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Review

Quantitative aspects of the application of capillary electrophoresis to the analysis of pharmaceuticals and drug related impurities

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ABSTRACT

Capillary electrophoresis (CE) is a collection of associated electrokinetic techniques, the principal ones being free solution capillary electrophoresis (FSCE) and micellar electrokinetic capillary chromatography (MECC). Both FSCE and MECC have been utilised for the separation of drug species. Several reports have demonstrated that CE is an attractive complement or alternative to HPLC which is widely used for pharmaceutical analyses. CE methods have been validated and are now in routine use within a number of pharmaceutical companies. This review details both applications taken from within a working pharmaceutical analysis laboratory and also provides selected literature examples. Applications include the quantitative analysis of drug related impurities, determinations of drug content in formulations, chiral analysis, stoichiometric determinations, and micro-preparative CE.

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1. INTRODUCTION

Great demands are placed on the analytical methods that are used for the determination and assay of both the active ingredient and impurities in pharmaceuticals. Impurities may be present from either synthetic or degradative sources and these therefore may have widely differing structures and/or polarities. Impurity levels may be of the order of 0.05–1% area/area of the main component which necessitates a detection system with a suitable linear range. During the development of a drug candidate, and the subsequent quality control of the marketed pharmaceutical product a considerable number of samples will be analysed. This volume of samples therefore requires that the analytical method employed be relatively inexpensive, simple, quick and robust. Currently the majority of these analyses are conducted by HPLC which can offer all the above required features. Similarly CE is capable of meeting these requirements and is now being recognised as an important option within pharmaceutical analysis.

The work of Jorgenson and Lukacs [1] popularised the technique of CE and encouraged academic and industrial investigators to enter this area of research. Terabe *et al.* [2] developed micellar electrokinetic capillary chromatography (MECC). Both free solution capillary electrophoresis (FSCE) and MECC offer different selectivity principles compared to each other and to chromatographic techniques such as HPLC. CE based methods are being increasingly applied to supplement and complement chromatographic generated data, in a wide and diverse number of application areas.

FSCE separates solutes by virtue of their different electrophoretic mobilities. A more mobile species travels along the capillary faster than a less mobile analyte. Under a given set of operating conditions the electrophoretic mobility has a fixed electrochemical value and the migration time is characteristic of the test solute. High peak efficiencies can be obtained using CE which can allow discrimination of closely related species. For example CE has been used for the separation of benzoic acids containing isotopically substituted oxygen atoms [3] and also positional isomers [4].

MECC is the most popular of the variants of electrokinetic chromatography, others being electro-osmotically driven chromatography [5] and solvophobic association electrophoresis [6]. In MECC a micellar solution of surfactant is used as the electrolyte. This combination permits resolution on both an electrophoretic and chromatographic basis enabling the resolution of neutral compounds which cannot be achieved by FSCE.

Highly automated PC-controlled CE instrumentation is now commercially available from a number of suppliers. These systems incorporate [7] sample introduction devices, UV absorbance detectors, a high-voltage supply, and an autosampler. CE detectors may be an order of magnitude less sensitive as compared to those available for HPLC. This can in part be compensated for by the high peak efficiencies obtained (with resulting ease of integration and improved resolution) and the use of low UV detection wavelengths (down to 185 nm) where many solutes have enhanced UV activity.

Precision of injection, as measured in peak area reproducibility is generally poorer in CE than in HPLC, typical values being 1–2% and 0.5–1% R.S.D., respectively. This failing is due to the technical difficulties involved in forcing a tiny sample volume into a narrow bore capillary.

There are a number of FSCE parameters than can be varied to achieve a required separation. These include the use of various pH conditions, electrolyte strength and nature, additives such as cyclodextrins, urea, ion-pair reagents and organic modifiers. Nielen [8] has reported the effect of varying such parameters upon the separation of aminobenzoic acid positional isomers. A similar number of variations can be used for optimisation of an MECC method. However, in addition the surfactant type and concentration may also be varied to alter selectivity.

Kuhr and Monnig [9] have provided an excellent general survey of recent CE developments. CE has been employed for the analysis of a wide and diverse range of compounds. These include biomolecules [10,11] such as amino acids, peptides, proteins and nucleotides where traditional forms of electrophoresis are extensively currently employed. In these areas CE is being widely used as previously developed methodologies can

be transferred to the capillary format. However, conventional electrophoresis has had only limited application to the analysis of pharmaceuticals where the emphasis and experience has been concentrated on the use of chromatographic techniques.

The possibilities of applying MECC to the analysis of pharmaceuticals was earlier reviewed by Nishi and Terabe [12]. There have been a large number of pharmaceutical related reports subsequent to this paper. The application of FSCE to the analysis of drugs has not been comprehensively summarized to-date. Therefore it is useful to review the quantitative applications and capabilities of CE in the area of pharmaceutical analysis.

2. INSTRUMENT PERFORMANCE DATA

Commercial instrumentation became available in 1988 and equipment is now available from a number of suppliers either as complete systems or in modular form. The instrumentation employed has been described many times and comprises a high-voltage supply, detector, a length of capillary tubing and a means of introducing sample into the capillary. Good performance is required in terms of reproducible peak areas and migration times to allow both qualitative and quantitative analysis.

The following levels can typically be obtained on the commercial automated instruments currently employed within our laboratory. Detection limits in the low mg/l levels in solution may be obtained for typical pharmaceuticals. Related impurity levels can be routinely monitored at *ca.* 0.1% area/area of the main component. These detection levels may be improved to *ca.* 0.02% (w/w) of main component using external calibrations when the main peak is off-scale. The precision of migration time precision is *ca.* 1% R.S.D. which allows qualitative identification of components within a mixture. Peak area precision is in the order of 1–2% R.S.D. This figure can be improved by employing internal standards. Analysis times are similar to those used in HPLC (*i.e.* 1–30 min). The range of detection wavelengths (185–760 nm) is also similar to those available for HPLC detectors. Typical operating temperatures are in the order of 20–50°C.

3. APPLICATION AREAS

It is intended that reported application areas will be covered in detail, few aspects concerning background theory or method development options will be mentioned. Papers on these subjects have recently been published by McLaughlin *et al.* [13] and Swartz [14].

The viability of employing CE for the analysis of pharmaceuticals was demonstrated in 1987 using homemade equipment [15,16]. Since then reliable commercial equipment has become available and the number of investigators has dramatically increased. Examples from most of the major drug classes have been resolved by CE. A survey of the therapeutic areas covered and the nature of the sample is given in Table I.

The application areas described to-date fall into the following categories: (i) determination of related impurities, (ii) main peak assay, (iii) chiral analysis, (iv) micropreparative CE, (v) identity confirmation, (vi) stoichiometric determinations.

3.1. Procedures for performing quantitative analysis

The procedures for conducting quantitative analysis are similar to those employed in HPLC. Main drug determinations are performed by obtaining response factors from calibration solutions [13,14,38,40]. Drug contents are then calculated applying these response factors to the results obtained for the sample solutions. As in HPLC, internal standards can be incorporated to improve precision as this eliminates injection based errors.

Peak areas are generally employed in quantitative analysis as peak height increases are non-linear at high sample concentrations. It is recommended to employ relatively high sample loadings to obtain good peak area precision.

Levels of impurities may be calculated using procedures similar to those employed in HPLC. Levels of individual impurities may be calculated [42] using response factors obtained from calibration solutions of the impurities. Alternatively [14,60] impurity levels may be quoted as %area/area of the electropherogram. This second approach is the most widely employed method as

TABLE I

SUMMARY TABLE OF THE DRUG CLASSES THAT THE DIFFERENT MODES OF CAPILLARY ELECTROPHORESIS HAVE BEEN APPLIED TO

Class	Electrolyte[a]	Sample composition	Ref.
Antibiotics			
Penicillins	MECC, SDS	Test mixtures	14
Sulphonamides	FSCE, CD	Test mixtures	17
Benzylpenicillin	High pH FSCE	Formulations	18
Various	MECC, SDS and LMT	Test mixture	19
Cephalosporins	MECC, SDS + TAA	Test mixtures	20
Cefuroxime axetil	MECC, SDS	Formulations	21
Aspoxicillin	MECC, SDS	Plasma	22
Sulphonamides	FSCE, pH 3–7	Pork meat extracts	23
Cefixime (and metabolites)	FSCE, pH 7 +methanol	Test mixtures	24
Cefpiramide	MECC, SDS	Plasma	25
Amoxycillin and impurities	MECC, SDS	Drug substance	26
Neomycin	FSCE, various	Eardrops	27
Quinolone and impurities	FSCE, pH 2	Drug substance	28
Antimalarial agents			
Quinine	FSCE, pH 7	Test mixture	29
Antidepressants			
Various tricyclics	MECC	Test mixtures	30
Fluparoxan	MECC	Drug substance	31
Benzodiazepines	MECC	Urine	32
Dilitiazem and impurities	MECC, SDC	Tablets	33
Various	MECC	Test mixtures	34
Various	MECC	Test mixtures	35
Anti-ulcer			
Ranitidine	FSCE, low pH	Syrup formulation	21
Cimetidine	MECC	Plasma	36
Ranitidine and impurities	FSCE, low pH	Syrup formulation	37
Cimetidine	FSCE, pH 7	Formulations	38
Bronchodilators			
Inolin (and others)	FSCE, low pH	Test solution	39
Salbutamol	FSCE, pH 5	Formulations	40
Theopylline	MECC, SDS	Body fluids	41
Salbutamol impurities	FSCE, pH 2	Drug substance	42
Steroids			
Corticosteroids	MECC, SDS	Test mixtures	33
Corticosteroids	MECC, SDC	Test mixture	43
Various	MECC, SDS + CDs	Test mixtures	44
Various	MECC, SDC	Test mixtures	45
Testerone esters	MECC, SDS	Test mixtures	46
Antihistamines			
Various	MECC, SDS	Formulations	47
Anti-cancer			
Methotrexate and metabolites	FSCE, pH 7	Serum	48
Analgesics			
Various	FSCE and MECC, SDS	Formulations	7
Various	FSCE and MECC, SDS	Test mixtures	14
Paracetamol	MECC, SDS	Plasma	49
Various	MECC	Test mixtures	13
Various	MECC, SDS	Formulations	50
Mucolytic agents			
S-carboxylmethyl-L-cysteine	FSCE, pH 9	Urine	51
Drugs of abuse			
Various	MECC, SDS	Plasma	52
Barbiturates	MECC, SDS	Test mixtures	53
Various	MECC, SDS	Urine	54
Non-steroidal anti-inflammatories			
Various	MECC, SDS	Test mixtures	20
Various	MECC, SDS	Test mixtures	45
Various	MECC, SDS	Test mixtures	55
Various	MECC, SDS	Test mixtures	56
Anti-migraine			
Sumatriptan	FSCE, pH 2	Urine	57
Sumatriptan	FSCE, pH 2	Formulations	58
Radiolabelled pharmaceuticals			
$^{99}Tc^{m}$ complexes	FSCE, various	Test mixtures	59

[a] CD = cyclodextrin; TAA = tetra-alkylammonium salts (ion-pairing reagent); SDS = sodium dodecyl sulphate (surfactant); LMT = sodium N-lauroyl-N-methyl taurate (surfactant); SDC = sodium deoxycholate (surfactant); SC = sodium cholate (surfactant).

standards of all impurities may not be readily available.

Normalisation of peak areas with migration times in CE is essential prior to reporting impurity level results as %area/area. This requirement arises due to differential residence times of peaks within the detector. Unless area normalisation is adopted it makes the cross correlation of %area/area impurity results between CE and others separative methods such as HPLC impossible to perform accurately [61]. If unnormalised peak areas are employed for calculation purposes peaks migrating before the main component will be underestimated whilst those migrating later

will be overestimated [61]. This normalisation is relatively simple to perform and involves the division of the peak area of each peak of the electropherogram by its corresponding migration time.

Determination of drug counter-ion levels are performed by external standardisation. Calibration solutions are prepared from appropriate AnalaR grade ionic materials such as NaCl and $MgSO_4$ depending upon the analyte of concern.

3.2. Determination of drug related impurities

This area is currently considered to be the primary role for CE within pharmaceutical analysis as FSCE and MECC offer alternative separation selectivities to the widely used technique of HPLC. CE is therefore a useful option to confirm purity results as obtained by HPLC.

Typical limits of detection (LODs) for impurities may be set at 0.1%area/area with reference to the main component. This LOD is approaching the linear dynamic range of commercial CE detectors. Swartz [14] reported a LOD of 0.1%area/area for salicylamide related impurities by MECC with a precision of ~5% R.S.D. for low level impurities. Bile salt based MECC [33] has been used to determine levels of dilitazem impurities in tablets with a reported LOD of 0.1%area/area for related impurities.

FSCE, TLC and HPLC were simultaneously employed [60] for the determination of domperidone related impurities in drug substance samples (Table II). Impurity levels for individual impurities agreed well between the techniques, however CE resolved an additional impurity which apparently co-eluted with another impurity on both HPLC and TLC.

To improve detection limits to below 0.1%area/area CE can be obtained by employing variable sample loadings [62]. An initial analysis is performed to obtain a main component just within the operating range of the detector. The sample loading is then increased to a higher level by a preset factor (*i.e.* 10 fold increase). The analysis of the higher sample loading will result in a off-scale peak for the main component but will allow detection of trace impurities. These impurities are then quoted as a % of total peak area. The peak area of the main peak in the off-scale separation is calculated by multiplying the peak area obtained in the on-scale separation by the factor relating to the sample loading increase.

The determination of the impurities of ranitidine is used [62] to illustrate the performance gains when employing high-low sample loading. HPLC and TLC methods [63] are employed for the analysis of ranitidine, several impurities are present at low (<0.1%) levels which cannot be directly quantified by CE. Fig. 1a shows a 2-s injection of a solution of a degraded ranitidine sample with the main peak on-scale, an LOD of 0.1%area/area was obtained. Fig. 1b shows a 20-s injection of the same solution with the main peak off-scale the LOD is calculated as 0.02%area/area. Both 2- and 10-s injections of the sample were repeated to assess the precision and increase in detection levels (Table III). There are also clear improvements in the precision of determining low level impurities using this procedure.

TABLE II

DOMPERIDONE RELATED IMPURITIES IN DRUG SUBSTANCE BATCHES (%AREA/AREA)

From ref. 60.

Impurity	Batch 1			Batch 2			Batch 3		
	CE	LC	TLC	CE	LC	TLC	CE	LC	TLC
R45571	0.24	0.26	0.19	0.22	0.23	0.15	0.26	0.27	0.23
R48557	0.15	0.35	0.34	0.15	0.34	0.36	0.15	0.30	0.27
Unknown	0.17	–	–	0.24	–	–	0.18	–	–

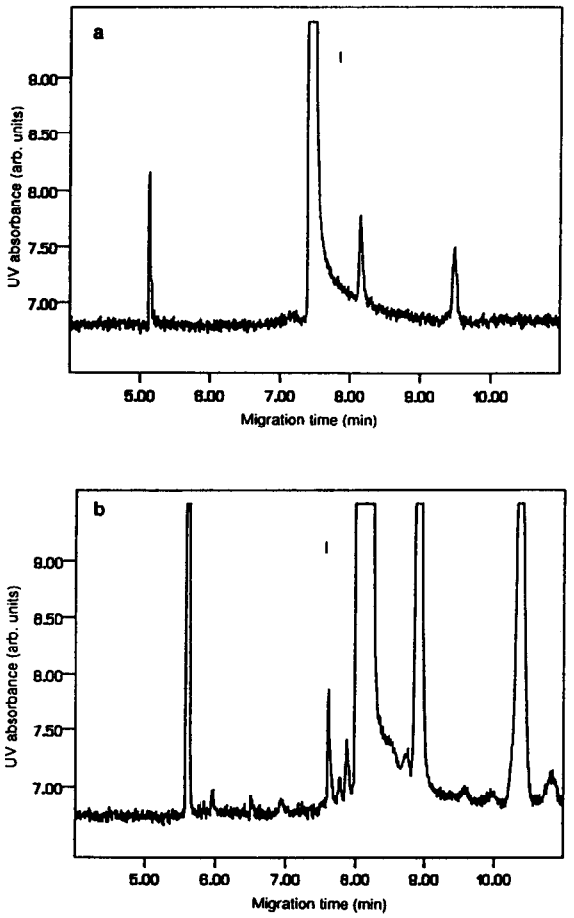

Fig. 1. (a) 2-s loading of degraded ranitidine solution; (b) 10-s loading. I = ranitidine. Reproduced with permission from ref. 62.

TABLE III

RANITIDINE RELATED IMPURITY DATA (%AREA/AREA) ($n = 5$)

O/S = Peak off-scale; RMT = relative migration time with reference to ranitidine; N/D = not detected. From ref. 62.

	2 s	10 s
Ranitidine peak area (R.S.D.)	399586 (0.8%)	O/S
Total impurity level (R.S.D.)	4.5 (2.9%)	4.4 (1.2%)
Number of impurities	4	8
Peak at RMT 0.69 (R.S.D.)	1.90 (4.0%)	1.68 (1.5%)
Peak at RMT 0.94	N/D	0.13
Peak at RMT 0.96	N/D	0.03
Peak at RMT 0.97	N/D	0.08
Peak at RMT 1.08 (R.S.D.)	0.13 (21%)	0.12 (2.2%)
Peak at RMT 1.10 (R.S.D.)	1.30 (9.3%)	1.25 (1.6%)
Peak at RMT 1.28 (R.S.D)	1.17 (5.0%)	1.16 (1.5%)
Peak at RMT 1.34	N/D	0.07

An alternative approach to improving detection limits is to employ high sample concentrations and to quantify impurities against external standards. FSCE has been employed [42] for the quantitative determination of 2 dimeric impurities ("dimer" and "bis ether") present in experimental salbutamol sulphate drug substance. To achieve the low detection level required a relatively high sample concentration (1 mg/ml) and a detection wavelength of 200 nm were employed. The salbutamol peak was off-scale and the impurities were quantified against external standards of the impurities. Linearity of response over the required impurity content range, and a detection limit of 0.02% (w/w) were obtained. A linear detector response (peak area) with bis ether content was obtained with a correlation coefficient of 0.999 and intercept of less than 1% of typical values. Precision data of <5% R.S.D. was obtained for response factors, which given the low levels being determined, were considered acceptable. Quantitative impurity results for salbutamol drug substance batches obtained by CE compared well [42] to those obtained by HPLC and TLC.

Typical analysis times reported in CE are similar to those encountered in HPLC, *i.e.* 10–40 min. However, the use of high voltages applied across short capillaries can greatly reduce the required times with only a marginal loss of separation efficiency [37]. A fluparoxan drug substance batch, prior to purification, containing high levels of related impurities was analysed by CE using both a standard length and a short capillary (Fig. 2a and b). This sample was analysed 5 times using both techniques to give the data in Table IV. The impurity profiles and levels are similar in both instances. The precision of main peak areas are 0.7 and 1.1% R.S.D. for conventional CE and high-speed CE, respective-

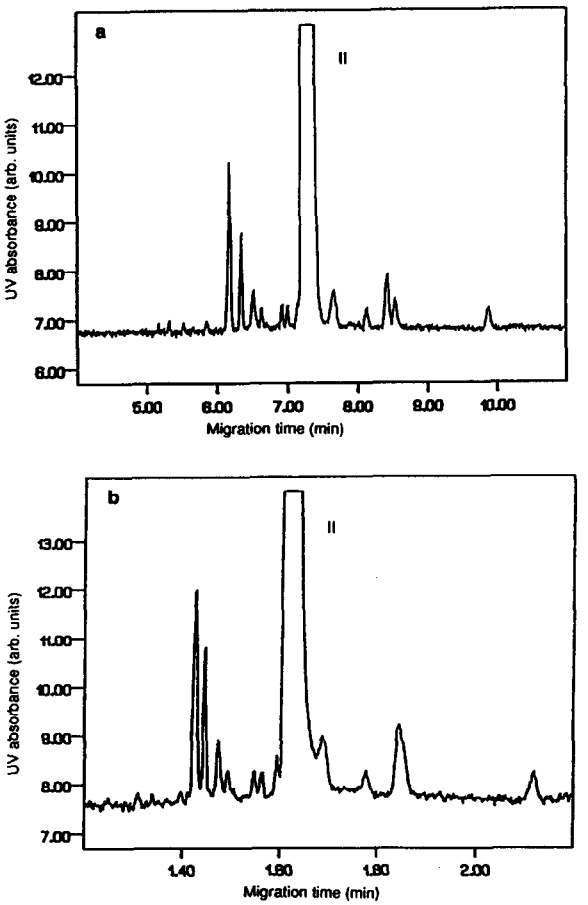

Fig. 2. (a) Normal-speed CE separation of fluparoxan related impurities; (b) High-speed CE separation of fluparoxan related impurities. II = fluparoxan. Reproduced with permission from ref. 37.

TABLE IV

COMPARISON OF CE AND HIGH-SPEED CE FLUPAROXAN IMPURITY DATA

No. of injections = 5. From ref. 37.

	CE	HSCE
Fluparoxan area	613792	750578
Fluparoxan area R.S.D.	0.7%	1.1%
Total no. impurities	15	14
Total %area/area (%a/a)	11.04	11.95
Total %area/area R.S.D.	1.8%	2.2%
Impurity RMT 0.85 %a/a	3.4	3.4
Impurity RMT 0.85 %a/a R.S.D.	1.6%	1.8%
Impurity RMT 0.87 %a/a	1.6	1.8
Impurity RMT 0.87 %a/a R.S.D.	4.0%	4.9%

ly, which indicates that high-speed quantitative determinations could also be considered.

Clearly CE has the ability to provide quantitative impurity data with an acceptable level of performance.

3.3. Main drug determinations

Increasing attention is being paid to the quantitative aspect of CE. Typical peak area R.S.D.s reported are in the region of 1–2% [7,13]. However, the use of internal standards permits improved quantitative precision to be obtained [64], as this compensates for any variability in injection volume.

CE has been applied to a number of drugs in a variety of formulations. Hoyt and Sepaniak reported [18] the first quantitative analysis by using CE to determine the penicillin G content of tablets employing phenol as an internal standard. The cimetidine content of several formulation presentations were determined by FSCE [38] and fair agreement was obtained with the label claim. Weinberger and Albin [56] employed SDS-based MECC to determine non-steroidal anti-inflammatories in tablets and obtained data equivalent to the label claim with good precision.

The techniques of CE and HPLC were simultaneously used [40] to obtain drug content results in brochodilator formulations. Good correlation between the three techniques and label claim was achieved (Table V). Correlation coefficients of greater than 0.999 for CE detector linearity were obtained. Precision data for CE assay results were in the order of 1–2% R.S.D.

Exploiting indirect UV detection Ackermans *et al.* [27] were able to quantify the levels of selected aminoglycosides in eardrops with both good precision and linearity. FSCE has also been employed [47] to quantify levels of antihistamines in tablets.

Nishi *et al.* [50] employed MECC to determine the levels of analgesics in formulations, the results were concordant with the label claim. Plyum *et al.* [60] determined the domperidone content in three formulation types by both CE and HPLC. The results showed reasonable agreement in terms of assay and precision, an

TABLE V

QUANTITATIVE ANALYSIS OF FORMULATIONS BY CE AND HPLC (mg/dose)

From ref. 40.

Sample	HPLC	CE
Salbutamol		
Tablets (4 mg/tablet)	4.05, 4.00	3.94, 3.72
Infusions (1 mg/ml)	1.04, 1.02	0.99, 1.00
Syrups (0.4 mg/ml)	0.40, 0.40	0.41, 0.39
Terbutaline		
Tablets (5 mg/tablet)	4.92, 4.89	4.68, 4.67
Ampoules (0.5 mg/ml)	0.51, 0.51	0.52, 0.50
Syrups (0.3 mg/ml)	0.31, 0.30	0.30, 0.30
Fenoterol		
Tablets (2 mg/tablet)	1.84, 1.86	1.88, 1.97
Respirator (5 mg/ml)	4.98, 4.92	4.89, 4.91

internal standard was employed in both analytical methods.

A particular feature of CE is that the capillaries are sufficiently rugged to enable direct injection of particularly "dirty" samples which would require extensive sample pretreatment prior to HPLC analysis. Drug levels have been determined from direct injection of plasma [22,32,49], urine [21,55] and syrup formulations [21].

Demonstrations of quantitative CE assays have largely originated from academic laboratories. A working pharmaceutical analysis report has been described in which a migraine treatment, sumatriptan, was quantified by CE [58] in solutions for injection. An internal standard was used for quantitation and external sumatriptan standards were used to obtain response factors. The CE method gave good performance in terms of precision (<1% R.S.D. for peak area precision), linearity and inter-day repeatability of both injection and analysis. Fig. 3 shows two replicate analyses of a sumatriptan sample solution. Results were simultaneously generated by both CE and HPLC [58] for four on-going stability batches of (12 mg/ml) injection solutions and compared well (Table VI). The excellent cross-correlation of HPLC and CE results for sumatriptan content suggests that CE could

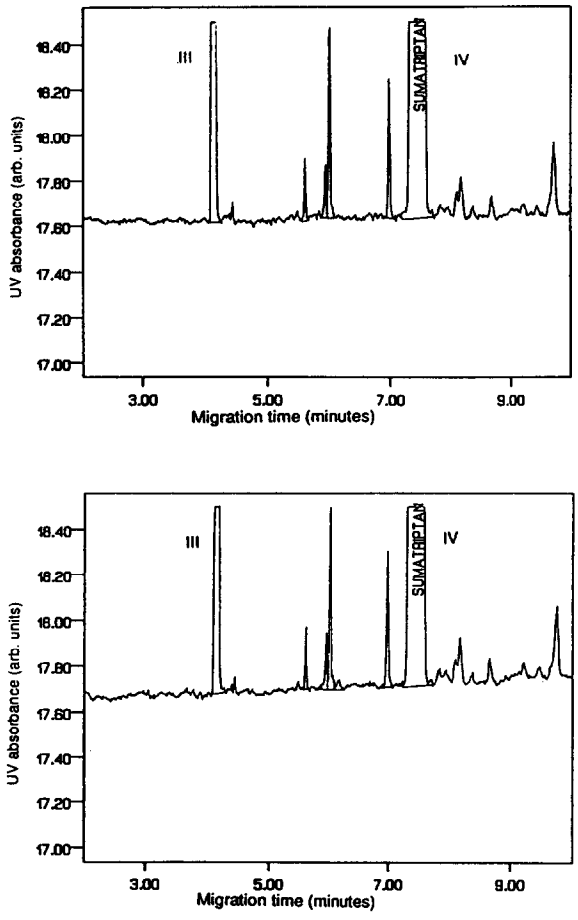

Fig. 3. Replicate CE separations of a sumatriptan sample solution. III = internal standard; IV = sumatriptan. Reproduced with permission from ref. 58.

be successfully employed for this and other quantitative main peak assays. In addition the good agreement between the results obtained by the two independent methods increases the validity of the results and could be considered as part of method validation for either method.

3.4. Chiral analysis

CE has been successful in enantioselectively separating several racemic pharmaceuticals. Several separation options have been reported and are given in Table VII.

A number of compounds have been separated including ephedrine, norephedrine [71], epinephrine [72], isoproterenol [71], terbutaline [73],

TABLE VI

DETERMINATION OF SUMATRIPTAN CONTENT BY CE AND HPLC FOR DIFFERENT FORMULATION BATCHES UNDER VARIOUS STORAGE CONDITIONS

From ref. 58.

Sample	Sumatriptan content (mg/ml)	
	CE	HPLC
Batch 2		
Condition 1 (aliquot 1)	11.5	11.6
Condition 1 (aliquot 2)	11.6	11.6
Condition 2 (aliquot 1)	11.6	11.7
Condition 2 (aliquot 2)	11.6	11.7
Batch 3		
Condition 1 (aliquot 1)	11.7	11.8
Condition 1 (aliquot 2)	11.8	11.8
Condition 2 (aliquot 1)	11.6	11.7
Condition 2 (aliquot 2)	11.6	11.7
Batch 4		
Condition 1 (aliquot 1)	11.7	11.8
Condition 1 (aliquot 2)	11.8	11.8
Condition 2 (aliquot 1)	11.7	11.7
Condition 2 (aliquot 2)	11.6	11.7

propanolol [66,67], trimequinol [70], dopa [69], and clenbuterol [68]. Similar levels of chiral recognision are often obtained in both HPLC and CE, however the superior peak efficiencies achieved in CE enables improved baseline separations to be obtained.

Peak area normalisation (section 3.1.) is required in chiral analysis as the later migrating enantiomer migrates more slowly through the detector giving an overestimation of peak area [61].

TABLE VII

MODES OF CHIRAL SEPARATION

Mode	References
FSCE with cyclodextrins (CDs)	65–68
FSCE with crown ethers	69
MECC with SDS and CDs	34
MECC with bile salts	70

3.4.1. *Performance of chiral CE*

The enantiomers of the bronchodilator clenbuterol have been resolved by CE [68]. A clenbuterol sample was injected 10 times and produced the performance data given in Table VIII.

The use of short capillaries for chiral high-speed CE separations is possible. The chiral FSCE separation of the racemate, of which picumeterol is the active enantiomer, has been reported [68]. A typical separation is given in Fig. 4a. This analysis was conducted utilising dimethyl-β-cyclodextrin as the chiral selector and applying 20 kV across a 57-cm (50 cm to detector) capillary. Fig. 4b shows the HSCE separation [74] of the racemate within a reduced analysis time of 3 min applying 13 kV across a 27-cm (20 cm to detector) capillary. Some resolution is sacrificed but this represents a reasonable and useful reduction in analysis time and could be applied to the monitoring of enantioselective synthesis or the enantiomeric purity of chiral drugs present in pharmaceutical formulations undergoing stability trials.

Nishi *et al.* [33] chirally resolved trimetoquinol using sodium taurodeoxycholate based MECC, an LOD of 1% of the inactive enantiomer was obtained. An LOD of 0.5% has been reported for 2′-deoxy-3′-thiacytidine (BCH189) [75].

TABLE VIII

PERFORMANCE OF CHIRAL SEPARATION OF CLENBUTEROL ($n = 10$)

The peak area data confirms the 1:1 ratio of the two enantiomers. From ref. 76.

	Precision (R.S.D.)
Migration time of enantiomer 1	1.3%
Migration time of enantiomer 1 relative to enantiomer	0.8%
Peak area for enantiomer 1	1.2%
Normalised peak area for enantiomer 1	0.8%
Peak area ratio of enantiomer 1 cf enantiomer 2	0.4%
Peak area ratio of enantiomer 1 and enantiomer 2	49:50

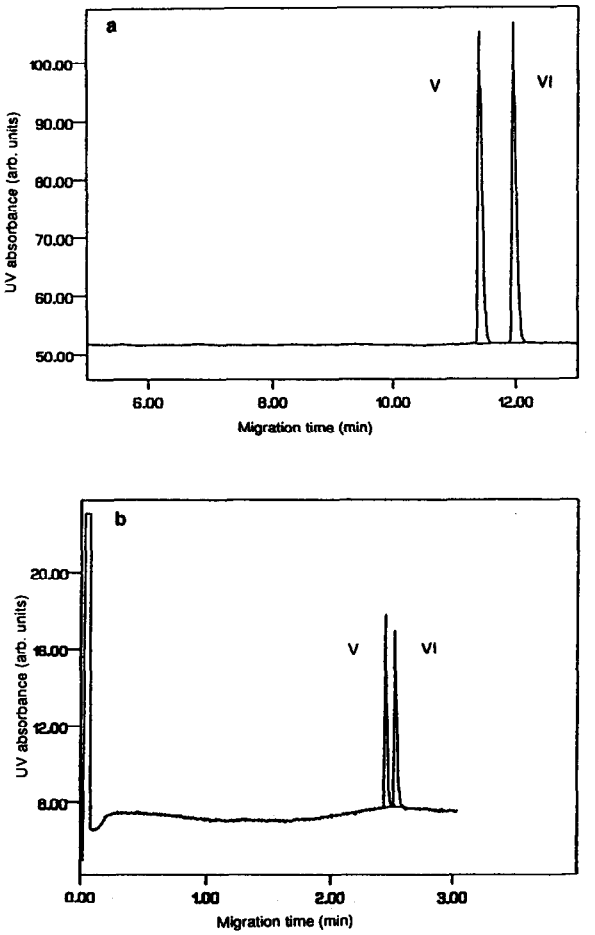

Fig. 4. (a) Chiral CE separation of picumeterol; (b) High-speed CE separation of picumeterol. V = (*R*)-picumeterol; VI = (*S*)-picumeterol. Unpublished data. Separation conditions: 50 m*M* borax with 30 m*M* dimethyl-beta-cyclodextrin (pH 2.2 with conc. H_3PO_4), sample concentration 0.5 mg/ml in water, 214 nm, 10-s pressure injection. Fig. 4a applying 20 kV across a 57-cm (50 cm to detector) 50-μm capillary. Fig. 4b as Fig. 4a except 13 kV applied across a 27-cm (20 cm to detector) 50-μm capillary.

3.4.2. Applications of chiral CE

FSCE has been reported [69] for the in-process control testing of the chiral purity of a precursor to a Sandoz drug with a limit of detection of 0.2% for the inactive enantiomer. Nishi *et al.* [33] employed MECC with sodium deoxytaurocholate micelles at pH 7.0 to determine the optical purity of five batches of trimetoquinol down to 1% of the *R* enantiomer in the presence of the *S*. Peterson and Trowbridge [72] performed quantitative chiral analysis of *l*-epinephrine in a pharmaceutical formulation using pseudoephedrine as an internal standard.

Rogan *et al.* [75] have also used CDs for the chiral separation of the anti-HIV drug BCH 189. The required (+) enantiomer is produced by an enzymatic biotransformation. The progress of biotransformation was monitored by chiral CE over a 51-h period. These CE data were used to calculate the reaction rate, order, and half-life.

Good agreement between the enantiomeric excess ratios for three batches of the single enantiomer compound picumeterol, as determined by both CE and HPLC has been obtained [76]. The HPLC method employed [68] for this analysis failed to give effective baseline resolution of the two enantiomers whilst the CE method enabled clear monitoring of the trace enantiomer (Fig. 5). An LOD of 0.1%area/area was obtained for the undesired enantiomer.

A CE method has been validated [77] for the enantiomeric purity determination of the enantiomers of fluparoxan. The method allowed determination of 1% of either enantiomer in the presence of its stereoisomer. Method validation showed adequate detector linearity over the required range. The method also gave good performance in terms of sensitivity for trace levels of the undesired enantiomers, injection precision and recovery.

An inter-company cross-validation exercise

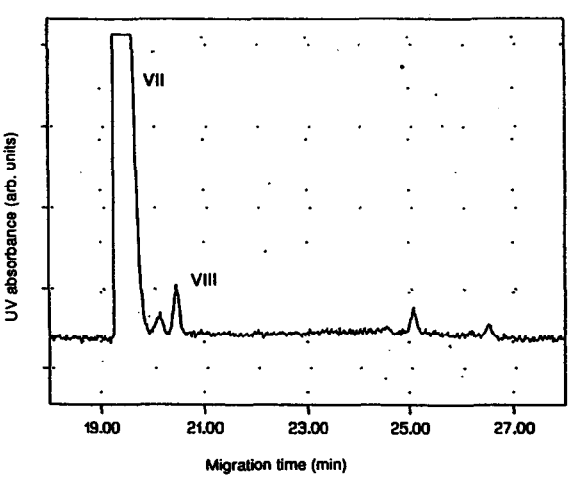

Fig. 5. CE separation of a single enantiomeric form of picumeterol from its stereoisomer. VII = (*R*)-picumeterol; VIII = (*S*)-picumeterol. Reproduced with permission from ref. 76.

has been conducted between seven pharmaceutical companies for a chiral CE method. Using a standard set of conditions each company was able to achieve baseline resolution, or greater, of the enantiomers of clenbuterol. Validation was simultaneously performed and acceptable levels of precision, accuracy, and linearity were achieved [78].

3.5. Micro-preparative CE

The micro-preparative use of CE has been reported to collect fractions, principally for protein and nucleotide separations [79–83]. The amounts collected are tiny, however sufficient material has been collected to enable protein sequencing.

Micro-preparative CE has been used [84] in pharmaceutical analysis to confirm peak homogeneity of a drug related impurity. An unrefined batch of fluparoxan drug substance was analysed by FSCE (Fig. 2b) and HPLC. Fractions containing a specific impurity were obtained by HPLC and CE. The fractions were then analysed by the alternative technique and peak identities confirmed by retention or migration time. Each fraction was shown to consist of a single peak at the position corresponding to the impurity of interest. CE and HPLC were used to quantify total impurity levels and levels of the selected impurity in three batches of fluparoxan drug substance and good agreement was obtained between the two techniques.

Due to the low amounts collected CE is not routinely considered for micro-preparative analysis. However, the advent of instrumentation in which numerous capillaries are employed in an array configuration [85] and the use of wider bore capillaries [86] may increase activity in this area.

3.6. Identity confirmation

Chemical identity confirmation is required following production of a batch of drug substance, or formulation. Typically, this is performed using a spectroscopic method and a separative method. Combinations of HPLC with NMR or IR are commonly applied testing regimes. The features of CE (section 4) make it an attractive alternative [87] to chromatographic methods for identity confirmation purposes. Use of a coinjection procedure can be employed [87] in a standard addition type operation to perform quantitative identity confirmation.

3.7. Stoichiometric determinations

Many drugs are converted to salts to produce the required solubility and physical properties. The extent of this conversion is typically determined by microanalysis or titrimetry. Capillary electrophoresis methods, exploiting indirect UV detection, have been developed [88] to determine inorganic anions and cations. Applying similar methodology it is possible [89] to determine levels of drug counter-ions such as chloride and sulphate with good precision, linearity and repeatability. Results obtained by CE are in accord with those generated by microanalytical techniques and ion exchange chromatography.

4. FEATURES OF CE METHODS

The relative merits of CE, HPLC and SFC have previously been considered [90] in depth. The main drawbacks and advantages of CE based analysis are discussed below.

The principal disadvantages of CE are instrument based and mainly relate to the precision and detection levels achieved. Typically the precision of injection is 1–2% R.S.D. for the main component. This is compared to HPLC where R.S.D.s of <1% can be obtained. To obtain improved precision levels in CE it may be necessary to incorporate internal standards. In addition the detection limits obtained in CE may be up to an order of magnitude less sensitive when directly compared to HPLC at a common UV wavelength. However, in CE it is possible to employ wavelengths as low as 185 nm where significant enhancements in UV absorbance activity are often possible. The preparative options available in CE are limited by the tiny volumes involved.

Some of the favourable features of CE compared to HPLC are that high separation efficiencies can be obtained which may enable resolu-

tion of closely related species. There are minimal sample volume requirements in CE. Multiple injections from as little as 10 μl can be achieved as typical injection volumes are in the order of 10 nl. Solvent and reagent consumption are reduced as daily reagent consumption is typically 20 ml of electrolyte. This represents considerable savings in terms of solvent purchasing and disposal. Sample pretreatment requirements may also be minimised as sample solutions can often be directly injected. The cost of capillaries is low compared to the expense of HPLC columns.

Overall CE should be viewed as a complementary and alternative technique to HPLC and should be employed for applications when appropriate.

5. FUTURE DEVELOPMENTS

As discussed in section 4 the current principal limitations of CE are instrumentation based. It is anticipated that the next generation of CE instrumentation will address the identified weakness areas. Emerging technologies will include the increased use of electrochromatography [91]. This technique involves the use of an electric field to drive solvent through a capillary packed with reversed-phase material [5]. Due to the nature of this electrically driven flow, high separation efficiencies can be obtained. It is anticipated that the commercialisation of capillary array instruments will be of great importance where high sample throughput is of concern.

6. CONCLUSIONS

Without doubt CE is an established analytical alternative and complement to HPLC in the area of quantitative drug analysis. CE is capable of generating high quality data with acceptable levels of precision, accuracy and linearity. Methods are capable of undergoing validation and are in routine use within pharmaceutical companies and CE data has been submitted to, and accepted by, regulatory authorities. There are several features of CE compared to other separative techniques and the choice of technique should greatly depend upon the nature and requirements of the application.

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