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Review

Analysis of acidic compounds using capillary electrochromatography

K.D. Altria[a,*], N.W. Smith[b], C.H. Turnbull[a,b]

[a]*Analytical Sciences, GlaxoWellcome R & D, Park Road, Ware, Herts. SG12 ODP, UK*
[b]*SmithKline Beecham Centre for Analytical Sciences, Chemistry Department, Imperial College of Science, Technology and Medicine, Exhibition Road, South Kensington, London SW7 2AY, UK*

Abstract

Capillary electrochromatography, CEC, is a hybrid of CE and HPLC and is rapidly gaining interest as a potential complementary technique. This paper provides an overview of literature concerning the separation of acidic compounds by CEC which fall into three distinct groups. These groups are those performed using capillaries packed with novel or unique stationary phases designed for CEC, and a smaller group where standard HPLC stationary phases packings such as ODS has been used. The third group involves the use of surface coated capillaries. This paper reviews the separation of acidic compounds by CEC and also includes a number of novel applications to illustrate the separation approaches and the analytical performance possible.

Keywords: Reviews; Inorganic anions; Amino acids; Aromatic acids; DNA; Acidic pharmaceuticals

Contents

*Corresponding author.

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1. Introduction

A number of review articles [1–4] have discussed the development and uses of capillary electrochromatography (CEC). This review is intended to focus primarily on the application of CEC to the analysis of acidic compounds. The majority of the work that has been presented to date in CEC reports on the separation of polyaromatic hydrocarbons (PAHs) [1–8] and other neutral compounds such as steroids [9,10]. CEC offers a number of advantages over other techniques, due to the unique separation mode which utilises a combination of both chromatographic and electrophoretic principles. The ability to use columns packed with small particles (1–3 μm) allows for higher efficiencies than those routinely seen with high-performance liquid chromatography (HPLC). These smaller particles can be used as electroosmotic flow (EOF) is used to drive the mobile phase through the packed capillary. In pressure-driven HPLC, problems with high back pressures occur when small particle sizes are used. Other advantages are the possibilities of using low UV wavelength detection, as the short pathlength used in CEC allow wavelengths of 190 to 200 nm to be used. The high percentages of organic solvents routinely used in CEC allow for the effective separation of water-insoluble and neutral compounds. Capillary electrophoresis (CE) using the addition of micelles can separate these neutral compounds but these buffers are not compatible with mass spectrometric detection. However the coupling of CEC with mass spectrometry has been shown to be a useful [9,10] means of detection.

The analysis of water-insoluble drugs by CE is often problematic but the use of non-aqueous CE is gaining interest as an alternative method of analysis [11]. Non-aqueous CEC conditions have been used [12] in the separation of triglycerides using a ternary mobile phase with UV detection at 190 nm.

The technique of CEC is still in a development phase [13] and as such there are many areas such as column packing, mobile phase compositions and application ranges that remain to be fully explored.

The range of buffers used in CEC is similar to those employed in CE. Inorganic buffers such as phosphate and borate were a popular choice in early CEC work. However, many research groups now use zwitterionic buffers [14] such as 2-(*N*-morpholino)ethanesulfonic acid (MES) and tris(hydroxymethyl)aminomethane (Tris). The use of zwitterionic buffers reduces [14,15] buffer depletion effects. These zwitterionic buffers also generate low currents during separations and these low operating currents reduce the formation of air bubbles in the capillary. The production of air bubbles during operation is the main technical problem with CEC.

The reported separations of acidic species by CEC vary greatly and this paper examines the different column technology and approaches used. The quantitative use and application of CEC to the determination of acidic species are discussed and illustrated with novel applications. The types of acidic compounds that have been analysed include small anions, amino acids, DNA, aromatic acids and acidic pharmaceuticals.

2. Column technology and separation approaches

Broadly the types of packed column used in CEC can be divided into two groups. The first being commercially available standard HPLC packing materials types; whilst the second involves stationary phases specifically developed for use in CEC. The use of surface coated capillaries is a further option in the area of CEC although recent reports have tended to concentrate on packed capillaries.

2.1. CEC capillaries packed with standard HPLC packings

Originally [1–4] much of the pioneering research in CEC was performed using separations of uncharged PAHs on C_{18} stationary phases. These separations were accomplished using high pH electrolytes and standard ODS type packings. High pH values are used to ensure a fast EOF which reduces the analysis times.

2.2. "Ion-suppressed mode"

It is difficult to separate acidic compounds using

standard stationary phase. The negative charge on the silanols on the packing material repels the negatively charged analyte. Therefore there will be little tendency for the compound to be retained by the stationary phase. The anionic ionised acid will therefore attempt to migrate towards the anode (Fig. 1a). If the EOF is sufficiently strong then the acids will eventually be detected with long retention times. However, if the acid has a sufficiently high mobility, or the EOF is slow, then the peaks will be undetected. To overcome these problems CEC has been operated in ''ion-suppressed'' mode where low pH electrolytes are used. The pH of the buffer should be well below the pK_a value for the acidic compounds. In these circumstances the compounds are uncharged (Fig. 1b) and interact with the stationary phase. However the EOF rate is poor at low pH which results in long analysis times. These analysis times can be reduced if high voltages are applied across short capillaries, or if short packed lengths are used. For example [7] acids have been separated at low pH on ODS packing with only a 7 cm packed bed before the detector. This short packed bed approach has also been demonstrated [16] for the analysis of PAHs using a non-porous silica stationary phase. Fig. 2 shows the separation of eight acidic drugs using a short (7 cm) packed bed of ODS material in a short (27 cm) total length capillary. This separation was achieved in the ion-suppressed mode as the pH of the electrolyte used was well below the pK_a of the acidic functions on the drugs separated. A low UV wavelength of 200 nm was used to optimise sensitivity. Table 1 shows the structure of the compounds and selected pK_a values where available.

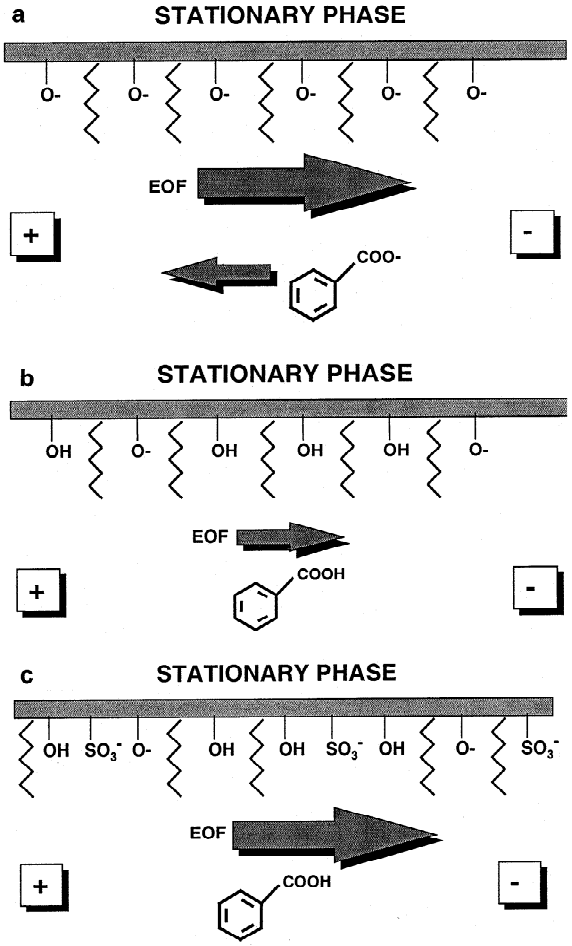


Fig. 1. Schematics of CEC separations using various pH values and stationary phases. (a) CEC separation of acids at high pH on standard phase. (b) CEC separation of acids at low pH on standard phase. (c) CEC separation of acids at low pH on mixed mode phase.

2.3. Ion exchange and ''mixed mode'' phases

Highly focused peaks have been obtained [17] for basic drugs when ion-exchange phases have been used in CEC. The SCX stationary phase used contains sulfonic acid functional groups, which are ionised and therefore negatively charged over a wider range of pH values. These columns therefore generate acceptably fast EOF rates at low pH and can be used successfully in the ion-suppressed mode. CEC specific phases have been developed in which the silica particles are coated with a mixture of sulfonic acid and alkyl chain moieties. These ''mixed mode'' columns offer the possibility of performing CEC separations of acids at low pH (Fig. 1c) with reasonable analysis times. A number of mixed mode stationary phase materials have been developed [18] included SCX/C_6, SCX/C_{18} and SCX/phenyl.

The repeatability of the analysis of a test mix of the eight acidic drugs separated in Fig. 2 was examined using a short end column and precision values of between 0.09 and 0.3% R.S.D. were obtained for relative migration time and 2–5%

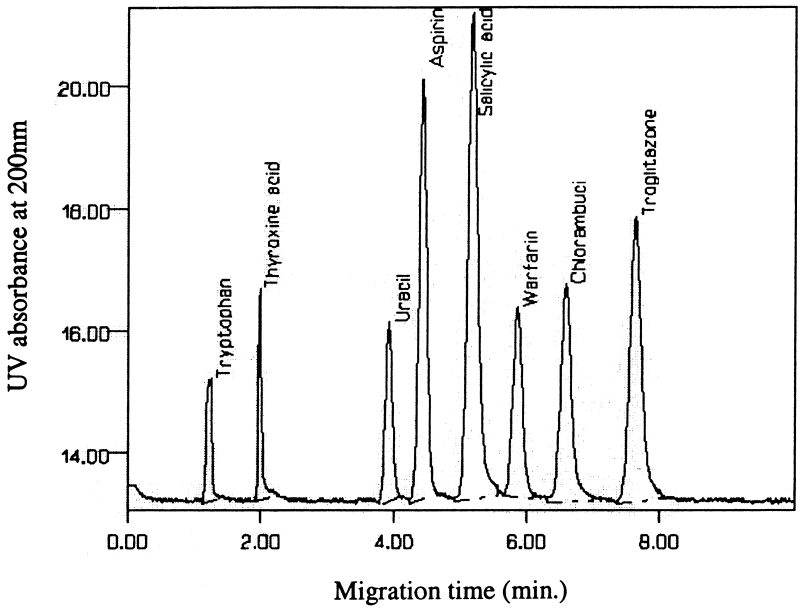


Fig. 2. Eight acidic pharmaceuticals separated at low pH. Separation conditions: column dimensions 7 cm×50 μm packed length (total length 27 cm) 3 μ ODS, supplied by Capital HPLC, injection: 3 kV for 2 s, separation 7 kV for 15 min at 200 nm, mobile phase acetonitrile–methanol–phosphate buffer (20 m*M*, pH 1.5 with phosphoric acid) (50:20:30, v/v). Sample dissolved in the mobile phase.

R.S.D. for peak area ratios. The eight acids could be separated in less than 8 min with a mobile phase at pH 1.5 using a 7 cm packed bed of SCX/C_{18}. This compared to an analysis time of 15 min in the standard C_{18} ODS phase.

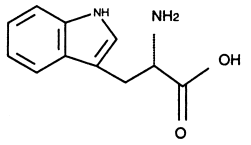
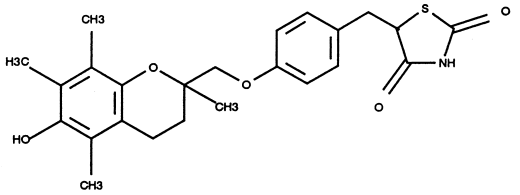
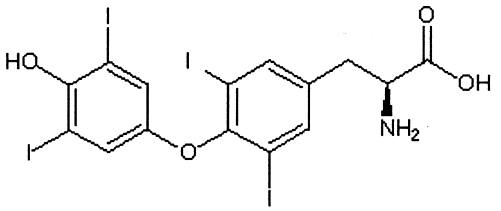
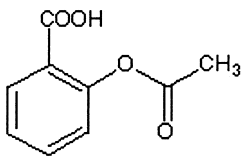
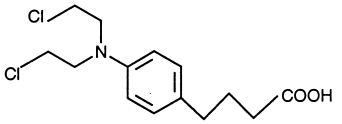
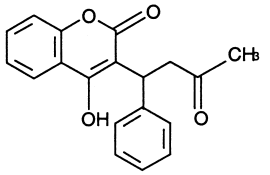
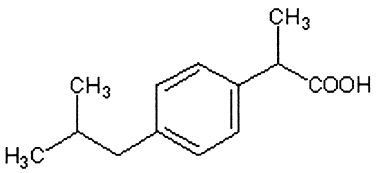
2.4. Chiral stationary phases

One of the earliest examples of chiral separations in CEC was performed using a specifically developed β-cyclodextrin stationary phase [19]. A number of anionic species were chirally resolved which included selected dansylated and dinitrophenyl amino acids using a pH 4.7 mobile phase containing 10–20 m*M* triethylammonium acetate (TEAA). A comparison was made between using phosphate or TEAA as the buffer. Phosphate buffer gave faster and more efficient separations but with reduced resolution.

Chiral separation of amino acids was also performed [20] using molecularly imprinted polymer filled capillaries. These polymers are prepared using solutions containing monomers and a ''print molecule''. The print molecule is removed after polymerisation. This removal leaves holes in the polymer, which are the shape of the print molecule. These holes will then be selective to inclusion of the print molecule. Therefore if the L-form of a chiral compound is used then the D-form will be less retained and this can lead to the possibility of achieving chiral resolution. Enantioselective polymers using L-aromatic amino acids as the print molecule were prepared at three different temperatures: 4°C, 40°C and 60°C. The polymers were then ground and sieved, and packed into 75 μm I.D. capillaries. It was observed [20] that the temperature at which the polymerisation was initiated directly affected the resolution of the separation of the D- and L-enantiomers, with the lower preparation temperature giving the best resolutions.

Macrocylic antibiotics such as vancomycin and teicoplatin have been used as chiral stationary phases in HPLC. Miyawa and Alasandro [21] chirally resolved ibuprofen and phenylalanine by CEC using a custom-packed 10 μm d_p teicoplanin column. These columns were used in both reversed- and

Table 1
Acidic pharmaceuticals analysed by CEC

Pharmaceutical	pKa	Use	Structure
Tryptophan	pK_1 2.83 pK_2 9.39	Treatment of neuropsychiatric disorders	
Troglitazone	pK_1 6.1 pK_2 12	Anti-diabetic	
Thyroxine acid	n/d	Thyroid hormone	 L-form
Acetylsalicylic acid (aspirin)	pKa 3.49	Analgesic, anti-inflammatory	
Chorambucil	n/d	Antineoplastic	
Warfarin	n/d	Anti-coagulant	
Ibuprofen	n/d	Analgesic, anti-inflammatory	

normal-phase modes and gave different enantioselectivities.

2.5. Coated columns

The focus of recent CEC investigations has centred on the use of packed capillaries. However early CEC work was also performed on internally coated capillaries. The use of coated capillaries is attractive in that their cost of manufacture is significantly lower than packed capillaries. Packed capillaries suffer from problems such as high back-pressures and air bubble formation, which can be avoided using coated capillaries. Coated capillaries however suffer from limited sample capacity, which could lead to sample overloading and reduced efficiency when attempting impurity determinations. This limited sample capacity on packed CEC capillaries has been identified [22] as being an issue.

There have been a number of examples of acids separated using coated CEC capillaries. One of the earliest papers in this field was in 1991 when capillaries coated with a polymer were used [23] in conjunction with ion-pair reagents in the mobile phase to separate five amino-naphthalenesulfonic acids. A cryptand polysiloxane coated capillary with pH 6 buffer has been used [24] to separate acidic proteins.

Enantiomeric separations on coated columns have also been performed. A capillary coated with Chirasil-Dex was used [25] to separate four racemic acidic non-steroidal anti-inflammatory drugs, ibuprofen, flurbiprofen, cicloprofen and etodolac. Capillaries coated with various celluloses [26] have been used to separate various acidic racemates such as warfarin and ibuprofen. CEC capillaries have been coated [27] with molecular imprinted polymer using *S*-(+)-2-phenylpropionic acid as the print molecule. These coated columns were used [27] to separate successfully the *R* and *S* enantiomers of phenylpropionic acid.

3. Applications

As mentioned previously the majority of CEC literature has focused on the separation of neutral compounds and there have been only limited examples of specific applications. These have included applications such as the determination of steroids [9] and drugs [28] in plasma, and drug seizures [29]. The application of CEC to acidic compounds includes inorganic anions, amino acids, aromatic acids, DNA and acidic pharmaceuticals.

3.1. Inorganic anions

Ion-exchange (IE) stationary phases have been used in CEC to separate small inorganic anions. The use of a strong anion-exchange CEC column was [30] compared to CE in the separation of a test mix containing iodide, iodate and perrhenate. Selectivity differences were obtained between the two techniques, with iodate and perrhenate giving reversed separation order. This was presumed due to a greater interaction of perrhenate with the stationary phase. The advantages of the separation by IE-CEC were that the efficiencies were higher than those obtained using CE. Another advantage was the lower limit of detection which was found to be approximately 20-times lower in IE-CEC than in CE. However, the analysis time were significantly faster in CE.

Various anion-exchange resins have been used [31] to separate sulfate, sulfite and thiosulfite anions using a mobile phase containing 0.15% 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES). In this case both pressure and voltage were applied across the packed capillary. Elution order was varied by changes in the level of voltage applied. This selectivity optimisation ability allowed determination of a low level of malonic acid in the presence of the major component, sulfuric acid.

3.2. Amino acids

Separation of the enantiomers of several amino acids has been achieved using [32] molecularly imprinted polymer as the stationary phase material. The polymer was prepared on-column and L-phenylalanine was used as the print molecule. The prepolymerisation solution also contained methacrylic acid and 2-vinylpyridine. One of the main advantages offered by this type of column is the absence of a retaining frit, thus helping to reduce the problem of bubble formation in the column. As well as varying the functional groups, the effect of varying the

mobile phase and the field strength was also studied. The group found [32] that using acetonitrile gave satisfactory results with water and acetic acid added to act as the electrolyte.

Isocratic and gradient elution has been used [33] to separate mixtures of dansylated amino acid mixtures on Zorbax ODS stationary phase. Isocratic elution was able to separate some of the amino acids. However, improved resolution (and better peak shape), especially of the strongly retained amino acids was obtained using gradient elution. The effect of temperature on the isocratic amino acids separation was examined [33] and EOF increased almost linearly between 25–53°C. Borate and phosphate buffers were used which gave pH values in the range 7–8.5.

Polyacrylamide gels are often used as a sieving media in CE especially for the separation of DNA. These gels have been used [34] in CEC as a stationary phase to separate dansylated amino acids. Linear (non-polymerised) and cross-linked gels were used to separate a test mix of five amino acids with no EOF present. A high pH Tris–boric acid electrolyte was used to generate EOF and to achieve a rapid separation of 12 dansylated amino acids.

3.3. Aromatic acids

A column packed with 6 μm Zorbax ODS was used [35] to separate a test mix containing *p*-toluic acid, cinnamic acid, *p*-terbutyl benzoic acid and *o*-dichlorobenzene in less than 3 min. A 4 m*M* borate buffer, which has a pH of 8.5, was used to generate a high EOF.

3.4. DNA

DNA has been successfully separated [36] using CEC with mass spectrometric detection. PAH adducts of DNA were formed in vitro and on-column focusing allowed the introduction of dilute samples suitable for this detection method. A ''step-gradient'' technique was employed in order to improve the speed of their separations. In this automated step approach the voltage is paused at some point during the separation and the separation vials are replaced with vials containing a higher level of solvent. The EOF generated by the application of the voltage draws the new electrolyte into the capillary resulting in the gradient formation.

3.5. Acidic pharmaceuticals

CEC has been extensively applied [1–4] to the analysis of neutral pharmaceuticals such as steroids. There have been limited demonstrations of how CEC can be used to afford the separation of acidic pharmaceuticals. Examples of acidic pharmaceuticals include [18,37] selected steroids, analgesics, anti-inflammatories, barbiturates and narcotics [29].

Fig. 3 shows the separation of a number of cephalosporins and penicillins on an ODS packing using a pH 3 mobile phase. Interestingly the same selectivity was obtained in CE using the same mobile phase in an unpacked capillary. Cephaloridine and cephalexin contain basic groups which are ionised at this low pH, whilst cefuroxime axetil is neutral and elutes at the EOF front. Cefuroxime and phenoxymethyl penicillin have low pK_a values and are negatively charged at this low pH. Therefore the separation that is occurring is predominantly effected by the electrophoretic migration of the compounds. This case highlights that CEC of charged compounds is achieved by a combination of partitioning and electrophoretic migration.

A number of acidic pharmaceuticals have been resolved [18] on standard C_{18} columns. A mobile phase containing 40% buffer at pH 2.3 was employed in order to obtain the separation of *p*-hydroxybenzoic acid, bumetamide and flurbiprofen (Fig. 4). This group showed [37] the advantage of using mixed mode columns by performing identical separations on both SCX/C_{18} and C_{18} ODS packed capillaries. The separation times was reduced from 13 min to 4 min when using the mixed mode capillary.

The use of low pH and ion-suppression was also shown [18] to be important in the separation of a steroid test mixture. At pH 7.8 only the four steroid peaks were detected [37] but when the separation was performed at pH 2.3 a fifth peak was observed due to an acidic component. At the high pH value used the acidic component had successfully migrated against the EOF and was therefore undetected.

Chiral separations, of which there have been relatively few in the pharmaceutical field, are obvi-

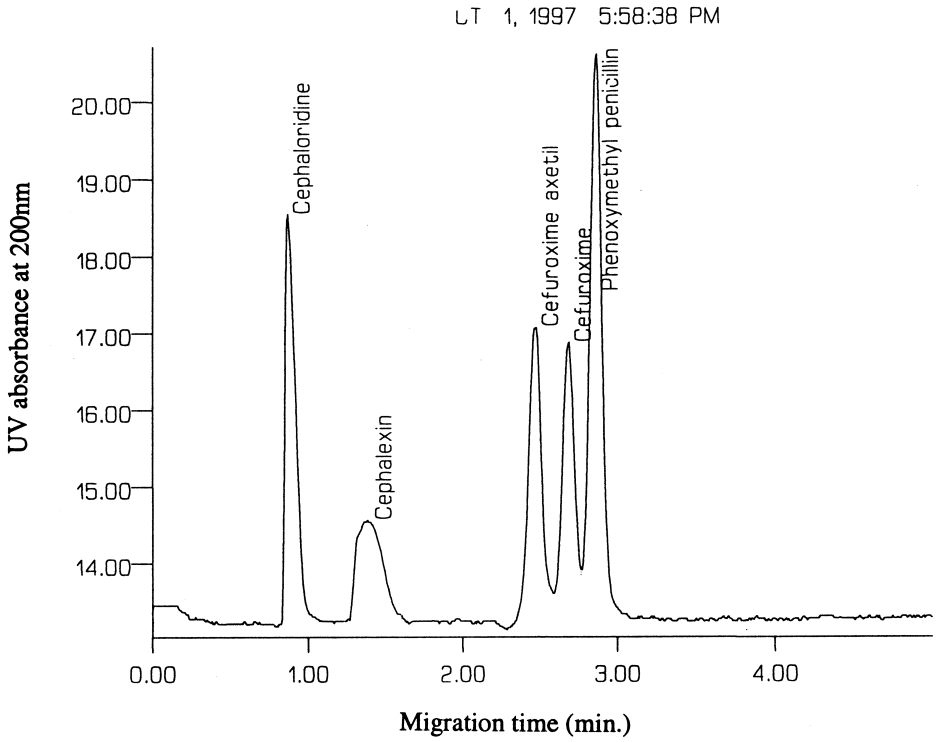


Fig. 3. Antibiotics separated by CEC. Separation conditions: column dimensions: 7 cm×50 μm packed, column packed with ODS and manufactured by Capital HPLC. Injection: 3 kV for 5 s. Separation: 10 kV, 200 nm, Mobile phase: acetonitrile–methanol–water (7.5 m*M* NH_4OAc, pH 3 with AcOH) (64:11:25, v/v). Samples at 0.2–0.3 mg/ml concentration dissolved in the mobile phase.

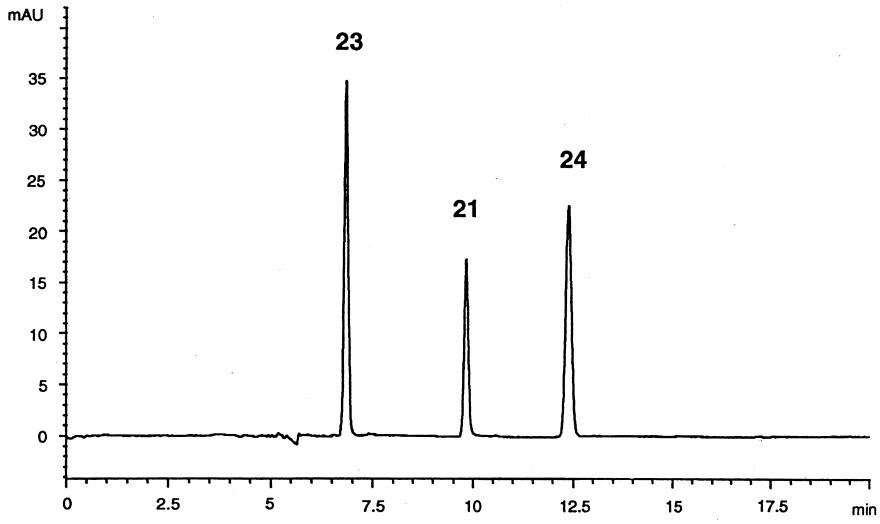


Fig. 4. Separation of acidic drugs at low pH in ion suppressed mode. Separation conditions: column dimensions: 23 cm×50 μm capillary packed with 3 μm C_{18} material and manufactured by Hypersil, injection: 5 kV for 15 s, separation: 30 kV, 230 nm, mobile phase: acetonitrile–50 m*M* NaH_2PO_4 (pH 2.5)–water (40:20:40, v/v). Samples at 0.2 mg/ml concentration dissolved in acetonitrile–water (50:50). Peaks: 23=*p*-hydroxybenzoic acid, 21=bumetanide, 24=flurbiprofen. Reproduced with permission from Ref. [18].

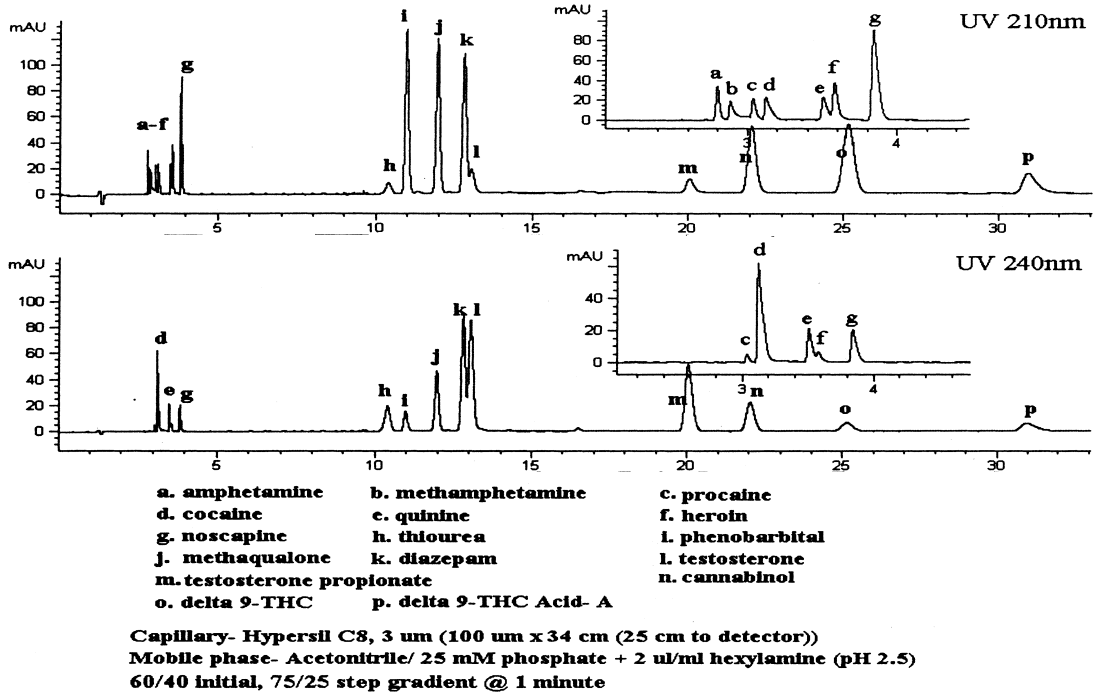


Fig. 5. Separation of a forensic drug screen. Reproduced with permission from Ref. [29].

ously of great importance with the development of new compounds and for preparative work. The main emphasis again has been on separation of neutral chiral compounds but the examples described in Section 2.4 show that the chiral resolution of charged compounds is also possible. One of the best examples of chiral separations in the literature [38] involves specially prepared chiral stationary phases. The best separation factor, α, achieved was 3.82.

The analysis of pharmaceuticals is one of the areas where the coupling of CEC to MS can be advantageous [9,10,28,33] and some examples of steroids, which have been analysed by this method, have been given in the literature.

Experimentally designed experiments have been used [39] to optimise the capillary electrochromatographic separation of related *S*-oxidation compounds in a Dupont Merck drug compound.

In probably the best application based work in CEC to date [29] the advantages of CEC over other techniques such as HPLC, gas chromatography (GC) and CE were demonstrated in the separation of cannabis substances. Low pH was also chosen for these separations in order to analyse mixtures of bases, neutrals and acidic cannabinoids. A number of factors such as the percentage acetonitrile, column length and temperature were optimised. A limit of detection for cannaboid impurities in marijuana and hash samples was found as approximately 0.0005%. A test mixture containing basic, neutral and acidic compounds was analysed (Fig. 5) using a mobile phase containing hexylamine (2 μl/ml). The hexylamine was added to reduce the tailing of basic compounds. The use of a step gradient was favourably compared to the isocratic separation to resolve all the test mix components.

4. Analytical performance of CEC

4.1. Sensitivity

The sensitivity of CEC is comparable to, if not better than [30] that of CE. A bubble cell [29] has been used to improve the sensitivity of CEC and gave a three-fold sensitivity increase compared to a

straight capillary. Sensitivity can also be improved [40] in CEC by utilising sample stacking or focusing, which can be produced by using a dissolving solvent of lower organic content than that of the mobile phase. Sample adsorption onto the stationary phase during injection can also allow [30] greater sample loading. The ability of CEC to be performed at low wavelength also aids the sensitivity and the analysis of impurities. The examples of impurities, which have been performed by CEC, are limited. The limits of detection for impurities in a tetrapeptide [41] were calculated at 0.05% impurity level.

Troglitazone is an acidic anti-diabetic drug which is routinely analysed for impurities using HPLC with gradient elution. It was of interest to us to compare the results of this gradient HPLC method to those obtained by a simple isocratic CEC method using a MeCN–MES, pH 5.5 (70:30) mobile phase. A Troglitazone test mix spiked with a number of impurities was used (Fig. 6) to demonstrate the selectivity of the CEC method. Levels of a specific Troglitazone impurity in a drug substance batch were determined by HPLC and CEC and were found to compare well at 0.15% and 0.12% area/area, respectively. Limits of detection of below 0.1% were obtained.

The most thorough application of CEC to the determination of drug-related impurities have been shown [42] for the determination of impurities in the neutral steroid norgestimate. Use of the CEC method reduced the analysis time by 50% compared to the equivalent HPLC method. The limit of detection for the impurities was less than 0.1%. Repeatability of less than 2% R.S.D. was reported for peak area and migration time precision.

4.2. Precision

As with CE, the injection precision in CEC is inferior compared to HPLC. In CEC the back-pres-

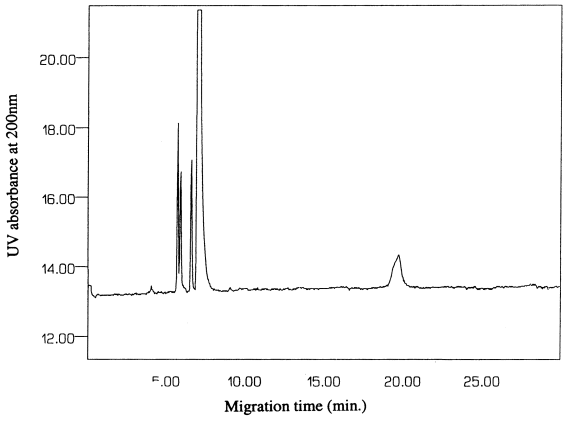


Fig. 6. CEC separation of troglitazone spiked with impurities. Separation conditions: column dimensions: 20 cm×50 μm packed with 3 μm C_{18} ODS manufactured by Innovatech. Sample concentration at 3 mg/ml troglitazone, sample dissolved in the mobile phase, injection 7 kV for 7 s, separation at 20 kV, 200 nm. Mobile phase: CH_3CN–MES (20 m*M*, pH 5.5) (75:25, v/v).

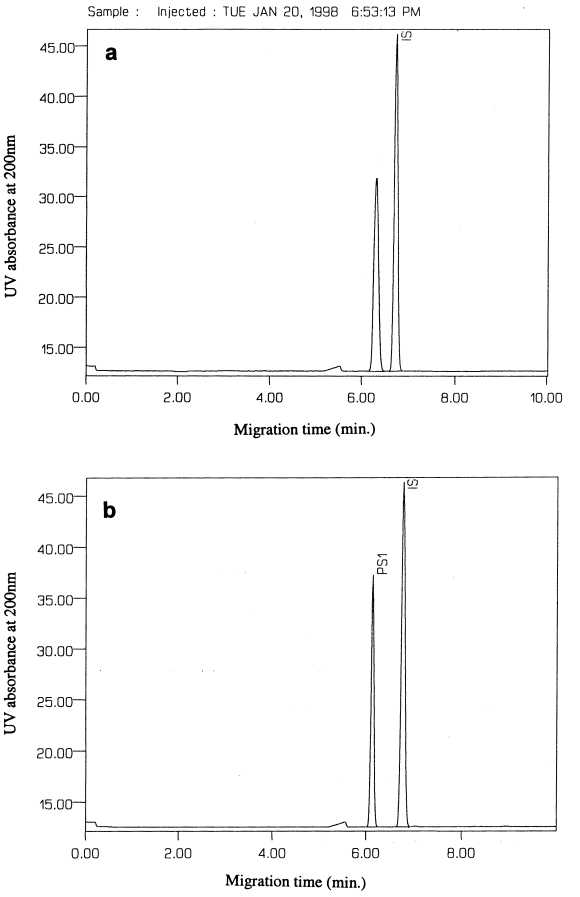


Fig. 7. Quantitative separations of pharmaceuticals by CEC. (a) Analysis of acetylsalicyclic acid (aspirin) by CEC. (b) Analysis of paracetamol by CEC. Separation conditions: column dimensions 20 cm×50 μm packed length packed with ODS and manufactured by Innovatech. Injection: 7 kV for 5s, separation at 15 kV, 200 nm, samples in acetonitrile sonicated and filtered, mobile phase acetonitrile–MES buffer (10 m*M*, pH 3) (80:20, v/v).

Table 2
Analytical performance of assay method

	Aspirin	Paracetamol
Precision R.S.D. (%) (n=10)		
Migration time (MT)	1.1	0.6
Relative MT	0.1	0.05
Peak area	3.0	1.6
Peak area ratio	0.3	0.34
Response factor	1.31	0.32
Linearity (50–150 ppm)	0.9995	0.9993
Label claim	299.3 mg/tablet	500 mg/tablet
Assay result	300 mg/tablet	497.5 mg/tablet

sure of the column is high and therefore pressure-based injections are difficult and electrokinetic injections are standard. Internal standards are recommended in CEC [2] to improve injection precision.

As far as we are aware there are no examples of assay data given in the CEC literature for acidic compounds. The novel data presented below are assay results of the analysis of paracetamol and aspirin tablets by CEC. Fig. 7 shows the separations obtained. A low pH buffer was used to suppress ionisation of the aspirin. Injection repeatability and linearity were examined and are given in Table 2. The acceptable precision obtained was due to the combined use of an internal standard (benzamide) and high sample concentrations. Unacceptable data was obtained when the results were calculated without use of the internal standard. The use of an internal standard in CE improves [43] detector linearity data as it reduces imprecision in the data points. Calculation of the solute migration time relative to the migration time of an internal standard also improves (Table 2) precision data. The assay results obtained by CEC were in good agreement (Table 2) with the label claim.

5. Comparison of CEC with HPLC and CE for the analysis of acidic compounds

Table 3 shows a graded comparison of the attributes of HPLC, CE and CEC. The table shows that CEC has the benefits of miniaturisation in terms of reduced operating costs compared to HPLC. CEC can also offer a similar degree of selectivity to that obtained in HPLC but with higher separation efficiencies. This improved efficiency means that faster or more resolved separations can be achieved in CEC compared to HPLC. However, in comparison to HPLC, both CEC and CE have poorer injection

Table 3
Comparison of CEC with HPLC and CE for the analysis of acidic compounds

	HPLC	CE	CEC
Operating parameter			
Solubility restrictions	+++	++	+++
Analyte range	+++	+++	+
Operational pH range	++	+++	++
Detection options	+++	+[a]	+[a]
Column fragility	+++	++	+
Column cost	++	+++	+
Set-up time	++	+++	++
Performance			
Separation efficiency	++	+++	++/+++
Sensitivity	+++	++	++
Precision	+++	+[b]	+[b]
Ruggedness	++	++	+
Economic considerations			
Operational costs	++	+++	+++
Solvent costs	+	+++	++
Equipment cost	++	++	++[c]
Training requirements	++	++	++

[a] Many detection methods investigated but not routinely employed.
[b] Routinely improved by using an internal standard.
[c] Needs a HPLC pump to pack/flush capillaries but runs on commercial CE equipment.
+ Poor, ++ acceptable, +++ good.

precision and reduced sensitivity. The major disadvantage with CEC is in the operation difficulties that can occur such as breakage of the fragile capillaries or generation of air bubbles in the columns which results in separation failures. The cost of CEC columns are similar to HPLC, which is high, compared to an inexpensive CE capillary. The reported analyte range of CEC is more limited than both CE and HPLC as strong bases represent a difficulty in CEC due to peak tailing. However, work [29] on the use of mobile phase additives is beginning to address these issues. A recent survey of a panel of CEC experts from both academia and industry highlighted that without the development of the columns and chemistry development of the technique will be hindered [13]. The panel broadly concluded that CEC was still a research technique but it offered tremendous scope for future development and applications.

6. Conclusions

CEC has been rapidly developing over the past few years. The technique offers the possibility of becoming a useful complement to HPLC, CE and other separative techniques. CEC can produce both highly efficient and rapid separations and benefits from being a miniaturised technique. There are currently technical difficulties associated with its operation but it is anticipated that developments will occur which will resolve these problems. This paper highlights the usefulness of the technique in a number of acidic applications and we have demonstrated a novel use of CEC for assay work with good results.

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