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Review

Determination of drug-related impurities by capillary electrophoresis

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Abstract

The use of capillary electrophoresis (CE) to determine drug-related impurities is becoming established within industrial pharmaceutical analysis laboratories. Increasingly CE is being viewed as an alternative for, and complement to, high-performance liquid chromatography (HPLC). This paper comprehensively reviews the progress of CE in drug impurity determinations subdividing the reports into low pH, high pH and MECC applications. The section covering method performance and validation clearly shows that CE methods are capable of validation in this area and can often give equivalent performance to HPLC methods. Possible benefits of adopting CE for this testing include reductions in costs and improved robustness. Potential developments are covered including the use of electrolyte additives, instrumental developments and the increased implementation of electrochromatography. It is concluded that the current status of CE is sufficiently strong to allow the analyst to view CE as a viable and attractive alternative to HPLC.

Keywords: Capillary electrophoresis; Drug-related impurities; Reviews; Pharmaceutical analysis; Drugs

Contents

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1. Introduction

Currently the vast majority of drug-related impurity determinations are performed by high-performance liquid chromatography (HPLC), which can offer the desired sensitivity for trace level determinations and offers a high degree of automation. A wide variety of stationary phases and operating modes makes HPLC applicable to all drug classes. The typical detection limits for drug-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. It is common practise to employ a secondary support analytical technique to verify HPLC impurity data. In the past this secondary testing has been largely performed by thin-layer chromatography (TLC). However, the high degree of automation that HPLC offers is not so readily available except in highly sophisticated TLC systems.

The advent of fully automated commercially available capillary electrophoresis (CE) systems has presented the pharmaceutical analyst with a new and viable analytical alternative to HPLC and TLC. The modern CE instruments are capable of the required sensitivity and precision, approaching that of HPLC. Autosamplers and on-line detection allow simultaneous assay of the main component and related impurities by CE, which is not a feature of TLC.

There is a distinct trend in drug discovery and development away from small organic molecules towards biotechnology derived products such as peptides, proteins and nucleic acid derivatives. These biomolecules have been traditionally analysed by gel electrophoresis and are particularly suitable for analysis by CE. Analysis of these biomolecules by CE is preferable to that by HPLC as CE can offer distinct advantages in terms of selectivity, sensitivity, speed and simplicity. Often pharmaceutical analysts are becoming familiarised with the operation, optimisation and performance of CE through their analysis of biotechnology products. This experience is then being applied to the analysis of conventional pharmaceuticals.

As in HPLC, there are distinct operational modes for CE and these are appropriate to specific drugs and drug classes. For instance free-solution CE (FSCE) using low pH electrolytes is appropriate for water-soluble basic drugs whilst FSCE with high pH (pH 7–10) electrolytes is appropriate for water-soluble acidic compounds. The chromatographic-type CE technique of micellar electrokinetic capillary chromatography (MECC) is applicable to all drug types including neutral and water-insoluble drugs.

Over the last four years a number of papers have appeared on the use of CE for the determination of drug impurities due to the advent of highly automated and sensitive capillary electrophoresis systems. Many pharmaceutical companies are investing heavily in capillary electrophoresis as this is viewed as a highly complementary technique to HPLC and the two techniques are often used in combination when assessing drug purity. The separation mechanisms are widely different in HPLC and CE; being partitioning and mobility differences, respectively. Therefore, a good agreement between HPLC and CE results strongly supports a comprehensive evaluation of a sample's purity.

This paper is the most comprehensive review to-date on the analysis of drug-related impurities by CE. Emphasis is placed on the various operational modes of CE for various drug types using a range of illustrative examples. The performance of CE is described in relationship to that routinely obtained by HPLC.

2. Background

Table 1 shows the wide range of drugs in which CE has been employed to determine related impurities. The three main separation mechanisms employed are low pH (for analysis of basic drugs), high pH (for analysis of acidic drugs) and micellar electrokinetic capillary chromatography (for the analysis of neutral and/or charged compounds). Various additional separation mechanisms have been employed and will be briefly discussed below for the three separation options.

Table 1
Reported application of CE to separation of drug impurities[a]

Compound(s)	Electrolyte composition	Sample	Comments	Ref.
Acetylcystein and impurities	FSCE, pH 8.6, 5% PEG 2000	Drug substance	Impurities detected at <0.05%	[1]
Alkaloids	FSCE, pH 4.5	Test mixtures	Detected by CE–MS	[2]
Amoxycillin and impurities	MECC, SDS	Drug substance	Deuterated and non-deuterated solvents compared	[3]
Anti-thromboxane agent and impurities	MECC, SDS with MeOH	Test mixture	High-temperature improved separation	[4]
Anti-viral and impurities	FSCE, pH 2.5	Drug substance	Experimental design for robustness studies	[5]
Atenolol and impurities	FSCE, pH 9.7	Tablets	Buffer depletion study	[6]
Benzylpenicillin and degradation imps	FSCE, pH 9	Gastric juices	Degradation measured with time	[7]
Capozide	MECC, SDS, MeOH	Drug substance	Z-cell used to monitor 0.01% impurity	[8]
Cefuroxime axetil and degradation imps	MECC, SDS	Formulations	Sample pretreatment reductions shown	[9]
Cephalexin and degradation impurities	MECC, SDS and ion-pair	Capsules	Stability indicating method	[10]
Codeine and by-products	FSCE, various pHs	Drug formulations	Impurities detected at 0.01% level	[11]
Dilitiazem and impurities	MECC, SDC	Tablets	Range of impurities monitored at 0.2–0.4%	[12]
Domperidone and related substances	FSCE, pH 4	Drug substance	Unknown impurity detected	[13]
Dothiepin	FSCE, pH 4.8, IPA and CD	Tablets	Experimental design used in method development	[14]
Enalapril maleate and rotamer	MECC, SDS	Tablets	Stability indicating method	[15]
Fluparoxan related impurities	FSCE, pH 2.5	Drug substance	Fraction collection of CE impurity peak	[16]
Fluparoxan and impurities	FSCE, pH 2.5	Crude drug substance	High-speed separation using short capillary	[17]
Fluparoxan and synthetic impurity	MECC, SDS	Drug substance	Separation of other drug substances and impurities within same report	[18]
Fluparoxan and 5 impurities	MECC, SDS and IPA	Test mixtures	Closely related impurities separated within 10 min	[19]
Fluticasone and impurities	MECC, SDS and MeOH	Test mixture	Additional impurity detected	[19]
Gentamycin and related impurities	FSCE, pH 9.4	Injection solutions	Additional impurities detected	[20]
Heroin impurities	MECC, SDS with DMSO or ACN	Drug seizures	Impurity profiles used for identification	[21]
Heroin impurities	MECC, SDS	Test mixtures	Range of impurities separated	[22]
Hydrochlorothiazide and chlorothiazide	MECC	Drug substance	Successfully validated to USP guidelines	[23]
Levothyroxine and impurities	FSCE, pH 2.5	Test mixtures	Various synthetic and degradative impurities separated	[24]
Minoxidil and impurities	FSCE, pH 3	Formulations	Impurity profile within 60 s	[25]
Opiod alkaloids and impurities	FSCE, CDs, pH 4	Crude samples and formulations	Electrolyte composition optimisation study	[26]

(*Continued on p. 46*)

Table 1 (*continued*)

Compound(s)	Electrolyte composition	Sample	Comments	Ref.
Pilocarpine and its degradation products	MECC	Test mixtures	Degraded samples analysed	[27]
Pyridine-4-carboxylic acid and impurities	FSCE, pH 3	Drug substance	0.1% LOD for related acids	[28]
Quinolone and impurities	FSCE, pH 2	Drug substance	Method validation performed	[29]
Ranitidine	FSCE, pH 2.5	Syrup formulation	Sample pretreatment reductions shown	[9]
Ranitidine and 6 impurities	FSCE, low pH	Drug substance and formulations	LOD of 0.01% for related impurities	[30]
Ranitidine and impurities	FSCE, low pH	Drug substance	High-speed separation using short capillary	[17]
Ranitidine and impurities	FSCE, low pH	Drug substance	Peak area normalisation study	[31]
Ranitidine impurity	FSCE, low pH	Solution stability	Solution stability study with time	[32]
Ranitidine	FSCE, low pH	High–low	Impurity determination at 0.01%	[33]
Remoxipride and related compounds	FSCE, pH 3, CDs and ion-pair	Drug substance	Impurities monitored at 0.1% level	[34]
Riboflavin-5-phosphate and impurities	FSCE, pH 9	Test mixtures	Range of electrolytes evaluated	[34]
Proptriptylene and others		Drug substance	20 mg/ml solution injected, 0.01% impurities determined	[35]
Salbutamol impurities	FSCE, pH 2	Drug substance	Cross-correlation between CE and HPLC	[37]
Salbutamol impurities	FSCE, pH 2	Drug substance	Peak identification by co-injection procedure	[38]
Salicylamide	MECC, SDS	Drug substance	Impurities determined at <0.1%	[39]
Sumatriptan and impurities	FSCE, pH 2	Injection solutions	Range of degradation and synthetic impurities	[40]
Tetracycline and degradation products	FSCE, pH 3.9, and EDTA	Test mixtures	Unknown impurities detected	[41]
Various impurities	MECC, SDS	Drug substance	Diastereoisomeric impurities detected at 0.005% level using Z-cell	[42]
Various drugs	FSCE, pH 7, and CD	Drug substance	Batches of drug substance analysed	[43]
Various antibiotics and impurities	FSCE and MECC	Drug substances	Suppliers identified by impurity profiling	[44]
Vasotec (antihypertensive)	MECC with Brij 35	Formulations	Stability indicating method	[45]
Xanthine and derivatives	MECC, SDS	Tablets	Impurities at 0.1% level and below	[46]

[a] FSCE = free solution capillary electrophoresis; MECC = micellar electrokinetic capillary chromatography; CD = cyclodextrin; SDS = sodium dodecyl sulphate (surfactant); EDTA = ethylenediaminetetraacetic acid; MeOH = methanol; IPA = isopropanol (propan-2-ol); DMSO = dimethylsulphoxide; ACN = acetonitrile; Brij35 = non-ionic surfactant.

2.1. Low pH

The majority of drugs are bases, being the chloride, hydrochloride or sulphate salts. These are generally water soluble and are ionised as cations at low pH. The use [5,16,17,24,29–32] of simple electrolytes such as phosphate or citrate at pH values of 2–3 exploits mobility differences of the impurities compared to each other and to the main drug. Often a simple 50 m*M* NaH_2PO_4 buffer pH adjusted to pH 2.5 with concentrated H_3PO_4 gives a useful initial separation. Analysis of basic drugs by HPLC can represent [47] a problem due to peak tailing. This problem does not occur so frequently in CE. For example, Fig. 1 shows separation of a range of synthetic and

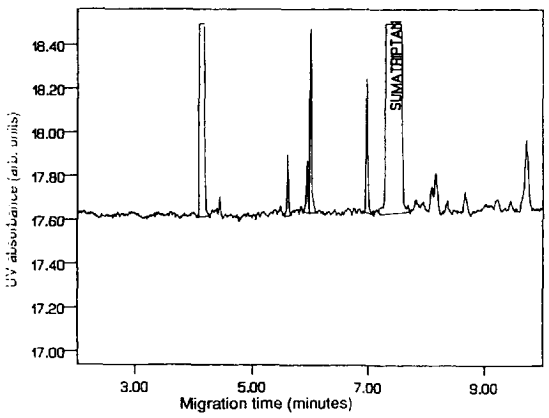

Fig. 1. Separation of sumatriptan and related impurities at low pH. Conditions: 50 m*M* borax, pH 2.2 with concentrated H_3PO_4; sample concentration, 0.5 mg/ml in water; 214 nm; 10-s pressure injection, 10 kV. (Reproduced with permission from Ref. [40].)

degradative impurities of sumatriptan, an anti-migraine compound. The large peaks are due to an internal standard and sumatriptan. The majority of degradation impurities and synthetic intermediates tend to be smaller than the drug compound and therefore migrate before the main peak. In addition, dimeric impurities may be doubly charged [38,40] at low pH and again migrate before the main peak, unlike in HPLC, where dimeric impurities are generally strongly retained. An additional advantage of these simple electrolyte systems is that they have low background UV absorbance and operation [29,38] at low UV wavelengths such as 190–210 nm is possible, where many drug compounds have significantly enhanced UV absorbance coefficients. Indeed, many small synthetic intermediates such as imidazole are very difficult to analyse by HPLC but present few problems [43] in CE at low pH with detection at 200 nm.

Various complexing agents may be added to the electrolyte to suitably alter the migration speed (mobility) of the drug and its impurities. The most frequently employed being the addition of millimolar quantities of cyclodextrin [14,17,26,34] into the electrolyte. The migration times obtained then reflect both the solute's electrophoretic mobility and its partitioning with the cyclodextrin. EDTA has also been employed [41] to suitably adjust the separation order of various tetracyclines by on-capillary derivatisation with the EDTA. The anionic complexes were resolved and directly quantified [41] by UV absorbance detection.

Other factors that can be optimised in method development include the addition of ion-pair reagents [34] and the ionic strength [34] of the electrolyte. Both of these influence the shape of the main peak and appropriate optimisation may allow resolution of a closely resolved impurity. For instance, various types and concentrations of ion-pair reagents were employed [34] in conjunction with addition of cyclodextrin in the optimisation of the separation of remoxipride. Addition of organic solvents [14] such as iso-propanol and methanol to the electrolyte can be useful but generally have a limited effect on resolution.

When attempting separation of acidic compounds, optimal resolution is often achieved at a pH close to the pK_a value of the various acids. For example, resolution of impurities of pyridine-4-carboxylic acid was maximised [28] at pH 3.

Water-insoluble basic compounds may often dissolve in a 1:10 dilution of the buffer or pH adjusted water. Amphoteric compounds may [29] be beneficially dissolved in a high pH solution, but analysed using a low pH electrolyte. Impurities have been determined in a water-insoluble amphoteric quinolone antibiotic [29]. The compound was only soluble at pH extremes of less than 2 and greater than 10. The sample was dissolved in NaOH solution and analysed with a pH 1.5 electrolyte. Generally samples should be dissolved in pure water if possible as this maximises resolution and peak efficiencies [13,16,29–32].

2.2. High pH

At high pH the migration direction of acidic components is against the electroosmotic flow (EOF), which maximises mobility differences. Operation with standard electrolytes [6,7,20,43] such as phosphate (pH 7) or borate (pH 9.5) often leads to useful initial separations for acidic compounds. As in low pH separations, selectivity

can be altered by addition of cyclodextrins, ion-pair reagents and organic solvents. Addition of organic solvents leads to a decrease in EOF and reduced ionisation of acids, which may be beneficial. Alternatively, the EOF can be reduced by increasing the viscosity of the electrolyte by the addition [1] of polymeric substances such as polyethylene glycol (PEG) or cellulose.

2.3. Micellar electrokinetic capillary chromatography (MECC)

This approach would be adopted when dealing with uncharged solutes or mixtures of charged and neutral species. This approach may also be considered when simple mobility differences prove insufficient in free-solution CE. The selectivity can be manipulated in a similar fashion as with those parameters employed in reversed-phase HPLC, and these include addition of cyclodextrin (CD), ion-pair reagents [10], organic solvents [4,9,21]. Additional selectivity manipulation can be achieved by varying the type and concentration of surfactant [12,36] or employing combinations [45] of surfactants.

Water-insoluble compounds are generally analysed using MECC. Samples can be prepared in 100% organic solvents, but this can produce problems of out-gassing when employing extended injection times. To minimise the potential difficulties it is best to prepare the sample in a solvent containing the minimum percentage of organic required to solubilise the sample. The sample should be soluble in the electrolyte used or on-capillary precipitation can occur. For example [36], levels of impramine N-oxide hydrochloride impurities were determined at the 0.01% level using a low pH electrolyte containing levels of the neutral surfactant Tween.

Fig. 2 shows separation [39] of a range of salicylamide impurities using an MECC method employing sodium dodecyl sulfate (SDS). A detection wavelength of 214 nm allowed detection of trace impurities at the 0.01% level.

2.4. Data handling

When calculating impurities as percent area/area it is necessary [31] to divide the peak area of each peak by its corresponding migration time prior to calculation. This accounts for the differential migration speeds of the peaks through the detector. This calculation is not required when quantifying impurities as percent w/w using response factors from standard solutions. If this correction is not performed, then peaks migrating before the main peak will be underestimated whilst later migrating peaks will be overestimated. This was experimentally demonstrated [31] using a solution of ranitidine spiked with a known amount of an impurity.

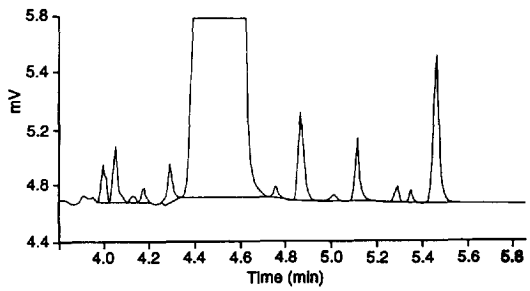

Fig. 2. Separation of a salicylamide solution by MECC. Electrolyte, 0.02 *M* sodium phosphate pH 11.0, 0.075 *M* SDS; capillary, 60 × 50 μm; voltage, 20 kV; sample concentration, 0.1 mg/ml in water; injection, 10 s. (Reproduced with permission from Ref. [39].)

3. Method performance and validation

3.1. Sensitivity

Much of the attention of reported applications has focused on attaining specific detection limits of 0.1% or less. Given the small injection volumes and on-capillary detection, sensitivity is generally not as good as with HPLC. Detection at 0.1% or lower can be optimised using low UV wavelengths, high sample concentrations, high–low injections, and wide-bore capillaries (100 μm or greater) or modified capillaries. For example, salbutamol impurities at 0.02% were monitored [37] by CE employing a detection wavelength of 200 nm. Levels of hydrochlorothiazide impurities were monitored [23] using a 100-μm capillary. Levels of xanthine impurities were determined [46] by MECC using injections of a 20 mg/ml solution with detection at 214 nm. Impramine

N-oxide hydrochloride impurities at the 0.01% level were measured [36] by injection of 20 mg/ml sample solutions.

Many of the sensitivity limitations are due to the limited linear dynamic range of the detector. To overcome this detector problem, as in HPLC, high–low injections may be performed [33]. In this procedure a "low" injection (short injection time or dilute sample) is conducted [33] in which the main peak is on-scale and its peak area can be recorded. A "high" (longer injection time or more concentrated sample) injection is then performed in which low-level impurities can be detected and the main peak is off-scale. These impurities are quantified against a calculated peak area. Using this high–low approach, levels of 0.01% area/area could be detected for impurities of fluparoxan and ranitidine. Fig. 3 shows both a 2- and 10-s injection of a ranitidine solution. The main peak was on-scale for the 1-s injection and off-scale for the 10-s injection. The impurity detection limit [33] for the off-scale injection was calculated as 0.01% area/area.

An extreme sensitivity of 0.0005% w/w was reported [35] for a strongly fluorescent impurity of remoxipride detected by laser-induced fluorescence. Levels of less than 0.05% were detected [1] for acetylcystein impurities using a high sample concentration with an overloaded peak. Use of a Z-cell capillary enabled [8] improved detection levels of 0.01% to be obtained for capozide.

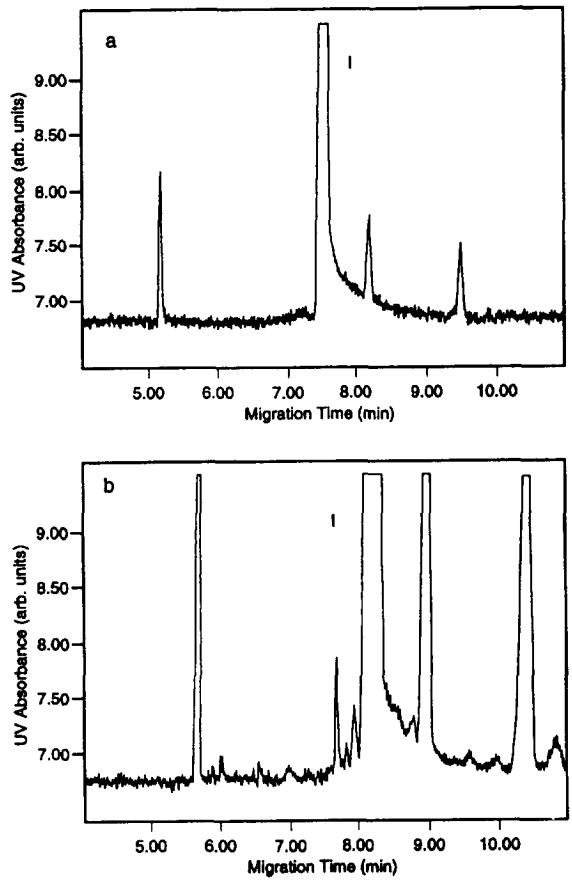

Fig. 3. Injections of 2 and 10 s of a ranitidine sample solution. (a) For 2 s loading of degraded ranitidine solution; (b) 10 s loading. Peak I = ranitidine. Electrolyte: 50 m*M* borax pH adjusted to 2.5 with H_3PO_4 and 2 m*M* hydroxypropyl-β-CD. (Reproduced with permission from Ref. [33].)

3.2. Linearity

Acceptable detector linearity of detector response with impurity level has been demonstrated for several compounds such as a quinoline antibiotic [29] and salbutamol [38]. This is measured by spiking drug substance with known amounts of the isolated impurity.

3.3. Precision

As in HPLC, the precision of peak area is highly related to sample concentration; therefore a high R.S.D. value would be expected for trace-level impurities. This is indeed the case, with typical R.S.D. values of ca. 10% obtained for salicylamide [39] and ranitidine [33] impurities at 0.1% or lower levels.

Migration-time precision of 1% R.S.D. for repeated injections has been shown [23,29,46], which is essential to allow confirmation of the exact identity of individual impurities present. Relative migration times generally give a better repeatability, with R.S.D. values of less than 1% reported. For instance, during the validation of a CE method for determination of impurities in a quinoline antibiotic [29] a single sample was injected 10 times and precision values of 0.4% and 0.6% R.S.D. were obtained for migration time and peak area, respectively. Use of an electrolyte with good buffering capacity was

Table 2
Precision data for ten injections of different xanthine derivatives

Compound ID	MT (%R.S.D.)	Area (%R.S.D.)	NA (%R.S.D.)
9	0.36	0.56	0.55
2	0.10	0.40	0.38
10	0.16	1.09	1.13
1	1.03	0.63	0.67
7	0.91	0.68	1.07
12	0.95	1.12	1.07
11	0.97	1.48	0.85

$n = 10$. MT = migration time. NA = normalised area (i.e. area of peak divided by its corresponding migration time). (Reproduced with permission from Ref. [46].)

shown [6] to give good precision values for atenolol and related impurities when determined in tablet preparations. Table 2 shows precision data [46] for analysis of xanthine derivatives.

It is important to employ an electrolyte with sufficient buffering capacity to obtain these consistent migration times and selectivities. Repeated analysis using the same electrolyte vials can cause a build-up [6,48] of electrolysis products, which may cause a change in both pH and composition which may alter selectivity (this effect is known [6] as buffer depletion). In some instances this may necessitate use of individual electrolyte vials [23] for a set number of injections.

3.4. Robustness studies

Robustness testing has been performed [5] on a CE method used to determine impurity levels in an anti-influenza drug. The robustness testing was performed [5] using appropriate experimental designs to statistically evaluate the effect of factors such as pH, electrolyte concentration, injection time and temperature, over predetermined ranges. A number of other CE methods have been examined for determination of hydrochlorothiazide [23], gentamycin [20] and atenolol [6].

3.5. Cross-validation

The results generated by CE are generally compared [13,16,32,37] with those of HPLC and/or TLC in order to demonstrate accuracy. Good agreement has been reported for ranitidine [32], an undisclosed drug substance [42], fluparoxan [16] and salbutamol [37]. Ten batches of salbutamol sulphate were analysed (Table 3) by CE and HPLC for levels of specific dimeric impurities at levels ranging from 0.05–0.5% w/w. Statistical analysis of the results [37] showed them to be equivalent.

Table 3
Levels of salbutamol impurities in drug substance determined by CE and HPLC

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

Reproduced with permission from Ref. [37].

However, CE and HPLC results did not agree for analysis of domperidone [13] and various tetracyclines [41] as CE resolved previously co-eluting impurities. CE also resolved previously unobserved impurities of the steroid fluticasone propionate [19] and gentamycin [20]. In the case of gentamycin the USP registered HPLC method involves a pre-separation derivatisation which is not selective to all impurities. The gentamycin CE method employed direct detection at low UV wavelength, which allowed quantitation of all impurities present.

3.6. Peak identity confirmation

Generally this is performed by matching the relative migration time of an impurity in the sample to that of a standard of the impurity. 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 [38] by on-line spiking with a solution of the specific degradation impurity. This can be programmed into the separation method. Fig. 4a shows the separation [38] of a salbutamol sample solution which also contains two early migrating dimeric impurities. Fig. 4b shows the separation obtained from a 5-s hydrodynamic injection of the salbutamol sample solution followed immediately by a 5-s hydrodynamic injection of a solution of the impurity of interest. Following both injections the voltage was applied and the separation given in Fig. 4b was produced. The impurity identity is clearly confirmed and no loss in resolution is observed from this dual-injection procedure.

Diode-array detection has also been utilised [49] in impurity peak identification. Fig. 5 shows the use of this detector to measure the spectra of a 0.1% impurity. In conjunction with the on-line spiking procedure discussed this would give added confidence in peak assignments.

The linking of CE to mass spectrometers allows [2] determination of molecular-mass information regarding impurities. Preparative use of CE allows collection [16] of the impurity, which can then be analysed further by HPLC or an additional analytical technique.

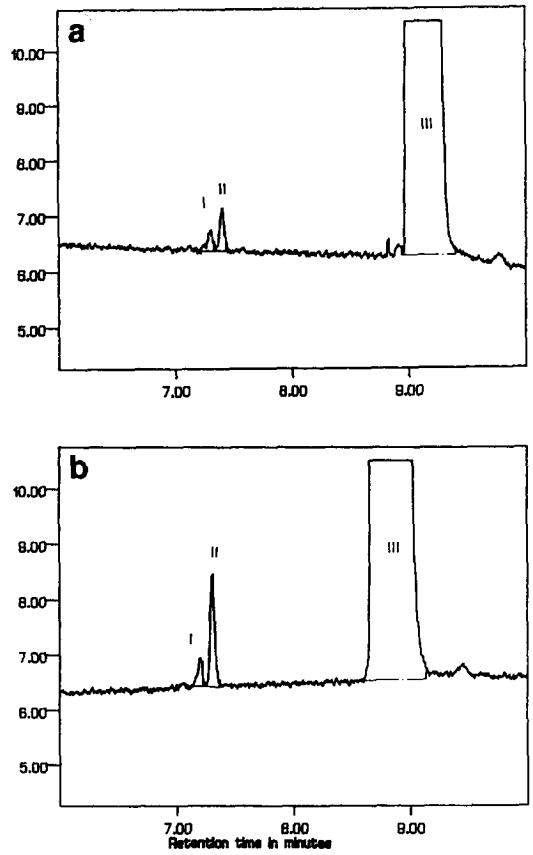

Fig. 4. Determination of salbutamol impurities by co-injection. (a) CE separation of a 1 mg/ml salbutamol solution, (b) separation of a 1 mg/ml salbutamol solution spiked with 0.5% (w/w) bis-ether by co-injection. Electrolyte, 20 m*M* Na citrate pH 2.5; voltage, 30 kV; detector, 200 nm; capillary, 75 × 57 μm. I = Side-by-side impurity; II = Bis-ether impurity; III = Salbutamol. (Reproduced with permission from Ref. [38].)

4. Comparison of CE with TLC and HPLC

CE can have considerable benefits for analysis of related impurities. Table 4 shows a comparison of CE, HPLC and TLC. Specific points from the table are discussed below.

Combinations of HPLC, TLC and CE give widely differing selectivities and present a good overall assessment of product purity. Preconditioning of CE capillaries is relatively rapid and is in the order of 2–3 min. Often a single capillary can be used to analyse a range of different compounds which are separated using similar electrolytes. Preparation of mobile phase for

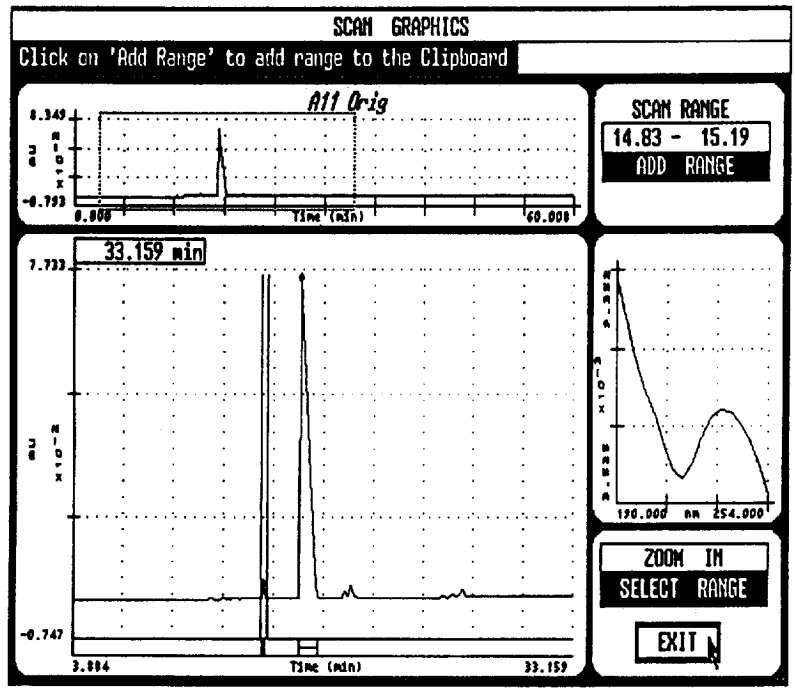

Fig. 5. Spectra of 0.1% impurity determined using a diode-array detector. Separation conditions: electrolyte, 50 m*M* NaH_2PO_4 pH 2.1; injection, 10 s; detection, 230 nm; voltage, 10 kV; temperature, ambient; capillary, 50 μm × 47 cm; concentration, 1 mg/ml of a basic drug dissolved in water; Beckman P/ACE 5100. (unpublished work).

Table 4
Comparison of HPLC, CE and TLC

	HPLC	CE	TLC
Sensitivity	+++	++	++
Precision at low levels	+++	++	+
Low UV wavelength detection	+	+++	+
Indirect UV detection	–	++	–
Preparative isolation	++	–	++
Running costs	+	+++	+
Sample preparation needs	++	++	++
Main peak assay	+++	++	–
Automation	+++	++	+
Robustness	++	++	++
Analysis time (1–5 samples)	+	++	+
Analysis time (5–50 samples)	++	++	++
Column etc. costs	+	+++	+
Experience	+++	+	+
Selectivity	++	++	++

Key: – poor; + acceptable; ++ good; +++ excellent.

TLC and HPLC can be a time-consuming daily task whilst CE electrolyte can be pre-prepared and stored for three months in many cases. As in HPLC, the use of short columns can considerably reduce analysis time. The typical CE capillary length is 40–60 cm. Reducing this to 20–25 cm dramatically reduces analysis time as the field strength (V/cm) is increased whilst the distance along the capillary to the detector is considerably shortened. Shortening the capillary [17] increases internal Joule heating and minimises time for resolution. Both factors lead to a decrease in separation performance, as shown in Fig. 6 for analysis of fluparoxan using a 57- and 27-cm capillary. Some resolution is lost [17] but the short capillary gives a good indication of purity and would be useful as a quality control type analysis or an in-process check.

The capillary format makes operation robust to the nature of the sample matrix and composition. The capillary can be rinsed with acid and/or base between injections to remove un-

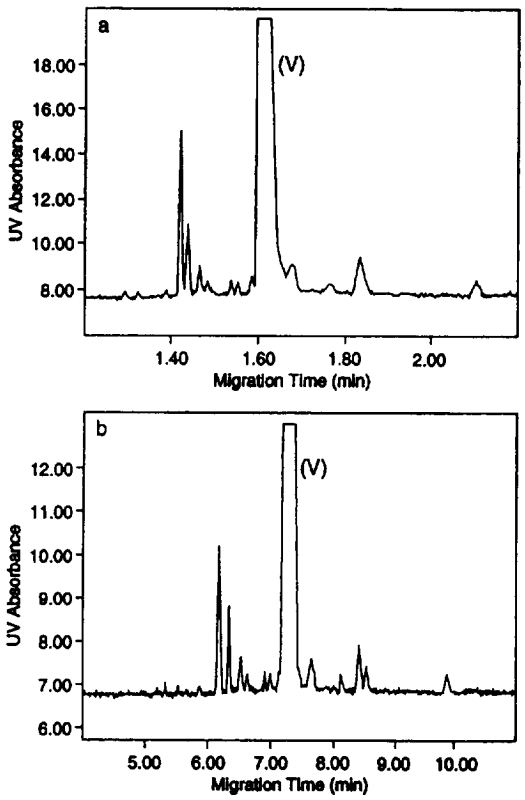

Fig. 6. Analysis of fluparoxan using capillaries of different lengths. Conditions: 50 m*M* borax, pH 2.2 with concentrated H_3PO_4; sample concentration, 0.5 mg/ml in water; 214 nm; 10-s pressure injection, 10 kV. Peak V = fluparoxan. (a) 27-cm capillary, (b) 57-cm capillary. (Reproduced with permission from Ref. [17].)

quantified sample components. Strongly retained sample constituents on HPLC may require use of gradients or extensive analysis times. For example, ranitidine impurities have been determined [9] in syrup formulations where the samples are simply diluted with water and analysed at low pH. Under acidic conditions ranitidine and its impurities are protonated and migrate along the capillary, the excipients are uncharged and are removed from the capillary by incorporating a rinse step between injections. Also, as discussed previously, operation at low UV wavelengths allows direct monitoring of compounds and impurities having only limited chromophores. This possibility can eliminate [20] the time-consuming need to derivatise samples.

The methods generally employ aqueous buffer systems with daily buffer requirements of ca. 20 ml. This compares very favourably [50] to the cost of purchase and disposal of the quantities of organic solvents employed in HPLC. Capillaries are generally plain fused-silica and cost a fraction of that of an HPLC column. Since the selectivity is independent of the capillary, method transfer is independent of the capillary, and method transfer is easier as highlighted by a series of successful inter-company method transfer exercises [51–53].

5. Applications

A recent survey [54] of a number of pharmaceutical companies in both the UK and US showed that determination of drug impurities constituted 24% of the workload of CE within pharmaceutical companies. The survey also indicated [54] that CE methods had been successfully included in marketing submissions to regulatory authorities.

The majority of attention within the literature has been paid to the purity testing of batches of drug substance such as domperidone [13], hydrochlorothiazide [23] and salbutamol [37]. Levels of related impurities have also been established in pharmaceutical formulations containing various drugs including atenolol [6], codeine [11], ranitidine [30], dilitazem [12] and sumatriptan [40].

Analysis of both stored drug substance and formulations constitutes a considerable workload within pharmaceutical analysis laboratories. A number of stability indicating CE methods have been reported and applied [23,45] to both stored drug substance and various formulations.

The use of CE in quality control has been shown [23] and the possible replacement of existing HPLC methods current used for testing was discussed.

CE has also been used to profile the constituents of heroin seizures [21] with good cross-correlation with HPLC. Levels of individual heroin impurities were shown [21] to be indicative of the synthetic route and source of the

heroin material. In a similar activity CE has been used [43] by FDA investigators to chemically profile bulk pharmaceuticals from various manufacturers. Batches of various drug substances such as antibiotics were obtained from different pharmaceutical suppliers and analysed by CE [43]. The impurity profiles in terms of the number of impurities and their respective levels were indicative of the suppliers.

In situ solution stability has been performed [32] in which a sample solution is placed on the autosampler tray and repeatedly analysed. The rate of solution degradation was followed for a specific impurity of ranitidine. The solution was placed on the CE autosampler and sequential injections programmed. The reaction was followed [32] unattended over 9 h until only 2% of the original component remained. A similar approach using off-line sampling has been conducted [7] to monitor decomposition of benzylpenicillin in gastric juices. It is also possible to control temperature on selected autosamplers, therefore temperature controlled reactions could be performed.

6. Future developments

There are three immediate developments within CE that are likely to assist in the increased use of CE for drug-related impurity determinations. These are increased selectivity options, instrumental improvements and the development of electrochromatography.

The selectivity that is often required for resolution of very closely related impurities from each other and the main component is often beyond simple FSCE. Therefore, the use of additives is becoming increasingly popular. Many of these additives are specifically manufactured for use in CE and include [55] chemically modified cyclodextrins. The addition of triethanolamine to low pH electrolytes has been shown [56] to be beneficial in reducing solute adsorption problems and improving peak symmetry. Fig. 7 shows an expanded section of the separation of a basic drug (having structural similarities to sumatriptan) using a phosphoric-triethanolamine electrolyte. The limit of detection for the trace impurities was established as 0.01% of the main peak. Other selectivity options also include [57] coated capillaries.

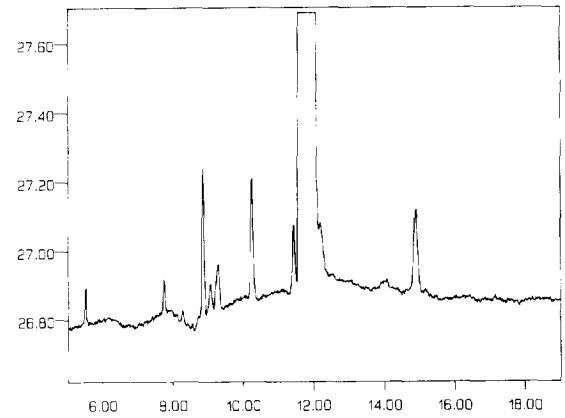

Fig. 7. Separation of the impurities of a basic drug using a triethanolamine-phosphoric electrolyte. Conditions: 50 m*M* phosphoric adjusted to pH 2.5 with triethanolamine; sample concentration, 0.5 mg/ml in water; 230 nm, 2-s pressure injection, 10 kV; 37 cm × 50 μm capillary; Beckman P/ACE 5100 (unpublished work).

Instrumental developments will also urge development and increased utilisation. Commercial instruments have only been available since 1988, but already second generation instruments have become available with improved sensitivity and diode-array options [49]. Capillaries with increased path lengths are also commercially available [8] to provide improved sensitivity.

Electrochromatography is a hybrid between CE and HPLC and involves [58] application of high voltages across CE capillaries which are filled with HPLC packing material. The superior flow profile obtained in CE can result [58] in very high separation efficiencies achieved with similar selectivity to HPLC. Fig. 8 shows the separation [58] of a prostaglandin from six of its related impurities. The number of reports and experience with this technique are somewhat limited, as work is currently performed using home-packed capillaries. However, it is anticipated that the advantages that come with use of electrochromatography will make it a viable addition to drug-related impurity determination methods within the next 5 years.

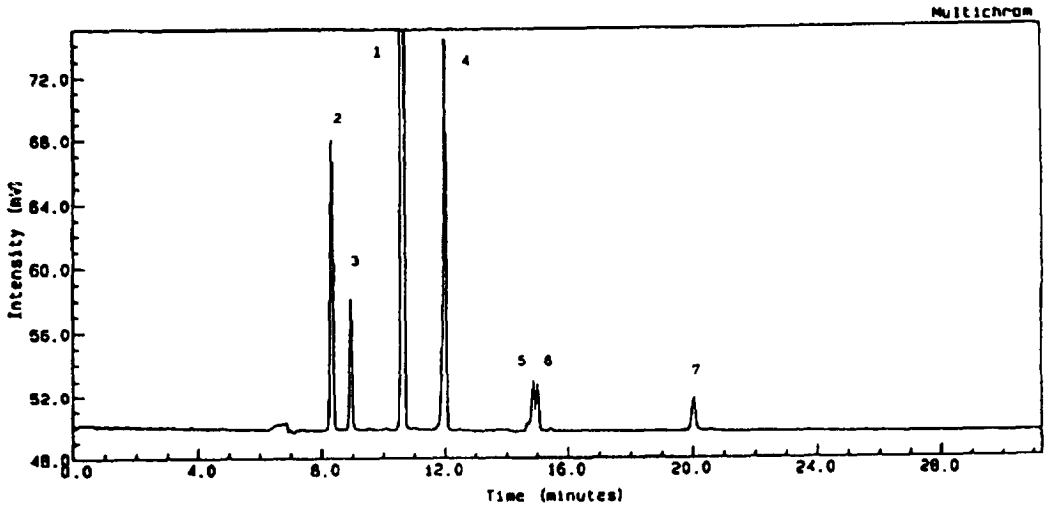

Fig. 8. Separation of a prostaglandin from six related impurities by electrochromatography. Conditions: 70% ACN–30% 10 m*M* Na_2HPO_4 (pH 9.9); 40 cm × 50 μm capillary filled with 1.8 μm Zorbax SBC8; 270 nm; 30 kV. (Reproduced with permission from Ref. [58].)

7. Conclusions

Capillary electrophoresis based methods of analysis have become established as useful alternatives and supplementary methods to HPLC in the assessment of drug-related impurity levels. Combinations of HPLC and CE provide a valuable confirmation of results as their selectivities are based on different principles, unlike HPLC–TLC combinations or combinations of different HPLC methods. Well-controlled CE methods can give performance characteristics equivalent to the HPLC method. This parity enables the analyst to critically choose between the two techniques dependent upon their specific performance and the requirements of a particular application.

The benefits of CE in this area compared to HPLC may include reduced analysis time, improved robustness, reduced sample pre-treatment requirements and savings in terms of consumables and purchase and disposal of organic solvents. CE methods have been successfully included in applications for regulatory authorities. Undoubtedly CE will become more firmly established within pharmaceutical analysis for related impurities determination assay as familiarisation and training becomes more widespread across the pharmaceutical industry.

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