally, the protein excipient is present at a much higher concentration than that of the product. Quantification of the protein product has been determined routinely by enzyme-linked immunosorbent assay (ELISA) because the protein excipients can interfere with conventional analytical assays such as UV spectrophotometry and HPLC.

Park and co-workers (9) from Amgen Inc. (Thousand Oaks, California, USA) developed a CE method for quantifying interferon alfacon-1 to facilitate development of the formulation. The major challenge was to detect interferon alfacon-1 in the presence of the human serum albumin excipient, which was at a concentration at least two orders of magnitude higher. Three interferon alfacon-1 isoforms were detected by the CE method, and these isoforms were clearly resolved from the human serum albumin peak. Interday precision of the migration times ranged from 2.6% to 3.6% relative standard deviation (RSD) for the three isoforms, and interday precision for total peak area was 7.6%. The sensitivity of the method was adequate to analyse formulations that contained 10–30 mg/mL of interferon alfacon-1.

Park and co-workers (9) used the CE and ELISA methods to determine the recovery of interferon alfacon-1 in various human serum albumin formulations immediately after filling a Mini-Med external pump catheter (Applied Medical Technology Ltd, Cambridge, UK) and incubating the sample in the pump for one day at 37 °C. Results from both methods were comparable, although the CE method showed better precision. The recoveries of interferon alfacon-1 immediately after filling the pump were 95% and 89% by CE and ELISA, respectively, when the formulation contained 0.25% human serum albumin. Higher concentrations of human serum albumin did not result in improved recoveries. After incubation for one day at 37 °C, the recovery of interferon alfacon-1 by either method was approximately 50% at all tested concentrations of human serum albumin. To overcome the adsorption effect observed at 37 °C, Park and colleagues (9) showed that adding 0.01% polysorbate 80 to the formulation resulted in excellent recovery of interferon alfacon-1.

In addition to quantification data, the CE method can also provide information about the qualitative degradation profile of the product; for example, aggregation, cleavage and deamidation. Thus, not only is CE a much simpler method to perform, it provides several advantages over the conventional ELISA method.

Research from Immunex: Jochheim and colleagues (10) from Immunex Corp. (Seattle, Washington, USA) used a capillary isoelectric focusing method to analyse Enbrel (tumour necrosis factor receptor-Fc [TNFR-Fc]), a homodimer of 467 amino acids that comprise the tumour necrosis factor receptor and IgG Fc molecule linked by three interchain disulfide bonds. TNFR-Fc contains oligosaccharide moieties at several sites on the protein backbone, and isoelectric focusing gels of TNFR-Fc show considerable charge heterogeneity. Jochheim and co-workers showed that the capillary isoelectric focusing profile of TNFR-Fc was comparable to the densitometric scan of its isoelectric focusing gel pattern. When analysed under optimized capillary isoelectric focusing conditions, the major TNFR-Fc peak focused near a pI of 4.8 with less than 1.5% RSD for pl determination. Isomorph species with pls that differed by 0.05 pl units were also resolved clearly.

Jochheim and colleagues (10) showed that the capillary isoelectric focusing method could detect changes in TNFR-Fc

![Figure 2: CE analysis of rat brain extracts. Shown are (a) blank rat brain spiked with drug, (b) rat brain after drug infusion and (c) blank rat brain homogenate. CE conditions were the same as in Figure 1. Peaks: 1 = drug, 2 = internal standard.](image-url)
subjected to various conditions that cause a pH change. For example, treatment of TNFR-Fc with sialidase generated a shift in the capillary isoelectric focusing profile towards higher pH values. Similarly, fractions obtained from preparative isoelectric focusing showed that the TNFR-Fc isomers could be separated into several defined regions. They also analysed these preparative fractions by isoelectric focusing gels, which provided comparable results, although the capillary isoelectric focusing method appeared to show more species. These data confirmed that the capillary isoelectric focusing method could detect and differentiate charged isoforms similar to the capabilities of isoelectric focusing gels. Then they analysed TNFR-Fc samples manufactured under various process changes and showed that the capillary isoelectric focusing method could detect differences among these samples. They concluded that the capillary isoelectric focusing method provides a valuable asset for evaluating product quality and consistency, and the method is now used routinely in process development applications at Immunex.

Research from Genentech: Ma and Nashabeh (11) from Genentech discussed several CE applications that are used for the analysis of protein pharmaceuticals. They used CE–sodium dodecyl sulfate (SDS), which is analogous to SDS–polyacrylamide gel electrophoresis (PAGE) separation of proteins, to provide a size-based fingerprint of a given product in terms of related size variants such as aggregates, desired product and fragments when compared with a well-characterized reference material. The fingerprint provided information about product purity and manufacturing consistency. Validated CE–SDS methods are currently used in this manner for lot release and stability testing of several marketed and to-be-marketed Genentech protein products.

In their studies, Ma and Nashabeh (11) emphasized the critical nature of sample preparation, because the traditional method of using elevated temperature to facilitate protein–SDS complexation can result in aggregation and fragmentation. They demonstrated artifacts and variability by using two temperatures (60 and 90 °C) for the complexation of SDS to a recombinant Fab molecule (comprising approximately a heavy and light chain of a disulfide-linked IgG molecule) with a 3–10 min range of incubation times. At 90 °C, the fragmentation of the Fab molecule to free light and heavy chains was approximately 5% at 3 min and grew to 10% at 10 min. At 60 °C, the extent of fragmentation remained relatively level at approximately 3.5% during the incubation period. By extrapolating the two temperature curves to time zero, they determined a true representation of the free fragment contained in the rhuFab to be 3.5%. Heating at 90 °C for 10 min would have resulted in greater than 10% fragmentation and a gross overestimation.

Ma and Nashabeh (11) also noted another difference in the CE–SDS method from SDS–PAGE. The oligosaccharide content of a protein influences migration time, and this property can be used in the analysis of recombinant monoclonal antibodies. Thus Fab, heavy-chain and Fc fragments of a monoclonal antibody are approximately 50 kDa in molecular weight, but they migrate in this aforementioned order in CE–SDS. These fragments are indistinguishable by SDS–PAGE. The anomalous migration in CE–SDS was attributed to the increase in hydrodynamic size as a consequence of glycosylation. Because the Fc fragment has two glycosylation sites, the molecule migrated at the slowest rate.

During formulation development for recombinant proteins, it is essential to determine the formulation that will provide appropriate stability for the protein product. SDS–PAGE has been extensively used for this purpose because it can detect aggregates and aggregates. Ma and Nashabeh (11) described a study that showed that CE–SDS has an advantage over conventional SDS–PAGE with detection by Coomassie or silver staining. In this example, they analysed a monoclonal antibody by labelling with a fluorescent dye, and detection was accomplished using laser-induced fluorescence following CE–SDS separation. When the monoclonal antibody was subjected to elevated temperature in five formulations, the Coomassie-stained gel was insufficiently sensitive to detect aggregates that could be detected by the silver-stained gel. Conversely, the silver-stained gel grossly over-estimated the amount of free light and heavy chains when compared with the results obtained by the Coomassie-stained gel. CE–SDS with laser-induced fluorescence detection, in contrast, could detect aggregates and a level of fragments comparable with Coomassie staining, thus combining the advantages of both SDS–PAGE detection systems. Adding known proteins to the protein product to simulate potential impurities, Ma and Nashabeh used CE–SDS with laser-induced fluorescence detection of fluorescently labelled proteins to show that protein impurities of approximately 45 ppm could be detected. This type of sensitivity is similar to that of SDS–PAGE methods using silver-stain detection. This method was used to monitor the production consistency of several marketed products with respect to size variants, aggregates and fragments, and to monitor host cell proteins.

Ma and Nashabeh (11) described two additional CE methods. In the first, CZE was used as a rapid means of performing an identity test. A single CZE method was used to differentiate between several monoclonal antibodies manufactured at Genentech. In the second, a CE method using 8-aminopyrene-1,3,6-trisulfonic acid derivatization of released oligosaccharides was validated and used for monitoring manufacturing consistency of terminal galactose in monoclonal antibodies.

Research from MedImmune: Schenerman and Bowen (12) of MedImmune Inc. (Gaithersburg, Maryland, USA) described the advantages of CE–SDS (also called capillary gel electrophoresis [CGE]) over SDS–PAGE coupled with densitometric scanning for the analysis of Synagis, a therapeutic monoclonal antibody. The SDS–PAGE method has traditionally been used to determine product purity. CE–SDS provides greater automation capabilities, and the system software is similar to conventional chromatography software. This similarity is an advantage because many methods used in the analytical control system require HPLC methods. The same parameters examined in an HPLC validation can be applied to the CE–SDS method. Schenerman and Bowen provided detailed information and guidance about validating a CGE method for quality control purposes. These authors demonstrated that the method compared favourably with the Coomassie-stain method, which has been used to demonstrate purity of recombinant proteins because of the linearity of its staining. The CE–SDS method is used routinely to analyse Synagis for lot release purposes at Medimmune.

These examples demonstrate that CE has progressed to routine use in protein drug development. However, these examples also show that analysts should use a correlation to an existing method to confirm the observations made by the CE method. After this correlation has been established, the CE method is usually faster and easier to perform, and, in many instances, it provides additional information unavailable from the conventional method.
CE in Routine Pharmaceutical Quality Control Analysis

The quality control analysis of pharmaceuticals is currently performed predominantly using HPLC. However, many pharmaceutical analysis laboratories have an increasing presence of CE instrumentation, so CE offers a real and attractive alternative to HPLC. CE in many instances can have distinct advantages over HPLC in terms of rapid method development, reduced operating costs and increased simplicity. In addition, a single set of CE operating conditions may be appropriate for a wide range of pharmaceuticals leading to very significant efficiency gains (13). The major strength of CE, however, is that the basic separation principles are different from those of HPLC and thin-layer and gas chromatography. Therefore, CE and HPLC used together make a powerful combination.

The scope of CE application in the area of pharmaceutical analysis is identical to that of HPLC, and users must often choose between the two techniques (14). Watzig and co-workers (15) prepared a very comprehensive article — with 840 references — that is useful additional reading. The application range includes determination of related impurities, determination of enantiopurity, main component assay, determination of inorganic ions, trace-level quantification, analysis of dissolution test sample solutions, and raw material testing (16). The performance of an optimized CE method is similar to that of HPLC methods.

Several routine application types are used for CE in the routine quality control environment, and they are individually covered with discussion and selected examples below.

**Determination of drug-related impurities:** As mentioned above, the combined use of CE and HPLC is powerful — this statement is best exemplified in impurity determination. For example, if independent HPLC and CE methods confirm the number and levels of impurities in a test sample, then analysts can view those results with confidence. Data such as these could form a pivotal portion of the validation data of either the CE or the HPLC method. Several publications have cross-correlated the impurity levels present in drug substance and pharmaceutical formulation samples (16, 17). The overriding requirement in impurity determination methods is their ability to quantify impurities present at 0.1% and lower levels. This quantification is possible using commercial CE instrumentation with standard capillaries. The analysis times for selective impurity determinations are similar to those of HPLC and are approximately 10–40 min.

**Assay of main component:** Analysts can obtain acceptable precision and accuracy using optimized and well-controlled methods. Using internal standards is recommended because the volumes injected in CE are strongly related to the viscosity of the sample and calibration solutions. Errors are also minimized by using relatively concentrated samples to generate large, easily integrated peaks.

Often, users can work with a single set of CE operating conditions to analyze a range of different drugs. For example, using a pH 2.5 phosphate buffer is suitable for the analysis of an extremely wide range of basic drugs (13). These drugs can be quantified accurately using internal standards such as imidazole, aminobenzoate and aspartame. Altria (13) determined chloride and sulfate contents of a drug counterion. CE has also been used to monitor levels of inorganic contaminants present in drug substance samples.

**Chiral analysis:** Chiral analysis is an attractive application because CE can have distinct advantages over HPLC in terms of reduced method development time, operating costs and method ruggedness (16). Detection levels lower than 0.1% of the undesired enantiomer are attainable, and these levels are equivalent to the performance of HPLC. Noroski and co-workers (20) successfully validated methods for determining chiral purity within several pharmaceutical companies.

Several selectivity options are available for chiral separations, including the use of cyclodextrins, crown ethers, antibiotics and proteins. Most quantitative applications for pharmaceuticals have required the addition of cyclodextrin to the separation buffer.

**Assay of enantiomer content:** Using indirect UV detection in CE enables the relatively rapid and simple determination of inorganic ions. Analysts can use this ability to quantify the levels of anions such as chloride, sulfate and nitrate or metal ions, such as sodium, potassium or calcium that are present in drug substance samples. This application is important because most drugs are ionic salts and they must be characterized fully. For example, basic drugs are often manufactured as chloride, hydrochloride, sulfate or maleate salts. Similarly, acidic drugs are often manufactured as sodium, potassium or magnesium salts. Altria (16) used CE to quantitatively determine the weight per cent content of a drug counterion. CE has also been used to monitor levels of inorganic contaminants present in drug substance samples.

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Altria (16) determined chloride and sulfate
levels in drug substance samples using an electrolyte that contained chromatate to provide the background UV signal for indirect detection. He added a cationic surfactant, tetradeyltrimethylammoniumbromide, to reverse the electroosmotic flow direction. He prepared calibration solutions using AnaLa® analytical-grade reagents (Merck Eurolab Ltd, Dorset, UK). Atria reported acceptable levels of precision (1–2%RSD), sensitivity (1 mg/L) and cross-validation with HPLC and the theoretical chloride or sulfate content.

Atria (13) used a standard low-pH electrolyte that contained imidazole to quantify sodium or potassium counterions in acidic drugs. The imidazole provided the background signal for indirect UV detection at 214 nm. Atria and colleagues (22) conducted an intercompany collaboration exercise using a formic acid-imidazole electrolyte to quantify the sodium in the cephalosporin sodium bromide, to reverse the electroosmotic flow. They repeated the separations and obtained accurate results confirming the theoretical weight per cent sodium content. The highly complementary nature of the two techniques means that CE and HPLC should be viewed as two kindred techniques rather than opponents. This “CE Currents” column has highlighted that the range of CE applications in pharmaceutical analysis is at least as extensive as that of HPLC. Adopting CE testing provides several distinct advantages, including faster analysis and method development, lower consumable expenses and easier operation. All these factors are important in chiral separations for which CE is often the technique of choice. The disadvantages of CE are not to be forgotten; they include poorer injection precision (hence the need to incorporate internal standards) and the limited number of staff members who are trained and experienced in using CE compared with the number who are competent with HPLC.

The highly complementary nature of the two techniques means that CE and HPLC should be viewed as two kindred techniques rather than opponents. This “CE Currents” column has highlighted that the successful, routine use of CE is common in many pharmaceutical companies. The diversity of applications include the support of early development work, such as bioanalytical studies, analysis of protein-based pharmaceuticals and routine quality control applications of marketed drugs. Many of the applications described in this column are in routine use, and their data have been submitted to regulatory authorities in submission dossiers.

**Conclusions**

The range of CE applications in pharmaceutical analysis is at least as extensive as that of HPLC. Adopting CE testing provides several distinct advantages, including faster analysis and method development, lower consumable expenses and easier operation. All these factors are important in chiral separations for which CE is often the technique of choice. The disadvantages of CE are not to be forgotten; they include poorer injection precision (hence the need to incorporate internal standards) and the limited number of staff members who are trained and experienced in using CE compared with the number who are competent with HPLC.

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**References**


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**Figure 3:** CE detection of 0.1% (R)-propranolol. Buffer: 25 mM Tris–phosphoric acid (pH 3) containing 5 mM CM–B-cyclodextrin and 8 mM DM–B-cyclodextrin; applied voltage: 15 kV (negative polarity); detection wavelength: 214 nm; injection time: 2 s; temperature: 25 °C. Peaks: 1 = (R)-propranolol, 2 = (S)-propranolol. (Reproduced with permission from reference 21.)