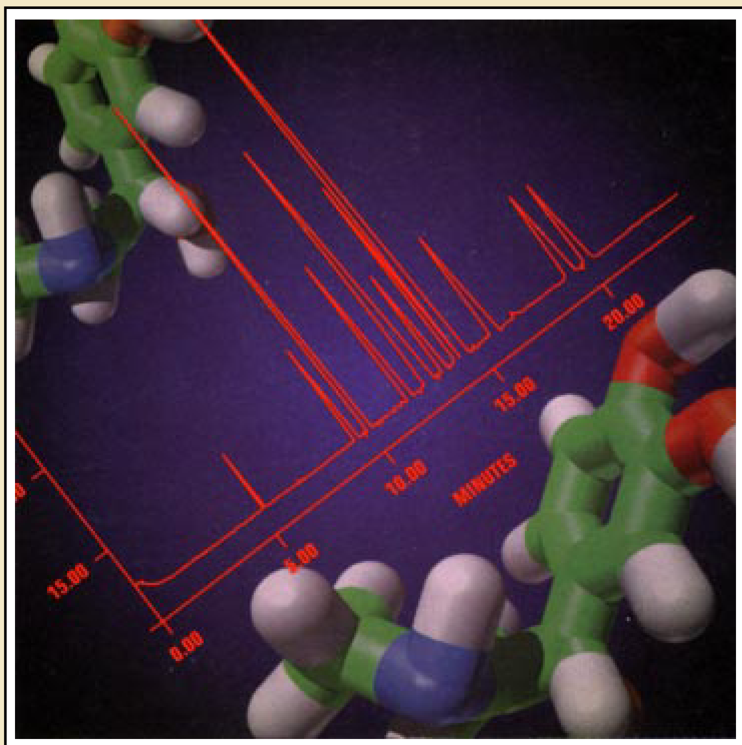
Introduction to Quantitative Applications of Capillary Electrophoresis in Pharmaceutical Analysis



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Introduction to Quantitative Applications of Capillary Electrophoresis in Pharmaceutical Analysis

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About the Authors

Kevin Altria commenced his Ph.D. studies at the University of London in 1986 studying various aspects of capillary electrophoresis. Highlights of his research include fundamental studies concerning electroosmotic flow, the first report of pharmaceutical analysis by CE, and the development of a novel radioactivity detector. Kevin has since joined Glaxo Research and Development and is currently concentrating on developing applications of CE for pharmaceutical analysis. He has authored or co-authored more than 40 CE-related publications and has edited a book on CE methodology and applications which is currently in press with Humana Press.

Manus Rogan graduated from Dublin City University with a B.Sc. in Analytical Science in 1989. Since then, he has been working as an Analytical Chemist at Glaxo Research and Development. Manus is near completion of part-time Ph.D. studies concerning use of CE for pharmaceutical analysis at the University of York. He has been a co-author on over 10 papers and chapters on CE and has a particular interest in the theoretical aspects of chiral CE.

The authors have previously written a primer on the theory and applications of chiral capillary electrophoresis.

Acknowledgment

Thanks are extended to Mrs. Larraine Horwood of Glaxo Research and Development for careful typing and continued patience.

Front Cover

Separation of 11 basic drugs by CE using a low-pH electrolyte with UV detection at 200 nm (Altria, 1993f).

Other Beckman primers (Volumes I, II, III, IV, and V) on capillary electrophoresis:

Title	**Beckman Part Number**
Introduction to Capillary Electrophoresis	360643
Introduction to Capillary Electrophoresis of Proteins and Peptides	266923
Micellar Electrokinetic Chromatography	266924
Introduction to the Theory and Applications of Chiral Capillary Electrophoresis	726388
Separation of Proteins and Peptides by Callipary Electrophoresis: Application to Analytical Biothechnology	727484

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Acronyms Used

The following acronyms are used in this book.

CE	capillary electrophoresis
CIA	capillary ion analysis
DAD	diode array detector
EDTA	ethylene diamine tetra acetic acid
EOF	electroosmotic flow
FSCE	free-solution capillary electrophoresis
GC	gas chromatography
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
IEC	ion-exchange chromatography
LIF	laser-induced fluorescence
LOD	limit of detection
LOQ	limit of quantitation
MECC	micellar electrokinetic capillary chromatography
MS	mass spectroscopy
ppb	parts per billion
ppm	parts per million (mg/L)
RSD	relative standard deviation
r^2	correlation coefficient
SDS	sodium dodecyl sulphate
TLC	thin-layer chromatography

1 Introduction

Capillary electrophoresis was popularized in the early 1980's by Jorgenson and co-workers (Jorgenson and Lukacs, 1983) who demonstrated that exceptional efficiencies could be obtained by performing electrophoresis in capillaries. Their early work on the electrophoretic separation of biomolecules spurred development of commercial instrumentation and the investigation of application areas where use of conventional electrophoresis was infrequent. One such area was that of pharmaceutical analysis where few applications of electrophoresis were in routine use due to the cumbersome and semi-quantitative nature of this technique. However, the introduction of the capillary format enables full quantitative and automated analysis to be conducted.

To ensure the safety and efficacy of the final marketed product, it is important to characterize drug substance material and formulations. Full characterization involves the assessment of the drug material by both physical and chemical methods. The measurement of chemical properties such as purity, assay, chiral purity, inorganic ion content, and identity confirmation is routinely performed by HPLC and other chromatographic techniques. Recently, capillary electrophoresis (CE) has been developed to perform these tests and shown to be a complementary and attractive alternative to the more established methods.

The majority of drugs are either acidic and/or basic water-soluble compounds. The basis for separation in free solution CE (FSCE) relies upon an exploitation of differences between the analytes' electrophoretic mobilities, which are related to the solutes' charge and size. Consequently, the separation of many drugs is possible by FSCE. For example, an acidic drug may be analyzed in its anionic form at high pH and basic drugs may be tested at low pH in their cationic form. The front cover depicts the efficient separation of 11 basic drugs using a low-pH electrolyte. Zwitterionic drugs (those containing both acidic and basic groups) may be analyzed at either end of the pH range. A mixture of neutral drugs would be unresolved by FSCE. However, ionic, charged micelles can be incorporated into the electrolyte solution to add a partitioning element to the separation. This is the basic idea behind MECC (Terabe *et al.,* 1984).

An important aspect of drug analysis involves the determination of drug-related impurities. This is generally performed by HPLC which has an established methodology and highly automated instrumentation available. It is important to ensure that a thorough and accurate impurity profile is generated.

This can be supplemented by cross-correlation of results obtained with those from TLC or an alternative HPLC method. In all cases, the selectivity relies upon a chromatographic interaction where co-elution or irreversible adsorption may occur. CE offers a completely different selectivity process and is, therefore, truly a complementary and orthogonal technique to HPLC.

Given that an analytical method may be developed for long-term use and thus may be applied to several thousand samples, speed, simplicity, and automation are key requirements. Both CE and HPLC can offer these facilities for many applications. Since the first application of CE to drug analysis in 1987 (Altria and Simpson, 1987; Fujiwara and Honda, 1987), there have been over 150 papers on this subject in the literature. This primer will cover aspects of the validation, application, and performance of CE methods in the analysis of pharmaceuticals as reported in the literature.

A basic introduction to the theory and various CE separation modes of CE is covered in "Introduction to Capillary Electrophoresis." Further primers give detailed discussion on MECC and chiral separations. Review articles of pharmaceutical analysis (Altria 1993a; Rabel and Stobaugh, 1993) and chiral separations (Kuhn and Hoffstetter-Kuhn, 1992; Rogan *et al.,* 1994) have recently been published.

2 Determination of Drug-Related Impurities

2.1 Overview

The determination of drug-related impurities is currently the principal role of CE within pharmaceutical analysis and presents a challenge to both selectivity and sensitivity. The main component and structurally related impurities have similar chemical properties and thus make resolution difficult. However, an advantage of CE over its chromatographic counterparts is that high separation efficiencies are achievable. The resulting peak sharpness often translates a small degree of selectivity to acceptable resolution. A detection limit of 0.1% area/area is widely accepted as a minimum requirement for a related impurities determination method. This 0.1% level is possible by CE. For example, Figure 1 (Swartz, 1991) shows determination of salicylamide-related impurities at 0.1% area/area and below. HPLC can routinely decrease this level by up to an order of magnitude (≈ 0.01%). Owing to the possibility that a formulation or

drug substance may contain a great number of low-level impurities, a method capable of resolving the required peaks within a defined analysis time is needed. The high separation efficiencies offered by CE means that this is now a real possibility.

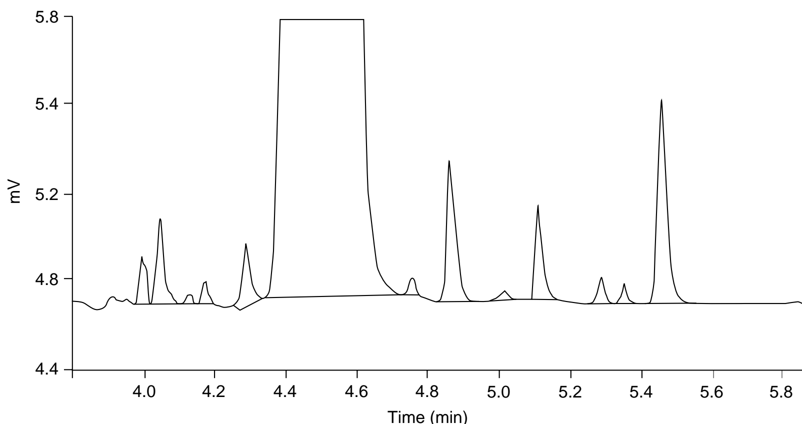

Figure 1. *Salicylamide CE impurity profile. Electrolyte: 0.02 M sodium phosphate, pH 11.0, 0.075 M SDS; capillary: 60 × 50 μm; voltage 20 kV; sample concentration: 0.1 mg/mL in water; injection: 10 s. Reproduced with permission from Swartz, 1991.*

When calculating impurity levels, it is necessary to divide the observed area of each peak by its migration time (Altria, 1993b). This normalization is necessary since faster-migrating peaks move through the detector at a greater speed than their slower counterparts. Therefore, faster-moving peaks have smaller peak widths and correspondingly smaller peak areas. The sum of these "normalized peak areas" is used to calculate impurities as % area/area. When impurity determinations are to be expressed as % w/w through the use of external standards, this normalization process is not required, providing the precision of migration time is acceptable.

2.2 Benefits of CE Methods for Related Impurity Determinations

When adopting a CE method for determination of drug related impurities, possible features include complementary data to HPLC, low wavelength detec-

tion, speed, and simplicity. Other facilities include the ability to carry out on-line spiking experiments and the use of UV diode array detectors.

2.2.1 Complementary Data to HPLC

The data generated on impurity profiling is often compared to that obtained by chromatographic methods (typically HPLC). The principles of separation in CE are entirely different to HPLC and, therefore, a good agreement between the two techniques strongly supports the integrity of the data. This technique combination is now established in many laboratories and has become a suitable replacement for the conventional use of TLC and HPLC in combination. Apart from routine investigations, this combined use is of particular importance during method validation.

The differences in selectivity between CE and HPLC can result in discrepancies in results with one technique showing an underestimation in impurity levels. This occurrence signifies that further method optimization is required. Critical events in the development of a formulation such as synthetic route or process changes or at key drug stability timepoints are times when this may occur. Literature examples include additional tetracycline (Zhang *et al.,* 1992) and domperidone impurities (Pluym *et al.,* 1992) resolved by CE. Figure 2 shows an impurity profile for domperidone in which peaks 1 and 2 co-eluted using both HPLC and TLC.

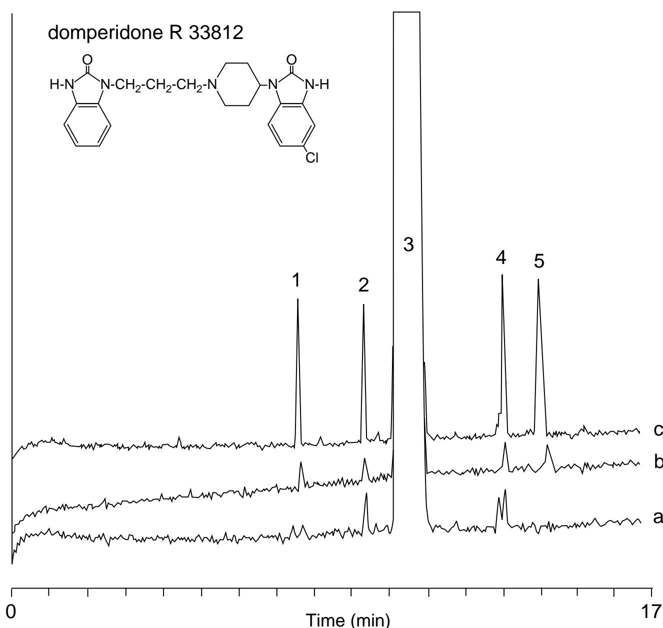

Figure 2. CE separation of Domperidone (R33812) and major known impurities. (a) Batch G1A041; (b) 0.1% reference mixture; (c) 1% reference mixture. Peak numbers: 1 = R29676, 2 = R45771, 3 = domperidone R33812, 4 = R48557, 5 = R52211. Separation conditions: citrate-phosphate, pH 4, +25kV, 30°C, 250 nm, 50 µm × 72 cm. Reproduced with permission from Pluym et al., *1992.*

2.2.2 Low Wavelength Detection

Aqueous-based electrolytes (which have low UV absorbance coefficients) are often employed in CE, allowing detection wavelengths such as 200 nm to be routinely employed. Many impurities or small intermediates have poor chromophores making their quantitation at traditional HPLC wavelengths difficult or impossible. This may be of particular importance for degradative processes where reactions may lead to the loss of the functionality providing the chromophore.

Alternatively, the use of low UV wavelengths may compensate for the inherent poor sensitivity in CE. For example (Altria, 1993c), when operating at

200 nm there is a ten-fold increase in signal for salbutamol and its impurities compared with 276 nm which is the HPLC wavelength.

2.2.3 Speed

Use of short capillaries coupled with high voltages can allow extremely short analysis times to be attained. For example, Figure 3a (Altria, 1993d) shows the separation of fluparoxan impurities within two minutes using a 27-cm capillary. Figure 3b shows the same separation using a 57-cm capillary. Although some degree of resolution is sacrificed, a good indication of the purity of the test substance is rapidly obtained.

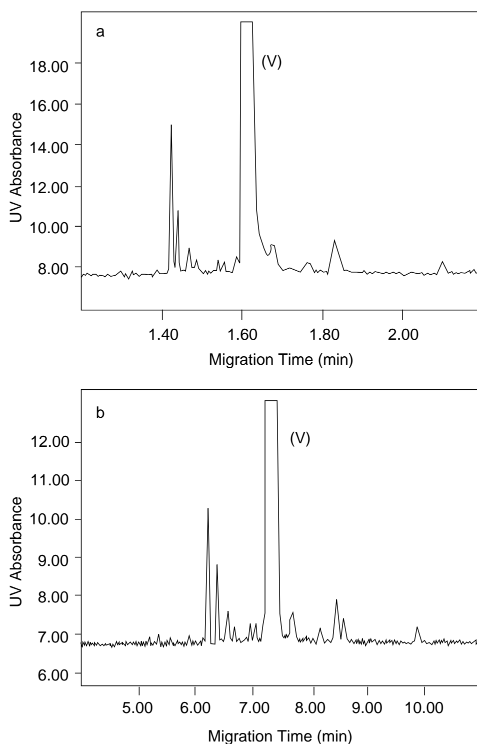

Figure 3. *High-speed separation of fluparoxan and related impurities. Conditions: 50 mM borax, pH 2.2, with conc H_3PO_4, sample concentration 0.5 mg/mL in water, 214 nm, 10 s pressure injection, 10 kV. Peak V = fluparoxan. Figure 3a: 27-cm capillary. Figure 3b: 57-cm capillary. Reproduced with permission from Altria, 1993d.*

2.2.4 Simplicity

The majority of methods employed involve use of aqueous-based electrolytes and uncoated fused-silica capillaries. This ensures that the methods are simple to operate and relatively easy to transfer between laboratories.

2.2.5 On-Line Spiking

For a complex impurity separation, relative migration times are not always sufficient for making a positive identification of a peak. Confirmation of peak identity can be achieved (Altria and Luscombe, 1993) by on-line spiking with a solution of the impurity. This can be programmed into the separation method. Figure 4a shows the separation of a salbutamol sample solution which also contains two dimeric impurities. Figure 4b shows the separation obtained from a 5-second injection of the salbutamol sample solution followed immediately by a 5-second injection of a solution of the impurity of interest. Following both injections, the voltage was applied and the separation given in Figure 4b was produced. The impurity identity is clearly confirmed and no loss in resolution is observed from this dual-injection procedure.

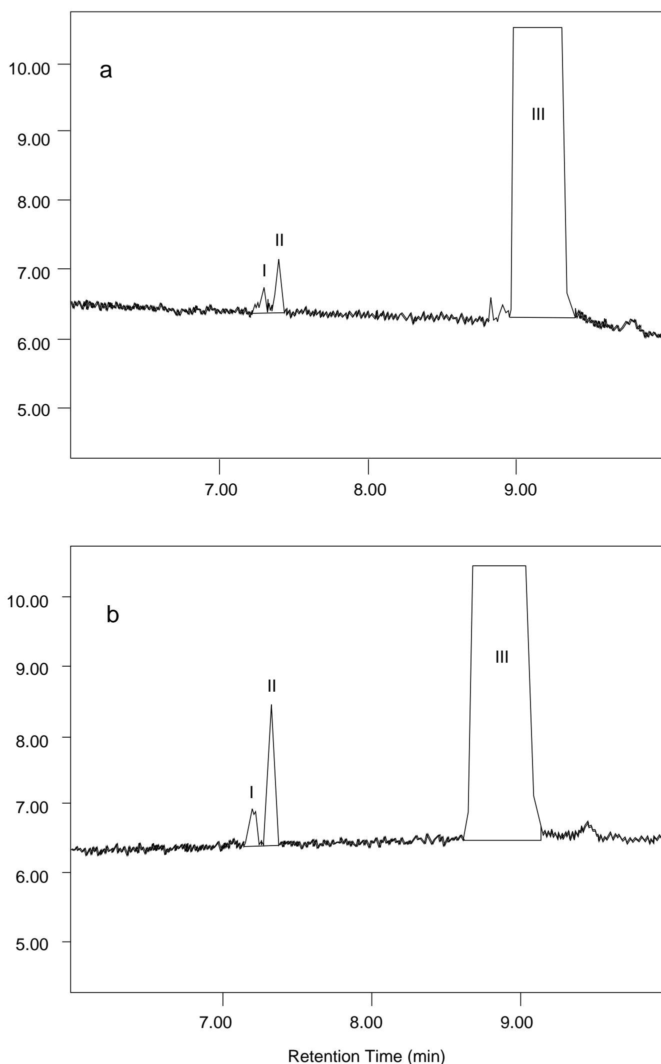

Figure 4. *Determination of salbutamol impurities. (a) CE separation of a 1 mg/mL salbutamol solution; (b) separation of a 1 mg/mL salbutamol solution spiked with 0.5% w/w/ bis-ether by co-injection. Electrolyte: 20 mM Na citrate, pH 2.5; voltage: 30 kV; detector: 200 nm; capillary: 75 cm × 57 µm. Reproduced with permission from Altria and Luscombe, 1993.*

2.2.6 UV Diode Array Detection

The recent advent of UV diode array detector (DAD) technology greatly assists in method development and peak assignment confirmation. Figure 5 shows the use of this detector to measure the spectrum of a 0.1% impurity. In conjunction with the on-line spiking procedure discussed previously, this gives added confidence in peak assignments.

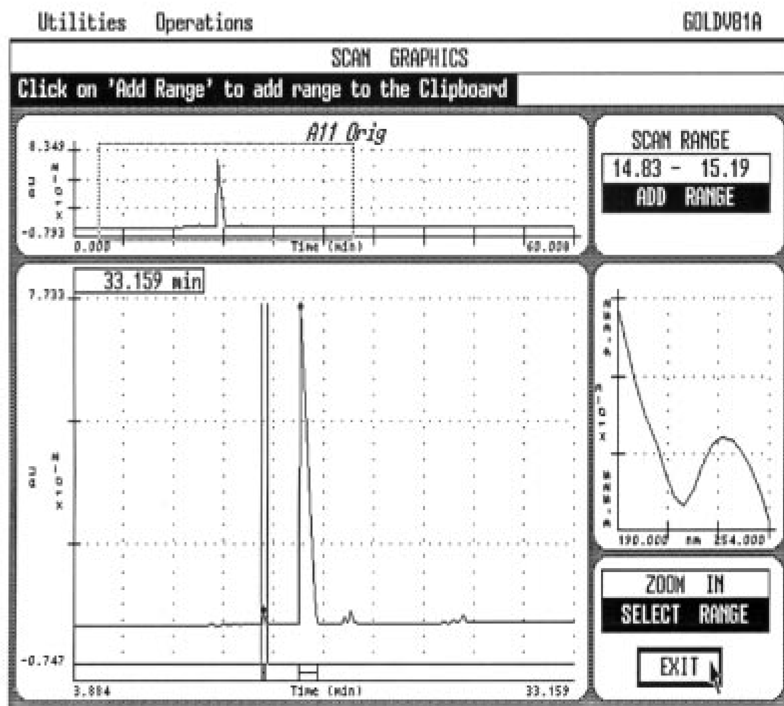

***Figure 5**. Spectra of an 0.1% area/area impurity. Reproduced with permission from Altria, 1994 (unpublished results).*

The principal disadvantages of the use of CE for determining related impurities are the possible requirements for higher sample concentrations. When operating HPLC and CE at the same wavelength, it may be necessary to use two to five times more concentrated samples for CE to obtain an equivalent limit of detection. This may represent problems for poorly soluble drugs.

Due to the small sample volumes employed in CE, the possibility of conducting micropreparative scale separations are very limited. However a number of reports have been published in the literature (Camilleri *et al.,* 1991; Altria and Dave, 1993).

2.3 Applications

A number of applications have been reported, many at the 0.1% area/area detection level. Figure 6 shows the impurity profile of ranitidine, using a two-second and ten-second injection. In this example (Altria, 1993e), a good correlation between CE and HPLC was obtained for the total number and level of impurities. Smaller impurities were quantified at the 0.1% level. Use of a higher sample loading can extend this detection limit even lower. If a short injection time (*i.e.*, one second) can be employed to produce a separation with the main peak on-scale, a longer injection time (*i.e.,* 10 seconds) will produce a separation with the main peak off-scale but with considerably enhanced sensitivity for the minor components. The peak area of the off-scale peak is then calculated by multiplying the peak area of the on-scale injection by the ratio of the injection times (*i.e.,* 10:1). Impurities are quantified as % area/area of the calculated area for the off-scale peak. This is demonstrated (Altria 1993e) in practice for fluparoxan impurities where the LOD for the off-scale separation is 0.01%.

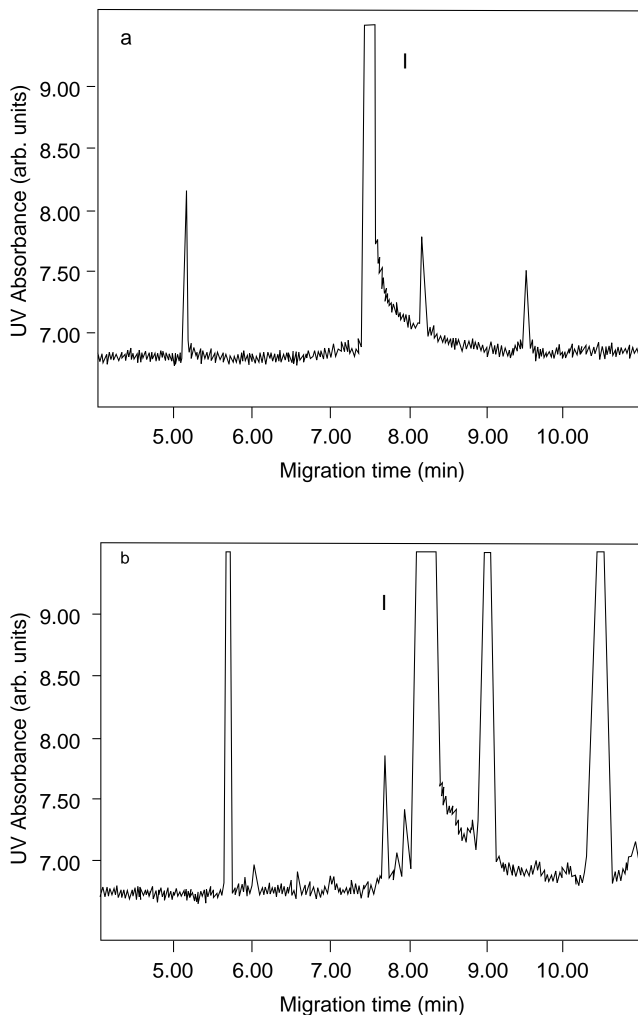

***Figure 6**. (a) 2-s loading of degraded ranitidine solution; (b) 10-s loading. I = ranitidine. Electrolyte: 50 mM borax, pH adjusted to 2.5 with H_3PO_4 and 2 mM hydroxypropyl-β-CD. Reproduced with permission from Altria, 1993e.*

The separation of a basic drug and its impurities at low pH can be achieved by exploiting fundamental differences in the charge and size of the components. If this selectivity is insufficient, incorporation of an additive such

as cyclodextrin (Ng *et al.,* 1992; Altria, 1993d) or an ion-pair reagent (Nishi *et al.,* 1990a) into the electrolyte may achieve the desired effect. Components will selectively interact with these additives, resulting in overall changes in separation selectivity. Figure 7 shows the separation of ranitidine and related impurities employing a low-pH electrolyte containing cyclodextrin. A 27-cm capillary was utilized and, therefore, a rapid analysis was achieved.

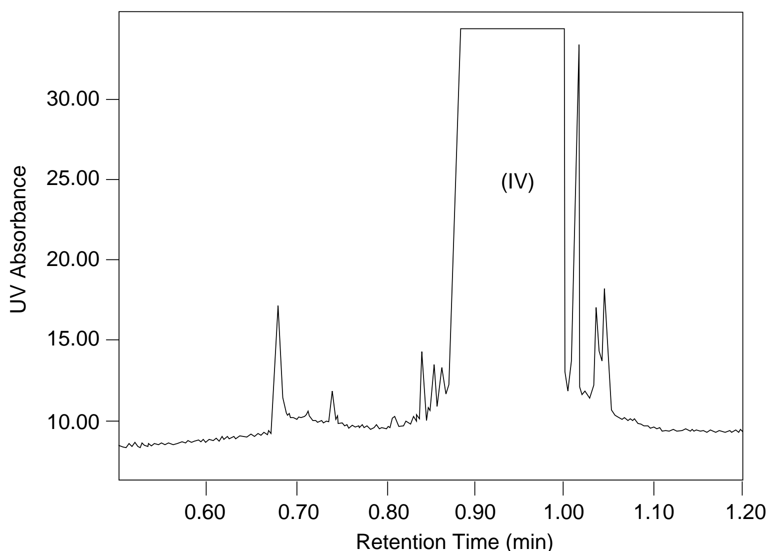

***Figure 7**. HSCE separation of degraded ranitidine syrup sample. Separation conditions: 25 mM borax with 2 mM dimethyl-β-cyclodextrin, pH 2.4, with conc. H_3PO_4; sample concentration, 15 mg/mL; 230 nm; 2-s pressure injection; 15 kV. Peak IV = ranitidine. Reproduced with permission from Altria, 1993d.*

The complementary nature of HPLC and CE was highlighted in a study (Altria, 1993c) of the determination of dimeric salbutamol impurities. These relatively large impurities were strongly adsorbed onto the HPLC column and therefore had lengthy retention times. However, in CE the dimeric impurities had a charge Z = +2 and therefore migrated before the salbutamol (Z = +1). Drug substance batches were tested by CE and HPLC using external standards of the impurities for quantitation. Table 1 shows the good correlation between the two techniques.

Table 1. Levels of Salbutamol Impurities in Drug Substance Determined by CE and HPLC

Good cross-correlation was obtained between the two techniques.

Batch	Bis Ether (%w/w)		Dimer (%w/w)	
	CE	**HPLC**	**CE**	**HPLC**
1	0.14	0.16	0.08	0.08
	0.14	0.16	0.08	0.08
2	0.10	0.11	0.06	0.07
	0.10	0.11	0.07	0.06
3	0.20	0.19	0.13	0.11
	0.20	0.19	0.14	0.10
4	0.12	0.13	0.07	0.06
	0.15	0.14	0.08	0.05
5	0.13	0.14	0.08	0.06
	0.12	0.13	0.07	0.05
6	0.31	0.28	0.18	0.17
	0.31	0.26	0.19	0.15
7	0.07	0.09	0.05	0.04
	0.08	0.10	0.06	0.03
8	0.38	0.38	0.20	0.18
	0.44	0.38	0.22	0.19
9	0.37	0.38	0.19	0.18
	0.37	0.35	0.19	0.19
10	0.75	0.66	0.39	0.33
	0.77	0.67	0.40	0.35

(Altria, 1993c)

A worker from the FDA demonstrated (Flurer and Wolnik, 1994) that higher levels of gentamycin impurities were detected using CE compared with those achieved by the USP-registered HPLC method. Gentamycin has a poor chromophore and, therefore, needs to be derivatized prior to HPLC analysis. However, not all impurities appear to be derivatized to an equal extent. CE allowed the direct analysis of gentamycin and impurities with low UV wavelength detection.

The time-dependent degradation of an impurity of ranitidine was monitored by CE (Altria and Connolly 1993). Sample solution was reinjected several times over the course of 9.25 hours. After this storage, less than 2% of the original substance remained. The initial sample solution was analyzed by both CE and HPLC (8.2 and 8.7% area/area impurity content, respectively).

A stability-indicating method for enalapril has been reported (Qin *et al.,* 1992) which gave detection limits of 0.2% for the monitored impurities.

Impurities have been determined in a water-insoluble quinolone antibiotic (Altria and Chanter, 1993). The compound was only soluble at pH extremes of less than 2 and greater than 10. The sample was dissolved in NaOH solution and analyzed with a pH-1.5 electrolyte. A detection limit of 0.1% was demonstrated during validation. Linearity was measured in two exercises (1 to 150% and 20 to 150% of target concentration); correlation coefficients of 0.9990 and 0.9997 were obtained, respectively. A single sample was injected 10 times and precision values of 0.4 and 0.6% RSD were obtained for migration time and peak area, respectively.

Resolution of acidic or neutral drugs and impurities requires operation at high pH and possibly the addition of a surfactant (SDS). Salicylamide impurities at levels below 0.1% area/area have been shown (Swartz, 1991) using SDS-based separation. Repeated analysis produced an RSD of 9.3% of the area of a peak relating to an impurity spiked at the 0.1% level.

Nishi and Terabe (1990) showed use of MECC to determine impurity levels in dilitazem drug substance. They reported detection limits of 0.1%.

Separation and quantitation of tetracycline impurities were achieved (Zhang *et al.,* 1992) by on-capillary derivatization with EDTA. The anionic complexes were resolved and directly quantified. No performance data was shown.

Levels of domperidone impurities were quantified by TLC, HPLC, and CE in drug substances (Pluym *et al.,* 1992). Table 2 shows the results from HPLC and CE for selected impurities determined in three drug substance batches. Good agreement between the three techniques was obtained for total impurity levels. However, CE resolved two additional components which co-eluted in HPLC and TLC determinations.

Table 2. Levels of Domperidone Impurities in Drug Substance Determined by CE and HPLC

CE resolved an additional unknown impurity.

Batch No.	R45571 Content		R48557 Content		Unknown Impurity	
	CE	HPLC	CE	HPLC	CE	HPLC
1	0.24	0.26	0.15	0.35	0.17	-
2	0.22	0.23	0.15	0.34	0.24	-
3	0.26	0.27	0.15	0.30	0.18	-

(Pluym *et al.,* 1992)

3 Main Component Assay

3.1 Overview

This is an important application area in drug analysis. Several reports have appeared concerning the successful correlation of CE and HPLC assay results indicating that CE produces comparable results. The major drawback to CE being more widely adopted in this area is the perceived lack of precision suggested in early papers (5 to 10% RSD). However, it should be recognized that this data was typically generated on homemade apparatus or using instrumentation which has subsequently been developed considerably further. Existing HPLC instrumentation can routinely achieve <1% RSD. The newly introduced commercial CE equipment can approach this level of precision (Watzig and Dette, 1993).

The major source of imprecision remaining when using commercial instrumentation is injection volume variability. Reproducible nanoliter sample volumes presents a formidable engineering challenge. Use of an internal standard will reduce this effect (Dose and Guiochon, 1991).

Injection precision is also related (Ryder, 1992) to sample concentration, being worse at low sample concentrations. This is due to increased variance contributions from factors such as integration errors and solute adsorption onto

the capillary surface. It is suggested, therefore, that a high sample concentration and injection volume should be employed. This has the effect of reducing separation efficiencies but improving precision. This use of high concentrations also allows the simultaneous determination of related impurities.

The volume of sample solution injected onto the capillary is related to the sample solution viscosity (Watzig and Dette, 1993). This does not represent a problem when analyzing drug substances since equivalent sample weights are dissolved in an identical matrix. Tablet excipients such as cellulose, starch, or cyclodextrins can considerably increase solution viscosity and, therefore, lower injection volumes and potency for tablet assay solutions. This would be highlighted by recovery experiments from tablet excipient mixtures. Employment of an internal standard will alleviate this problem.

3.2 Benefits of CE for Drug Assay

The features of adopting a CE method for main peak assay include generation of data complementary to HPLC, possible reductions in sample preparation, reduced operating costs, and simplicity.

3.2.1 Complementary Data to HPLC

As with related impurities determinations, CE can be used to confirm assay data generated by HPLC. Assay of identical samples by both CE and HPLC can play an important part of method validation or result confirmation for either technique. Several reports (Ackermans *et al.,* 1992a; Altria and Filbey, 1993; Pluym *et al.,* 1992; Tsai *et al.,* 1992) have shown equivalence between assay results obtained by HPLC and CE for a range of formulations. These results are discussed later in section 3.3, “Applications.”

3.2.2 Sample Pretreatment Reductions

Many formulations contain components which are strongly retained and may unduly affect the chromatographic performance of HPLC columns. Therefore it is often necessary to pretreat sample solutions prior to HPLC analysis. Typical procedures include solid phase extraction, filtration, and centrifugation. Use of guard columns can reduce this necessity.

However, in the CE analysis of a formulation containing a basic drug at low pH, the majority of excipients, being neutral, will not migrate. These excipients will remain at the injection end of the capillary and will be removed

during a rinse step. For example, direct injection of diluted syrup samples was possible, minimizing sample pretreatment (Altria and Rogan, 1990).

Components with poor chromophores are generally not suitable for direct analysis by HPLC without sample derivatization. However, the use of low UV wavelengths or indirect detection in CE can overcome this difficulty. See page 13 for an example involving the antibiotic gentamycin. Several aminoglycoside antibiotics including neomycin, streptomycin, and sisomycin were separated (Ackermans *et al.,* 1992b) by CE (Figure 8).

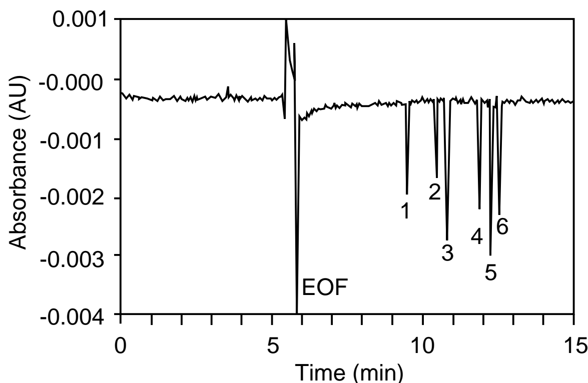

Figure 8. *Electropherogram for the separation of (1) dihydrostreptomycin, (2) lividomycin, (3) amigacin, (4) kanamycin, (5) tobramycin, and (6) sisomycin (all 1.0 mg/mL) in the anionic mode with the reversed EOF applying a background electrolyte of 0.1 M imidazole acetate at pH 5.0 containing the additive FC 135 (50 µl/mL). Capillary length, 67 cm; applied voltage, 12.5 kV; pressure injection time, 2 s; UV detection wavelength, 214 nm .Reproduced with permission from Ackermans* et al., *1992b.*

3.2.3 Operating Costs

In CE, consumable expense is relatively low. Typical methods employ aqueous electrolyte solutions with 10 to 20 mL being a common daily requirement. Costs are minimal when compared to HPLC solvent purchase and disposal. Uncoated capillaries are generally employed which are a fraction of the cost of a HPLC column.

3.2.4 Simplicity

Often a single set of operating parameters can be applied to a wide variety of drugs. Figure 9 shows the separation (Chee and Wan, 1993) of 17 basic drugs at a low pH. An additional 11 basic drugs are resolved (Altria, 1993f) using similar conditions (see front cover for separation).

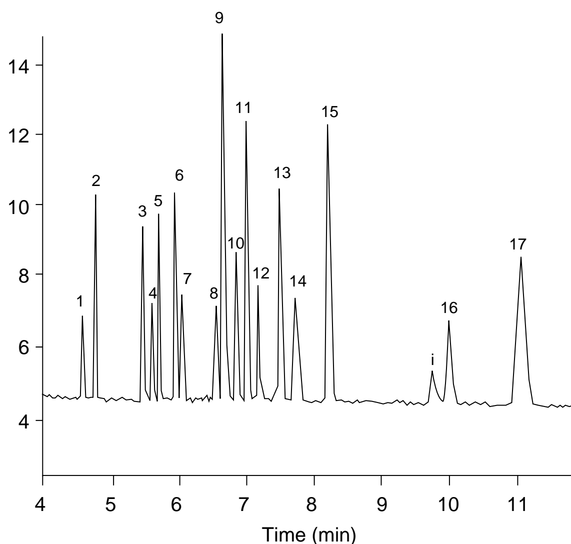

***Figure 9**. Electropherograms of a mixture of 17 basic drugs.*
(1) methapyrilene, (2) brompheniramine, (3) amphetamine, (4) methamphetamine, (5) procaine, (6) tetrahydrozoline, (7) phenmetrazine, (8) butacaine, (9) medazepam, (10) lidocaine, (11) codeine, (12) acepromazine, (13) meclizine, (14) diazepam, (15) doxapram, (16) benzocaine, (17) methaqualone. Separation conditions: 0.05 M sodium dihydrogenphosphate-phosphoric acid, pH 2.35; capillary: 60 cm × 75 µm; injection: 10 s; hydrostatic loading; 22 kV; detection: 214 nm. Peak i is an artifact of benzocaine (peak 16). Reproduced with permission from Chee and Wan, 1993.

Use of an MECC method to quantify seven ingredients of theophylline tablets (and two possible internal standards) has been reported (Dang *et al.,* 1993). Agreement between label claim and MECC results was demonstrated for several components simultaneously.

A pH-7.0 electrolyte was shown to resolve 16 common sulphonamides (Ackermans *et al.,* 1992c). Figure 10 shows the separation with an analysis time of 32 minutes. The method was applied to the testing of sulphonamide levels in pork meat.

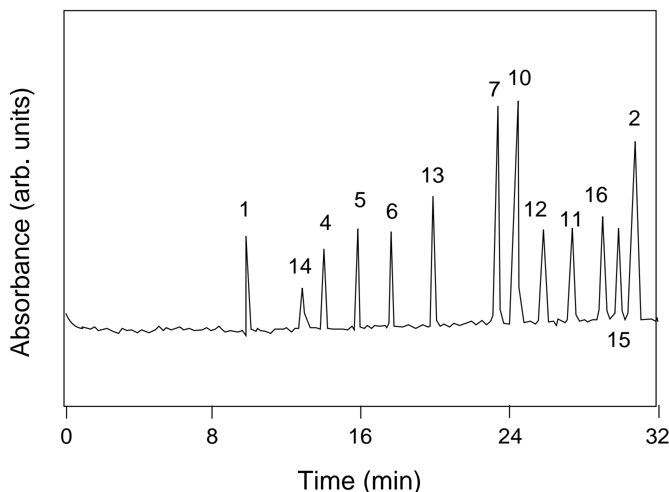

Figure 10. *Electropherogram of the separation of 16 sulphonamides (0.1 mg/mL) in the Beckman standard capillary and detection 50 cm, pressure injection time 2 s (39 nL), applied voltage 10 kV of standard mixture electrolyte 0.02 M imidazole acetate at pH 7. Reproduced with permission from Ackermans* et al., *1992c.*

3.3 Applications

Many reports have appeared and have been reviewed (Rabel and Stobaugh, 1993; Altria, 1993g) on the use of CE for quantitative analysis of drug formulations. CE has been applied to the testing of a wide variety of formulations including tablets, solutions for injection, infusion solutions, syrups, eardrops, creams, and rotacaps. The majority of reports have demonstrated equivalence between CE, HPLC, and/or label claim for the formulation.

Some noteworthy examples include the determination of three bronchodilators (fenoterol, salbutamol and terbutaline) in six different dosage forms (Ackermans, *et al.,* 1992a). Comparisons were made between the CE data, HPLC, isotachophoresis (an alternative electrokinetic separation tech-

nique), and label claim and all results obtained were deemed to be satisfactory. Correlation coefficients of better than 0.999 were reported between HPLC and CE results. Acceptable performance of the CE methods was also reported for linearity ($R = < 0.999$) and precision for migration times and peak areas ranged between 0.3 to 2.2% RSD.

MECC was used (Nishi and Terabe, 1990) to test paracetamol, caffeine and ethenzamide content in tablets from two manufacturers. The results obtained were between 99 to 102% of the label content with precision of 1.0 to 2.3% RSD for peak area and 0.5 to 0.9% RSD for migration time. An internal standard was employed to achieve this precision.

In general, CE is not the first technique of choice when faced with the need to separate non-water-soluble compounds due to problems with on-capillary precipitation. In some cases, MECC conditions can be appropriate. For example, the separation of benzothiazepines and corticosteroids has been reported (Nishi *et al.,* 1990b). Dilitazem content in tablets by MECC was found to be within 99 to 102% of label claim with area precision of 2.2% RSD. In this work, methanolic solutions of the samples (*e.g.,* as a cream formulation) and standards were employed and injected directly on capillary.

Ackermans *et al.* (1992b) quantified various aminogylcoside antibiotics using indirect UV detection. They reported correlation coefficients of < 0.9995 in the range 0.1 to 1.0 mg/mL.

Alendronate levels in formulations were determined by CE. Separation and detection was achieved by virtue of on-capillary complexation with Ca^{2+} ions in the running buffer (Tsai *et al.,* 1992). HPLC, involving a chemical derivatization, was also employed. An average recovery of 100.7% was obtained by CE in the range of 80 to 120% of target concentration. Equivalent assay results were obtained by both techniques. Ten tablets were analyzed by both HPLC and CE to produce average assay results of 2.47 and 2.45 mg/tablet, respectively, with precision data of 0.8 and 0.7% RSD, respectively. However, the sample analysis took 8 minutes using CE, whereas HPLC required 4 hours.

The content uniformity of enalapril-containing tablets has been assessed by a stability-indicating CE method (Qin *et al.,* 1992). Detection limits of $< 0.2\%$ were reported with good precision (0.6% RSD). Detector linearity over the range 50 to 150% of target concentration was shown ($R^2 = 0.999$).

Ackermans *et al.* (1991a) determined levels of water-insoluble dapsone in tablets using methanol as the sample-dissolving solvent and fenbendazole as an internal standard. Average assays of 105.2 mg/tablet were obtained for a 100 mg/tablet (no indication was available of possible tablet content overage values). Correlation coefficients of 0.999 were obtained for detector linearity in the range 10 to 100% of target concentration.

Various analgesics have been determined by MECC (Fujiwara and Honda, 1987). Recoveries of 98.7 to 101.0% were reported from excipients. Precision data for peak area ratios was shown to be 0.8 to 1.8% RSD. Assay of tablets gave precision data for drug content ranging from 0.9 to 1.4% RSD. The results obtained were in good agreement (99.3 to 100.6%) with the label claim for tablet content.

Levels of the anti-migraine agent sumatriptan in injection solutions were determined by CE and HPLC (Altria and Filbey, 1993). An internal standard was employed to provide improved precision. Results generated by the two techniques were in good agreement (Table 3). Detector linearity in the range of 5 to 150% of target concentration was 0.9993. Peak area ratio data was 0.1 to 0.8% and 0.5 to 0.7% RSD for sample and standard solutions, respectively. Inter-day repeatability gave assay data repeatability within 1%. A range of synthetic and degradative impurities was simultaneously separated and detection limits of <0.1% were possible.

Table 3. Sumatriptan Contents in Injection Solutions as Determined by CE and HPLC

Good agreement was obtained between the two techniques.

Sample	**Sumatriptan content (mg/mL)**	
	CE	**HPLC**
Batch 2 Sample 1	11.5, 11.6	11.6, 11.6
Batch 2 Sample 2	11.6, 11.6	11.7, 11.7
Batch 3 Sample 1	11.7, 11.8	11.8, 11.8
Batch 3 Sample 2	11.6, 11.6	11.7, 11.7
Batch 4 Sample 1	11.7, 11.8	11.8, 11.8
Batch 4 Sample 2	11.7, 11.6	11.7, 11.7

(Altria and Filbey 1993)

An inter-company collaboration program has been established to investigate the transfer and application of CE methods. Seven independent pharmaceutical companies are involved in this study. One exercise involved the quantitative analysis of the paracetamol content in a commercial capsule formulation containing both paracetamol and caffeine. Using an internal standard and an SDS-based MECC method, all seven participating companies were able (Altria, 1994a) to repeat the separation. Figure 11 gives three representative electropherograms showing the repeatability of the separation. All companies reported CE assay values equivalent to the HPLC data and the label claim (298.3, 302.5, and 300 mg/capsule, respectively). Relative migration precision data ranged between 0.3 to 0.8% RSD. The average precision data for the calibration response factor was 1.5% RSD, while the assay results gave an RSD of 1.0% for 56 individual determinations.

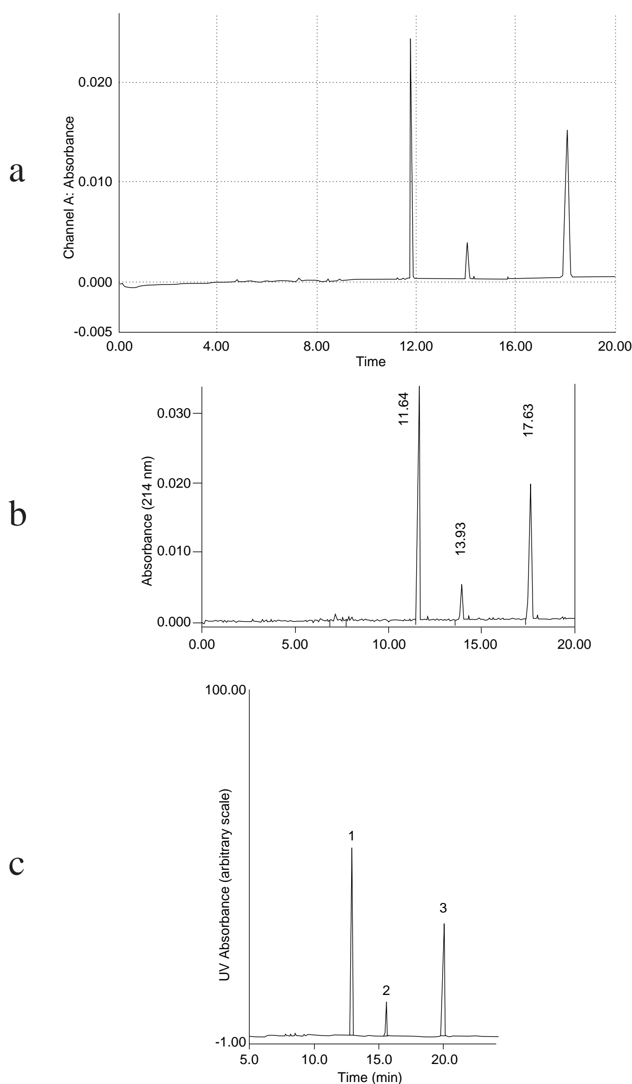

***Figure 11**. Separation of (1) paracetamol, (2) caffeine, and (3) 4-hydroxy-acetophenone (internal standard) by MECC. Instrument-specific settings: (a) ABI HT, (b) Beckman P/ACE, (c) Spectra Physics. Conditions: electrolyte, 40 mM disodium tetraborate, 125 mM sodium lauryl sulphate; detection, 210 or 214 nm; capillary, fused silica, 72 cm × 50 µm (a, c) 57 × 50 µm (b); temperature, 40°C. Reproduced with permission from Altria* et al., *1994b.*

A fully validated quality control method for hydrochlorothiazide and chlorothiazide drug substances has been reported (Thomas *et al.,* 1994). The MECC method also allowed the quantitation of a selection of synthetic impurities at the 0.1% level. Injection precision of < 1% RSD was obtained without an internal standard. Careful control of rinse cycles and buffer replenishment were necessary to achieve this performance. The method was shown to conform to USP validation guidelines. Validation included measurements of precision, repeatability between analysts, capillaries and instruments. Other measurements included robustness testing and linearity of detector response (R^2 = 0.995 to 0.999). Repeated analysis of various drug substance batches by different analysts showed a little variability between assay values.

4 Chiral Separations

4.1 Overview

This is an important application of CE in drug analysis as CE can offer distinct advantages over alternative techniques. Several approaches to achieving chiral separations by CE have been reviewed (Kuhn and Hoffstetter-Kuhn, 1992; Rogan *et al.,* 1994). These include the use of chirally selective cyclodextrins, micelles, proteins, and crown ethers. The separation principles and theory are described in depth in a previous primer.

4.2 Benefits of CE Methods for Chiral Analysis

Chiral pharmaceuticals are generally synthesized as racemates or single enantiomers. Analysis of a single enantiomeric form requires determination of levels of the undesired enantiomer at less than 1% or even 0.1% in certain circumstances. Quantitation at these levels presents a considerable challenge to current techniques since both appropriate sensitivity and selectivity are required. In fact, due to the poor peak efficiency and subsequent quantitative difficulties in this type of HPLC, chiral analysis by CE is one area where CE typically matches, if not outperforms, HPLC.

When compared to HPLC, CE has several attractive features for chiral analysis. These include the potential for speed of method development and analysis, robustness, simplicity, and cost. An example of the type of performance achievable by CE is shown (Altria *et al.,* 1994c) in Figure 12.

The undesired enantiomer of the BCH 189 racemate is detected at 0.3% by CE and the signal-to-noise ratio indicates a possible LOD below 0.1%.

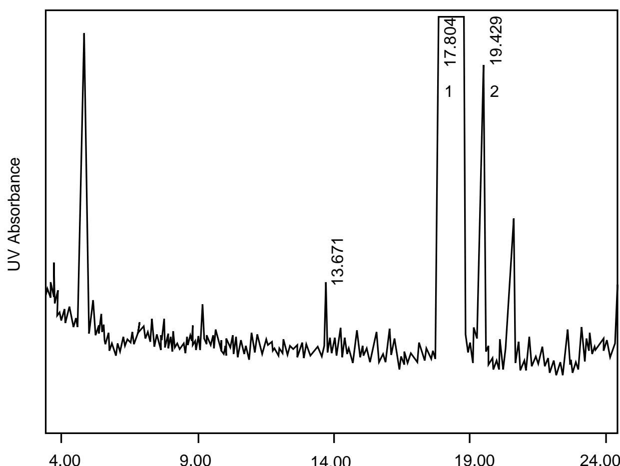

Figure 12. *Analysis of a BCH 189 drug substance batch containing 0.3% of the (-) enantiomer (2). Separation conditions: electrolyte, 50 mM dimethyl-β-cyclodextrin in 50 mM borax, adjusted to pH 2.5 with conc. H_3PO_4; detection, 214 nm; voltage, 13 kV; capillary, 47 cm × 50 µm. Reproduced with permission from Altria* et al., *1994b.*

4.2.1 Speed of Method Development

Chiral HPLC method development can be a time-consuming process and can involve laborious investigations of numerous stationary phase and mobile phase combinations. Extensive column equilibration may also be required. When operating in normal phase conditions, sample pretreatment may be required in HPLC to convert ionic drugs back to their base forms for reasons of solubility.

A particular attraction of the use of CE for chiral analysis is the simplicity and speed at which separation conditions may be assessed. With a knowledge of the aqueous solubility and ionizable groups of the compound, initial separation conditions can be selected (the previous primer on chiral separations contained sections on method development and optimization). Several potential

electrolyte compositions can be assessed in an overnight sequence and the most appropriate optimized further.

4.2.2 Speed of Analysis

Short analysis times are possible when operating with short capillaries and certain electrolyte compositions. Figure 13 (Sepaniak *et al.,* 1992) shows the resolution of phenylalanine enantiomers within 90 seconds. The enantiomers of the bronchodilator picumeterol have been resolved (Altria, 1993f) in 2.5 minutes using a 27-cm capillary.

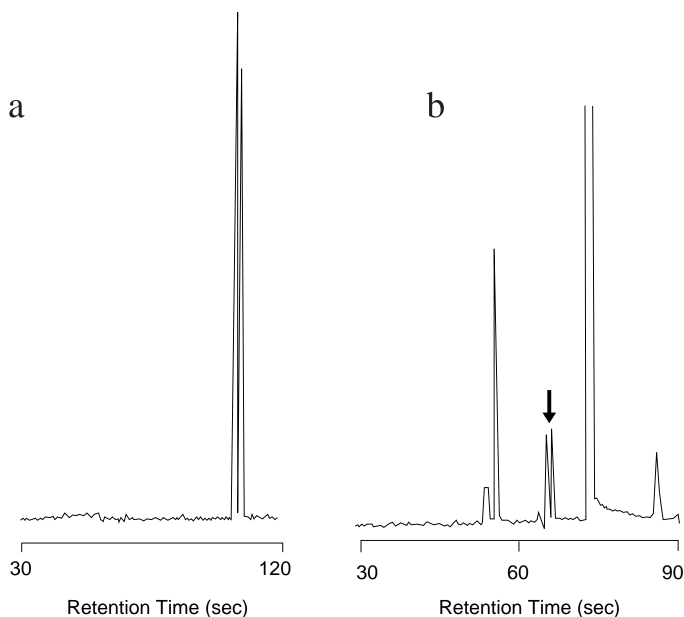

***Figure 13**. (a) Separation of DNS phenylalanine enantiomers from the components in a commercial formulation (35 kV applied). (b) Rapid separation of DNS phenylalanine (25 kV). Conditions: electrolyte, 0.01 m disodium phosphate, 0.006 M disodium borate (pH 9); detection, laser-induced fluorescence; capillary, 50 cm × 25 µm. Reproduced with permission from Sepaniak* et al., *1992.*

4.2.3 Robustness

Chiral HPLC is flexible in terms of the number of stationary phases and mobile phase additives that can be employed (Allenmark, 1988). However, the ruggedness of these methods can be questionable, especially when sensitive protein phases are employed.

In most reported circumstances, chiral separations in CE have been achieved using untreated fused-silica capillaries which are simple and reproducible. The robustness of chiral CE methods was highlighted (Altria *et al.,* 1993a) in an inter-company cross-validation of a method for the enantiomeric resolution of clenbuterol. The separation was achieved using a low-pH electrolyte containing a derivatized cyclodextrin. All seven independent pharmaceutical companies achieved baseline resolution, or better, for the clenbuterol enantiomers. Figure 14 shows three specimen separations. Acceptable data for linearity, migration time, and peak area precision was found in all cases. A peak area ratio precision of $< 1\%$ RSD and a 50:50 enantiomeric ratio were also shown.

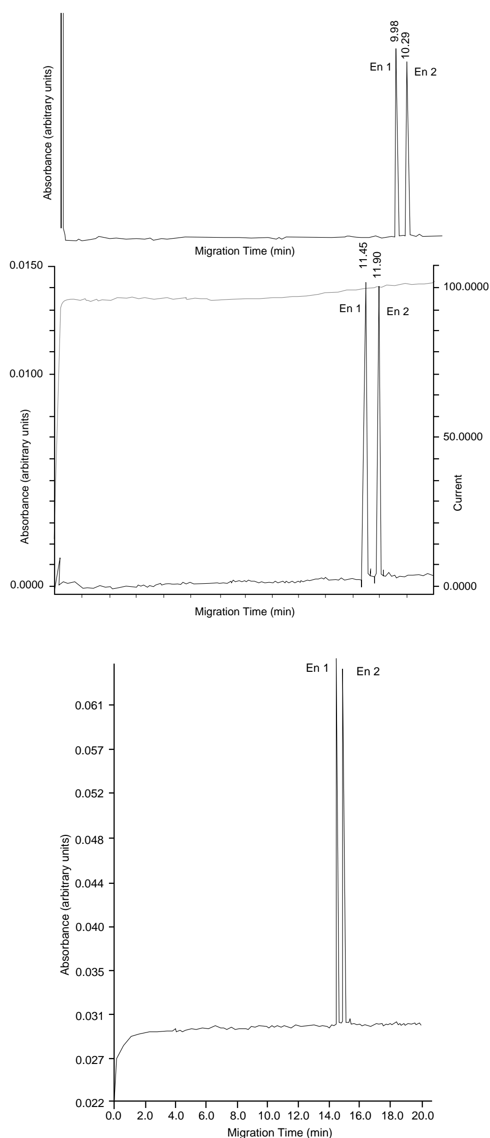

Figure 14*. Specimen chiral separations of clenbuterol achieved at three companies. Electrolyte: 30 mM hydroxylpropyl-β-cyclodextrin (typically 0.83 g per 20 mL) in 50 mM disodium tetraborate, pH adjusted to 2.2 with concentrated orthophosphoric acid.Reproduced with permission from Altria* et al., *1993a.*

4.2.4 Cost

Chiral HPLC columns generally employ specialized stationary phases and are therefore expensive (≈ $600). On the other hand, uncoated fused-silica capillaries are relatively inexpensive. Daily electrolyte requirements may be as little as 20 mL of electrolyte containing a chiral additive at millimolar concentrations. This compares favorably to liters of an organic solvent-based mobile phase.

The principal disadvantage when using CE for chiral analysis is the separation of water-insoluble compounds. Separations have been attempted with chirally selective bile salt micelles (Nishi *et al.,* 1990a) or with electrolyte containing both SDS and cyclodextrins (Terabe *et al.,* 1993).

4.3 Applications

The principal application area is the enantiomeric purity testing of drug substances. For example CE is used for the optical purity testing of Sandoz drug EN792 with an LOD of 0.2% for the undesired enantiomer (Kuhn *et al.,* 1992a). Similar separation conditions were employed (Nielen, 1993) for chiral separation of norephedrine and ephedrine and allowed the enantiomeric purity testing of both compounds at the 1% level with acceptable precision (1.2 to 2.5% RSD for areas and 0.2 to 1.5% RSD for optical purity results) and linearity better than 0.9997.

A chirally selective MECC method was developed for the optical purity testing of a trimequinol drug substance with an LOD of 1% (Nishi *et al.,* 1990a).

A CE method was used to monitor an enantioselective enzymatic biotransformation reaction (Rogan *et al.,* 1993). The reaction produced the required (+) enantiomer by selective deamination of the undesired (-) form using an enzyme. The reaction of the (-) was monitored over a 51-hour period and the final product found to contain less than 0.5% of the (-) enantiomer. Reaction rate and half-life information was generated.

A chiral CE method was shown (Soini *et al.,* 1992a) to be capable of determining 0.1% of the S-bupivacaine in the presence of R-bupivacaine.

CE and HPLC have been employed (Altria *et al.,* 1994c) to test the optical purity of picumeterol drug substance batches. Detector linearity of 0.9993 was obtained for peak area ratio data plotted against prepared mixtures of picumeterol enantiomers. Good agreement between the results was obtained con-

firming the accuracy of both techniques (less than 0.4% difference between techniques).

Enantiomeric ratios can alter following storage. For example L-epinephrine can undergo enantiomeric inversion to its D-isomer during storage. CE has been employed (Peterson and Trowbridge, 1992) to test enantiomeric purity of stored formulations containing L-epinephrine and acceptable precision (1.4 to 1.8% RSD for peak area ratios), linearity (0.9988 to 0.9998), and recoveries (99 to 101%) were obtained.

A validated method for the chiral analysis of fluparoxan drug substances has been reported (Altria *et al.,* 1993b). A typical separation of a test mixture of a fluparoxan enantiomer spiked with 1% of its stereoisomer is given in Figure 15. The test mixture was a synthetic mixture of 60:40% w/w of the (+) enantiomer: (-) enantiomer) and the peak area ratio obtained accurately confirmed the spiking level. Repeated analysis of mixtures containing enantiomers spiked at the 1% level confirmed this spiking (0.8 to 1.1% peak area). The validation of this method included precision peak area (1.6 to 2.0% RSD), linearity (0.994), and limits of detection (0.3%) and quantitation (1.0%). The method was capable of determining 0.3% of either enantiomer in the presence of the other.

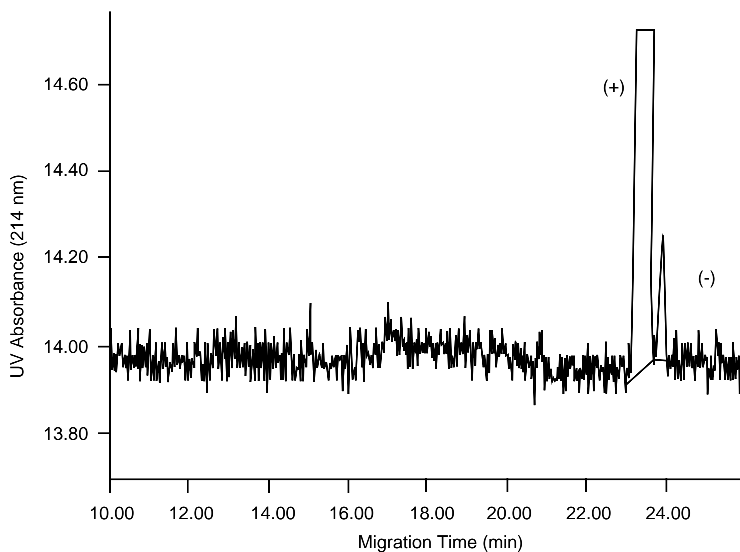

Figure 15. *Electropherogram of 1% (-) enantiomer in presence of (+) enantiomer of fluparoxan. Separation conditions: 57 cm × 50 μm; electrolyte, 10 mM borax, 10 mM tris, 150 mM β-cyclodextrin, 6 M urea-isopropanol (80:20) v/v; adjusted to pH 2.5 with H_3PO_4; temperature, 25°C; detection, 15 kV. Reproduced with permission from Altria, 1993b.*

Hohne *et al.* (1992) chirally resolved several aminoalcohols using electrolyte-containing crown ethers. They reported a detection limit of 0.5% for the inactive enantiomer of methoxamine.

An inter-company cross-validation (Altria *et al.,* 1993a) of a method for the enantiomeric resolution of clenbuterol between seven independent pharmaceutical companies showed good resolution (Figure 14). Linearity data for detector response was assessed over the range 10 to 150% of target concentration with correlation coefficients of 0.990 to 0.999. Precision for migration time and relative migration time was 0.2 to 1.3 and < 0.1% RSD respectively. Peak area and peak area ratio precision was found to be 0.8 to 2.5 and 0.2 to 0.9% RSD. An average 50:50 enantiomeric ratio was obtained by the seven companies with an RSD of 0.6%.

Chiral separations of drugs in biofluids have also been reported (Gareil *et al.,* 1993; Heuermann and Blaschke, 1993; Shibukawa *et al.,* 1993). The simplicity and robustness of CE make it particularly attractive in this area. Examples include the quantitation of dimethindene enantiomers in urine, following a single 4-mg dose (Heuermann and Blaschke, 1993), and the separations of leucovorin and its major metabolite at therapeutic levels in plasma (Shibukawa *et al.,* 1993).

In the body, enantiomers are often preferentially metabolized and a suitable method is required to monitor these reactions. A chiral CE method was used (Gareil *et al.,* 1993) to confirm that the (-) enantiomer of warfarin is preferentially metabolized in patients undergoing warfarin therapy. Figure 16 shows a typical separation with a LOD of 0.2 mg/liter for each enantiomer. A warfarin homologue was used as an internal standard and an RSD of 2.1% was obtained for peak area ratios for the standards.

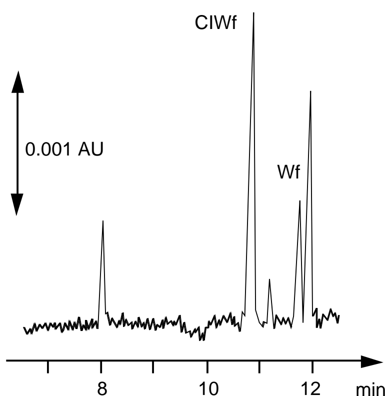

***Figure 16**. Electropherogram of a plasma sample of a patient under warfarin therapy. Conditions: electrolyte, 100 mM sodium phosphate buffer (pH 8.35), 8 mM Me-β-Cd-methanol (98:2, v/v); capillary, 72 cm × 50 μm ID; voltage, 20 kV (I=70 μA); temperature, 25°C; UV detection, 310 nm; hydrodynamic injection time, 1 s. The additional unlabeled peaks were not identified. The total warfarin concentration determined was 2.0 mg/mL with an S/R enantiomeric ratio of 34:66. Wf = warfarin; ClWf = 5-chlorowarfarin. Reproduced with permission from Gareil* et al., *1993.*

A validated method for the determination of cicletanine in both plasma and urine has been reported (Soini *et al.,* 1992b).

5 Stoichiometric Determinations

5.1 Overview

To modify the physiochemical properties such as solubility, bioavailabilty, and biocompatability, many drugs are prepared as salts. Typically, acidic drugs are prepared as their sodium or potassium salts. The range of salt forms available for basic drugs is more diverse, including both inorganic counter-ions such as chloride or sulphate and organic counter-ions such as succinate or maleate.

Many reports of the determination of inorganic ions or small organic acids by CE have appeared (Jackson and Haddad, 1993a) in what is commonly known (Jandik *et al.,* 1991) as capillary ion analysis (CIA). Generally, the methods employ indirect UV detection and have short (< 5-minute) analysis times. Figure 17 shows (Altria *et al.,* 1994b) the separation of a range of inorganic anions present at low ppm levels. Chromate is added to the electrolyte to provide the necessary background UV response and the detector polarity is reversed.

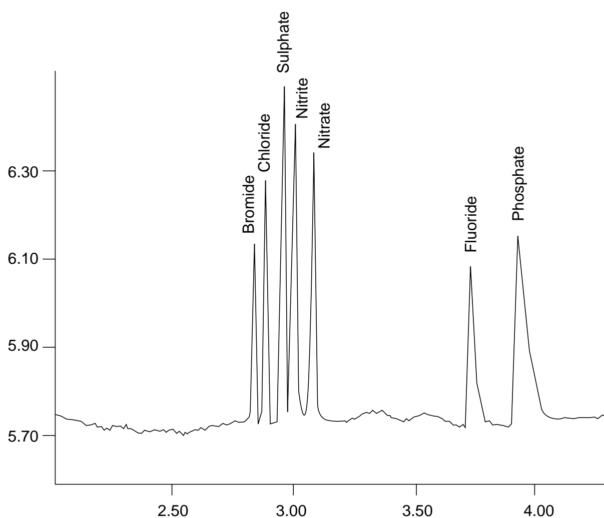

***Figure 17**. Separation of an anionic test mixture. Separation conditions: electrolyte, 5 mM chromate with 0.5 mM tetradecyl trimethyl ammonium bromide; capillary, 75 µm × 57 cm; voltage, -15 kV; detection, indirect at 254 nm. Reproduced with permission from Altria* et al., *1994b.*

5.2 Features of CE Methods for Stoichiometric Determinations

Drug:counter-ion stoichiometry is traditionally determined by ion-exchange chromatography (IEC) or titrimetry. CE represents an alternative technique and can offer advantages in terms of simplicity and cost.

5.2.1 Simplicity

In general uncoated fused-silica capillaries are employed in CIA which, as discussed in previous sections, are inexpensive. Aqueous-based electrolytes are used which can be prepared and stored for a long period of time prior to reuse. This compares to the preparation of IEC mobile phases and the use of IEC columns which often require regeneration procedures. This testing is performed on standard CE equipment. Automated testing and data handling is possible with both IEC and CIA, and both are therefore more desirable than titrimetry.

5.2.2 Cost

Both sample solutions and electrolyte are aqueous and volume requirements are minimal. The cost of a capillary, compared to a column, represents a further cost saving. A further advantage that may be overlooked is that the testing can be performed on standard unmodified CE instrumentation.

The principal disadvantage of CE compared to IEC may be limits of detection. Low ppm levels of both cations and anions are possible by CIA when employing standard injection techniques. Single figure ppb levels are possible (Jackson and Haddad, 1993b) when electrokinetic injection is used. This sensitivity issue is irrelevant in stoichiometric determinations as high sample concentrations (*i.e.,* 100 to 200 ppm) are employed.

5.3 Applications

This is a new CE application area and to-date few reports of the CIA analysis of pharmaceuticals have been reported. Undoubtedly more will appear as the technology matures. To illustrate possibilities, references to other application areas and, where possible, pharmaceuticals are given.

5.2.1 Anions

The two pharmaceutical application reports involve the determination of drug stoichiometry (Altria *et al.,* 1994b) and monitoring of inorganic contaminants in drug substances (Nair and Izzo, 1993). Other examples include the quantitative determination of inorganic anions in tap water (Motomizu, 1992) and bread (Ackermans, 1992c).

Stoichiometric testing of several drug substances has been conducted (Altria *et al.,* 1994b). Figure 18 shows separations of a batch of a drug prepared as a chloride salt. Clearly this batch largely contains chloride but also contains low levels of other inorganic and organic anions as contaminants.

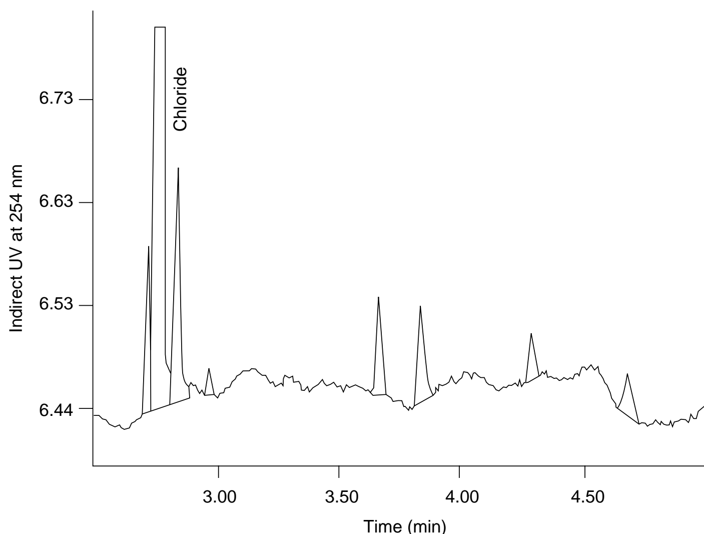

***Figure 18**. Electropherogram of chloride assay of GGR1 drug substance. Conditions as per Figure 17. Reproduced with permission from Altria* et al., *1994b.*

Table 4 shows results for chloride and sulphate content in three drug substances (denoted GRD1–3). Good agreement between CE, theoretical anion content, and microanalysis results are obtained (Altria *et al.,* 1994b). The method performance in terms of linearity ($R^2 > 0.999$) and RSD values of 1 to 2% for peak area and migration time was also acceptable.

Table 4. Levels of Anionic Counter-Ions in Drug Substances Determined by CE

Good agreement was obtained between CE, microanalysis, and theoretical content.

Sample	Theoretical Content	Microanalysis	CE Results
Chloride (%w/w)			
GRD 1 batch A	8.0	-	8.0 , 7.9
GRD 2 batch A	9.6	9.5	9.3 , 9.3
GRD 2 batch B	9.6	9.6	9.4 , 9.4
GRD 2 batch C	9.6	9.5	9.2 , 9.7
GRD 2 batch D	9.6	9.4	9.9 , 9.6
GRD 2 batch E	9.6	9.5	9.3 , 9.5
Sulphate (% w/w)			
GRD 3 batch A	16.6	-	16.7
GRD 3 batch A	16.6	-	16.8

(Altria *et al.,* 1994b)

When fully characterizing a drug substance, it is necessary to determine the inorganic ion content which originates from synthetic reagents. If present, inorganic ions will contribute to the mass of the substance and will need to be assayed. CE has been employed (Nair and Izzo, 1993) to determine a range of inorganic and organic ions present in drug substances. Limits of detection of < 0.1% w/w were reported. The method allowed the determination to be conducted on both water-soluble and insoluble drug substance materials. Insoluble drugs were dissolved in acetonitrile:water mixtures. Figure 19 shows the separation of a test mixture of inorganic ions dissolved in acetonitrile:water and analysis of a 1-mg/mL solution of deuterated pravachol. Various validation criteria were applied to the method including linearity (typically 0.999 in the range of 1 to 100 ppm), precision (1.3 to 6% RSD for anions spiked at the limit of quantitation, 0.1% w/w), and sensitivity (LOD of 0.05% w/w impurity in the drug substance).

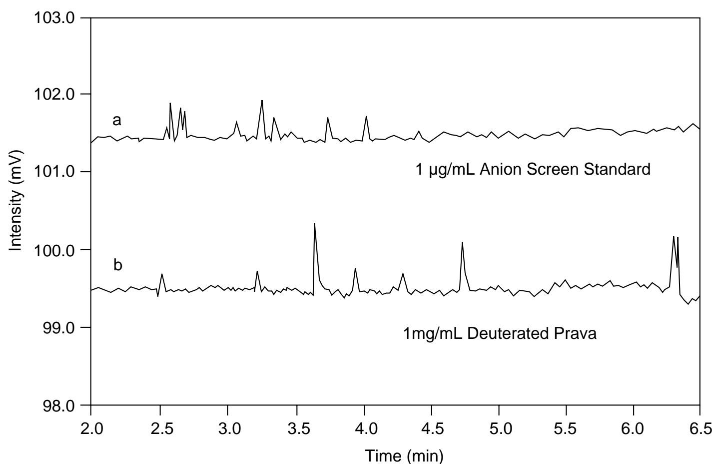

Figure 19. *Electropherogram of (a) 1 µg/mL anion screen standard. The migration order of the anions is bromide, chloride, sulphate, nitrite, citrate, fluoride, phosphate, carbonate and acetate. (b) 1 mg/mL deuterated pravachol. Separation conditions: electrolyte, chromate, dilute sulphuric acid and Waters' Anion-BT OFM; voltage, 20 kV; injection, 20 s, hydrostatic; detection, indirect UV at 254 nm. Reproduced with permission from Nair and Izzo, 1993.*

5.3.2 Cations

Typical separation conditions employed (Beck and Engelhardt, 1992) involve use of a low-pH electrolyte containing imidazole (or similar) as the UV background electrolyte.

Figure 20 shows a typical separation of a standard metal ion test mix used in the determination of metal ions in vitamin tablets (Swartz, 1993). The method allowed low-ppm LODs to be obtained. Aspects of validation successfully evaluated include linearity (better than 0.998), precision (< 1% for migration time and < 2% RSD for peak area), and sensitivity (1 ppm). Results from CE compared well with those obtained by ICP.

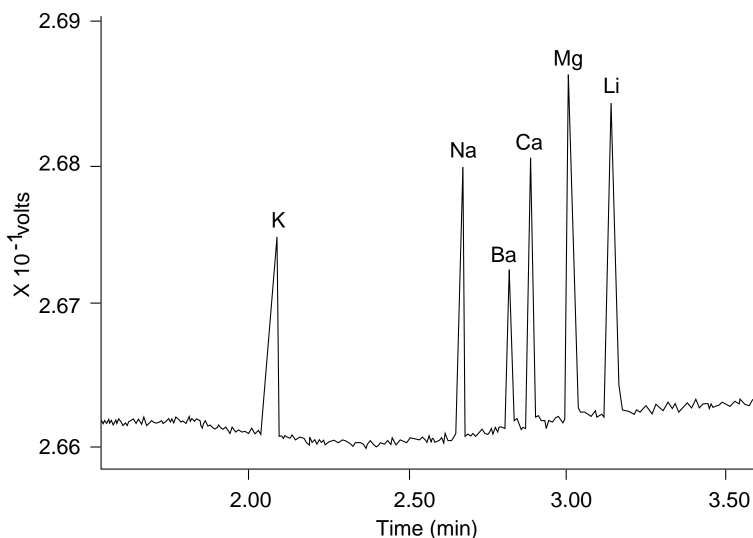

***Figure 20**. Separation of alkali metal and alkaline earth metal ions. Separation conditions: 5 mM imidazole, pH 4.5; voltage, 25 kV; sample concentration, 1 ppm (Swartz, 1993).*

Levels of potassium and sodium content have been determined in various acidic drug substances (Altria *et al.,* 1994b). Table 5 shows that the results obtained by CE for three drug substance materials (denoted GRD4–6) agree well with the theoretical and IEC results. Good method performance in terms of linearity (0.9999 over the range 10 to 500 mg/L), precision (0.3 to 1.5% RSD), and sensitivity (LOD of 1 ppm) was also obtained.

Table 5. Levels of Cationic Drug Counter-Ions in Drug Substances Determined by CE

Good agreement was obtained between CE, HPLC (IEC) and theoretical content.

Sample	Theoretical Content	HPLC	CE Results
Sodium			
GRD 4 batch A	5.2	-	5.2, 5.1
GRD 4 batch B	5.2	-	5.0, 4.8
GRD 4 batch C	5.2	-	4.9, 4.7
GRD 5 batch A	3.6	-	3.3, 3.4
Potassium			
GRD 6 batch A	6.0	6.1	6.1
GRD 6 batch B	6.0	-	5.9
GRD 6 batch C	6.0	-	6.0

(Altria *et al.,* 1994b)

Given the simplicity and robustness of the CE methods used for the determination of drug stoichiometry, it is expected that there will be a significant increase in application and perhaps even replacement of existing methods of testing.

6 Quantitative Procedures

6.1 Overview

The options for conducting quantitative analysis are similar to those adopted in HPLC and have been reviewed (Altria, 1993g). The output format is similar to an HPLC chromatogram *(i.e.,* a plot of UV absorbance versus time). Therefore, HPLC data handling and peak integration packages are generally applicable to CE. As discussed in the Introduction, it is important (Altria, 1993b) to normalize peak areas in appropriate circumstances. Some important considerations and reminders essential for achieving good quantitative data are detailed below.

6.2 Impurity Determinations

Impurity levels may be calculated and reported (Swartz, 1991) as a % of the total peak area of the electropherogram. This assumes an equal response factor for all known and unknown impurities. Alternatively (Altria, 1993c), if isolated standards of impurities are available, then external standards can be prepared and response factors obtained. These response factors are then used to calculate impurity levels as % w/w.

6.3 Main Component Assay

As with HPLC, sample solutions are analyzed against standards of known concentration. If an internal standard is employed as a means of increasing precision (Dose and Guiochon, 1991), then ratios of the drug and internal standard peak areas are used in the calculations.

6.4 Chiral Analysis

Enantiomeric content can be reported in several ways. The most common expresses the peak area of the undesired enantiomer as a percentage of total peak area. Alternative approaches determine the enantiomeric excess or use external standards of either enantiomer. Irrespective of which method is used, it is essential to use normalized peak areas (Altria, 1993b). This is exemplified by the data obtained for the repeated analysis of a racemic drug (Table 6). A peak-area ratio of 1.00 should be obtained for a racemic compound. The unnormalized areas indicate a ratio of 1.04, which suggests that more of the second detected enantiomer is present. However, the expected ratio of 1.00 is obtained (Altria, 1993b) when calculating using normalized areas. If low levels of undesired enantiomer are to be quantified, it may be necessary to employ sufficient sample concentration such that the main peak is off-scale. In this case, an external standard of either enantiomer at an appropriate concentration would be employed to generate a response factor.

Table 6. Effect of Peak Area Normalization on Peak Area Data for an Enantiomeric Separation

Normalization of the data confirms the correct area ratio for a racemic compound.

	Enantiomer 1	Enantiomer 2	Peak area ratio
Peak area (observed)	1199781	1246293	1.04
Peak area (normalized)	103827	103841	1.00
Migration time (min.)	11.55	12.00	

(Altria, 1993b)

6.5 Stoichiometric Analysis

Analar-grade inorganic materials such as NaCl are employed (Altria *et al.,* 1994b) as standards to generate a response factor for the ion concerned. Sample weights are adjusted to give an equivalent concentration of the ion being analyzed.

7 Method Validation Considerations

7.1 Overview

The approach to the validation of a CE method is similar to that employed for HPLC methods (Altria and Chanter, 1993). Aspects such as accuracy, precision, reproducibility, linearity, robustness, and method transfer are evaluated during validation. This section covers each of these criteria with illustrated examples and references.

7.2 Accuracy

Good agreement between CE results and the label claim or an alternative test method is often used to demonstrate accuracy. It is also recommended that recovery-type experiments are also performed, thus establishing the effect on quantitation of the matrix, if any.

7.3 Precision

Precision in CE, as with HPLC, is dependent on the type of method and its degree of optimization. Poor peak shape and low loadings will obviously result in unacceptable performance. Precision in CE is typically of the order 0.5 to 2% RSD for main peak assay, with or without internal standard (Altria and Filbey, 1993, Ackermans *et al.,* 1992a). For trace impurities, the precision would be expected to be < 10% RSD (Swartz, 1991; Altria and Chanter, 1993). Migration time and relative migration times should be about 1% RSD and < 1% RSD respectively. For example, in the transfer of a method for determining the enantiomeric ratio of clenbuterol (Altria *et al.,* 1993a), peak area precision was 1 to 2% RSD, peak area ratio precision was < 1%, and migration time precision was ≈ 0.1% RSD across seven companies.

7.4 Reproducibility

It is necessary to demonstrate that the method can be reproduced in a variety of situations. As with HPLC, reproducibility between capillaries, instruments, analysts, days, and laboratories is required. As the method may be employed on CE instruments from various manufacturers, it is important to demonstrate that acceptable performance can be attained using different sample introduction modes and capillary dimensions.

Examples of reproducibility studies include assessments of CE methods for the determination of sumatriptan (Altria and Filbey, 1993), and hydrochlorothiazide (Thomas *et al.,* 1994).

7.5 Linearity

It is important to show that a linear relationship between detector response and sample concentration exists within the working range required, in both direct and indirect modes. Typically, a range of 50 to 150% for main peak assay and of 0.1 to 10% for related impurities should be assessed. The literature contains numerous reports of the good linearity that may be expected for direct and indirect measurements. For example, correlation coefficients of better than 0.999 have been reported for sumatriptan (Altria and Filbey, 1993), several bronchodilators (Ackermans *et al.,* 1992a), and alendronate (Tsai *et al.,* 1992).

When determining impurity levels or trace enantiomer content, it is also necessary to demonstrate detector rectilinearity. This involves maintaining the main component at a constant concentration and varying the content of the minor component. Acceptable rectilinearities have been reported for impurities

of salbutamol (Altria, 1993c) and a quinolone antibiotic (Altria and Chanter, 1993). Correlation coefficients of > 0.99 have been reported (Altria *et al.*, 1993b) for fluparoxan enantiomer levels of 1 to 10% of the desired enantiomer content.

7.6 Robustness

Many definitions exist for this term but, in the context of this primer, it relates to the sensitivity of the method to small, deliberate deviations from the method. For instance, if the method states an operating temperature of 30°C, will acceptable performances be maintained at either 25°C or 35°C? Two approaches to method robustness are possible: uni-variate or multi-variate. The uni-variate approach involves systematically varying each parameter sequentially. This "one-by-one" approach has been performed (Thomas and Ghodbane, 1993) for the determination of enalapril content in tablets by CE. The multi-variate assessment involves simultaneous evaluation of several parameters using a predefined matrix. Typical experimental designs that may be employed include Placket-Burman (Vindevogel and Sandra, 1991) and Central Composites.

CE has been employed (Altria and Filbey, 1994) to determine levels of 4-guanidino-Neu5Ac2en and related impurities. Figure 21 shows separation of the main component from six impurities (denoted I1 to I6). Both fractional factorial and central composite designs were employed in the robustness testing of this method. Figure 21 also shows (Altria and Filbey, 1994) a Pareto plot of the resolution of 4-guanidino-Neu5Ac2en form I2 as a function of electrolyte pH and voltage.

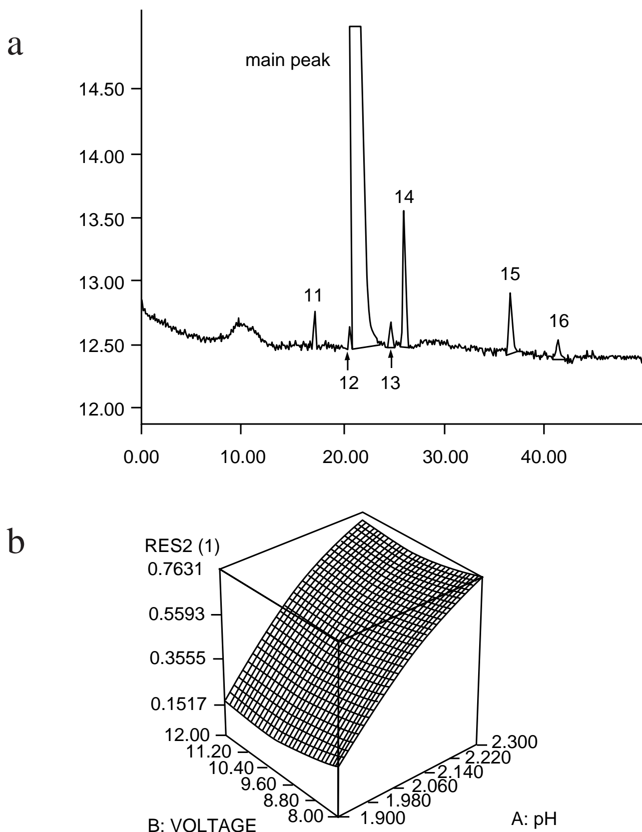

Figure 21. *Application of experimental design in robustness testing of a related impurity determination method. (a) Separation of 4-guanidino-Neu5Ac2en and related impurities. (b) Response surface showing the combined effect of pH and voltage on the resolution between the main peak and impurity I2. Separation conditions: electrolyte, 50 mM* NaH_2PO_4*, pH 2.1; injection, 10 s; detection, 230 nm; voltage, 10 kV; temperature, ambient; capillary, 50 µm × 47cm; concentration, 1 mg/mL. Reproduced with permission from Altria and Filbey, 1994.*

The need to demonstrate repeatability on instruments from at least two instrument manufacturers is stressed. Given the infancy of CE, the need for complete validation of methods is essential to generate further confidence and increased momentum.

8 Future Developments

8.1 Overview

There has been a rapid development of CE since the launch of commercial instrumentation. Improvements in detector sensitivity and the introduction of diode array technology are assisting continued developments. This section contemplates potential development areas that will influence the use of CE for pharmaceutical analysis. It is anticipated that the principal areas are new separation options, detection possibilities, capillary technology, and electrochromatography.

Currently, the separation options available in CE are not as extensive as HPLC. The introduction of new electrolyte additives and surface-modified capillaries are expected to have a major part to play. The flexibility and low cost involved in CE separations will facilitate these developments.

Some examples include reports on the use of ionizable cyclodextrins (Nardi *et al.,* 1993) and crown ethers (Kuhn *et al.,* 1992b). Ion-pair reagents and non-aqueous solvent systems will also extend the possibilities to water-insoluble drugs. The authors also recognize that the area of MECC is as yet relatively untapped. The variety of off-the-shelf surfactants and possible permutations are astronomical. Novel chiral selectors such as proteins (Li and Lloyd, 1993) will receive increased attention and will expand the area of chiral analysis further (Figure 22). These investigations will give the analytical chemist of the future many options for solving a separation problem.

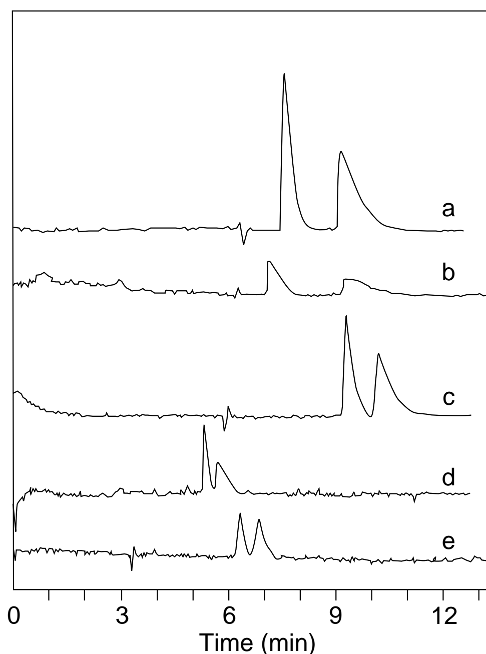

***Figure 22**. Electrochromatograms showing the enantiomeric separations of the following: (a) disopyramide (15% 2-propranol/4 mM phosphate, pH 6.8; potential, 12 kV; current, 2 μA); (b) pentobarbital (2% 2-propranol/2 mM phosphate, pH 5.5; potential, 20 kV; current, 2 μA); (c) hexobarbital (2% 2-propranol/2 mM phosphate, pH 5.5; potential, 18 kV; current, 2 μA); (d) cyclophosphamide (3% 2-propranol/2 mM phosphate, pH 6.5; potential, 25 kV; current, 2 μA); (e) benzoin (5% 1-propranol/5 mM phosphate, pH 6.5; potential, 15 kV; current, 3 μA). Reproduced with permission from Li and David, 1993.*

Surface-modified capillaries are also expected to become important. Initially, capillaries were coated to eliminate electroendosmotic flow, but increasingly specific coatings are being developed for selectivity purposes. For example, a CE column coated with immobilized cyclodextrin (Mayer and Shurig, 1993) has been used for chiral separations of various non-steroidal anti-inflammatories.

Beckman has developed an amine-coated capillary which offers interesting selectivity possibilities. The coating results in the capillary surface having a

positive charge, whereas an uncoated capillary is normally negatively charged due to dissociation of surface silanols. Application of a voltage across the amine coated capillary generates a strong EOF in the opposite direction to that obtained with an uncoated capillary and thus offers the potential for a reversal of migration order to that normally obtained. Figure 23 shows a test mixture of four basic drugs separated at pH 4.6 using an amine-coated capillary and a negative voltage. This separation could not be achieved on an uncoated capillary at the same pH. Undoubtedly the development and application of coated columns will expand the possibilities in CE method development.

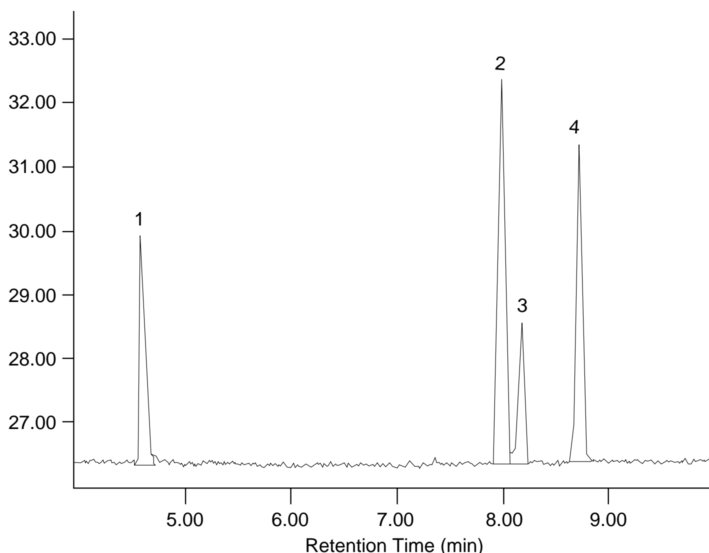

Figure 23. *Separation of 4 basic drugs on amine-coated capillary. 25 mM NaH_2PO_4 (pH unadjusted), 200 nm, 2-second injection, 50 cm × 37 cm amine-coated capillary, -10 kV. Altria, 1994, unpublished results.*

8.2 Detection

Currently the vast majority of CE applications in pharmaceutical analysis have involved the use of UV absorbance detection. Separation of pharmaceuticals having poor chromophores such as aminogyclosides (Figure 8) have involved the use of indirect UV detection and low UV wavelength detection.

New detection options on commercial instrumentation include diode array (DAD) and laser-induced fluorescence. DAD technology offers a number of benefits in areas such as peak identity confirmation and peak homogeneity testing.

Currently the use of laser-induced fluorescence (LIF) detection is somewhat limited in pharmaceutical analysis as few drugs have the required natural fluorescence. The additional steps of preseparative sample derivatization is unattractive from a routine method perspective. However, appropriate programming of the separation method (Houben *et al.,* 1993) can allow unattended on-column sample derivatization. The sample and the required derivatization reagents are injected into the capillary, allowed to react, and the derivatized sample components are then separated.

It is expected that more detection options will become commercially available to supplement those currently available. Most HPLC detection modes have been reported for CE and include electrochemical (Yik and Li, 1992) and radioactivity detectors (Altria *et al.,* 1990). The potential sensitivity and selectivity these offer will further widen the scope for application. The hyphenation of CE to MS is well established and a number of reports of CE-MS to drug analysis (Johansson *et al.,* 1991) have appeared. Again, significant progress in this area is expected.

8.3 Electrochromatography

Electrochromatography is a technique in which a CE capillary packed with HPLC stationary phase material is employed for separations. The capillary column is filled with electrolyte and a voltage applied. Application of the voltage causes electroendosmotic flow to occur which effectively pumps electrolyte through the column. Solutes separate by virtue of their chromatographic interactions with the packing and also by differences in electrophoretic mobilities. The technique is therefore essentially a combination of CE and HPLC and, as such, offers yet another dimension to method development options.

Electrochromatography can be operated on standard CE instruments and has many of the features of CE in terms of reduced reagent purchase and solvent disposal costs but with the added advantage of good peak efficiencies. Little attention has been paid to this area as yet and the reports are limited (Yamamoto *et al.,* 1992). Figure 24 shows the separation of israpidin which illustrates the high separation efficiencies that are possible using electrochromatography using 3 mm ODS particles.

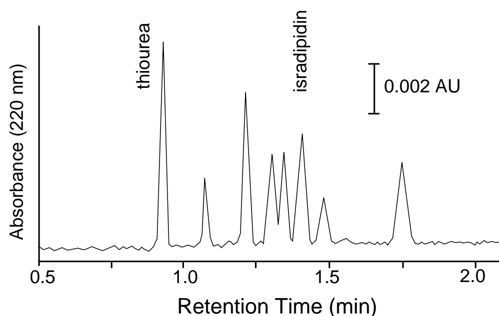

Figure 24. *Separation of isradipidin by electrochromatography. Reproduced with permission from Yamamoto* et al., *1992.*

9 Conclusions

Capillary electrophoresis is now firmly established as a viable option for the analysis of pharmaceuticals. Specific application areas include the determination of drug-related impurities, drug potency, chiral analysis, and determination of drug counter-ion content. CE is often viewed as an alternative or complementary technique to HPLC.

Validated CE methods are in routine use in many industrial pharmaceutical analysis laboratories. Validation criteria for CE methods are similar to those employed in evaluation of HPLC methods.

Use of CE for specific analysis such as chiral analysis can have benefits in terms of method robustness and ruggedness, cost, and time. Current disadvantages are largely poorer sensitivity when directly compared to HPLC and the limited preparative options. Undoubtedly, technological developments and advances in methodology will strengthen and endorse the position of CE within pharmaceutical analysis. Of considerable note are the possibilities that the further development of new detector options, coated capillaries, and electrochromatography may bring.

Without doubt, CE has been recognized as a valid means of testing pharmaceuticals. To date, over 150 references have appeared on the subject. This number will no doubt double or triple in the next two to three years as the number of application areas expands.

Practical Hints

The following section is intended to highlight a number of practical aspects which should be considered during the development and subsequent use of CE-based methods in pharmaceutical analysis. While these are by no means exhaustive, they serve to alert the user to potential problem areas, their possible remedies, and relevant literature references containing further details.

Electrolyte/Sample

Analyte peak shape can be dramatically improved by:

- matching the mobility of the analyte and the buffer co-ion (Mikkers *et al.,* 1979);
- having the sample a factor of 10^2 lower than the buffer concentration (Mikkers *et al.,* 1979);
- dissolving the sample in a low concentration buffer (water if possible) and analyzing with a high-concentration electrolyte (Chien and Burghi 1992);
- to avoid band broadening due to Joule heating effects, buffers with low specific conductance should be used (generally operation above ≈ 100 µA can lead to problems of internal heating in the capillary);
- buffers and samples should be filtered prior to use to remove particulates.

Sensitivity

The sensitivity of a method using standard CE equipment (*i.e.,* UV detector) can improved by:

- employing a “high-low” injection technique (see Section 2.3, Altria, 1993e);
- using low UV wavelengths such as 200 nm where many components have significantly enhanced UV activity (Altria, 1993d);
- employing large sample volumes (*i.e.,* long injection times) under stacking conditions (Chien and Burghi, 1992);
- using larger-bore capillaries than the standard 50 or 75 µm. Bores as high as 180 µm have been employed (Altria, 1993f) to dramatically increase sensitivity. However, deleterious heating effects may occur and the buffer concentration, voltage, and capillary length should be adjusted to give an acceptable level of current.

Quantitation

The following measures may improve peak area precision:

- methods should be run at constant temperature as the volume injected is proportional to temperature (Rose and Jorgenson, 1988).
- peak area normalization (Altria, 1993b) should always be conducted. The exception to this is where migration time precision is very good and external standardization is employed.
- internal standards can be used to increase precision (Dose and Guiochon, 1991).
- the volume injected onto the capillary is proportional (Rose and Jorgenson, 1988) to the viscosity of the sample and calibration solutions. Viscosity differences can be introduced when components such as cellulose are in the sample which may lead to apparent imprecision. Use of an internal standard eliminates this difficulty.
- high sample concentrations should be used to reduce the effect of integration errors.

Capillary Care

It is recommended that:

- the capillary is rinsed for 30 minutes with 0.1 M NaOH on its initial use;
- the capillary is flushed with 0.1 M NaOH (or acid) and equilibrated with the running buffer prior to each injection;
- the capillary is rinsed with water and air blown through it at the end of each day. This prevents buffer precipitation and subsequent capillary blockage;
- individual capillaries be dedicated to specific methods to avoid “memory effects” which may lead to non-reproducible separations;
- an initial injection of a blank, or sample solution, be performed prior to commencing an analytical sequence to allow the system to equilibrate.

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