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Capillary electrophoresis for forensic drug analysis: A review

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Abstract

This paper reviews recent applications of capillary electrophoresis to forensic drug analysis and covers the literature since 2001. A brief overview of capillary electrophoresis is followed by a discussion of analytical applications which have been categorized into two sections: (i) drug seizures and non-biological samples, and (ii) forensic toxicology and biological samples.

Keywords: Capillary electrophoresis; Forensic analysis; Review

1. Introduction

The characterization and quantification of chemical substances in a wide variety of matrices is a challenge faced by forensic scientists. Many forensic samples are complex mixtures, for example biological extracts or debris associated with the scene of an explosion, and analysis generally requires separation prior to identification of chemical species. This has traditionally been carried out using gas chromatography (GC) and high-performance liquid chromatography (HPLC) [1]. Capillary electrophoresis has been investigated as an alternative separation technique for forensic analysis. It has the potential to provide far more rapid separations than are generally achievable with HPLC, and can provide a separation where the analyte in question behaves poorly under GC analysis. This might be due to thermal instability, or at least high-temperature reactivity, as is the case with the drugs, LSD and psilocin (the active ingredient in "magic mushrooms"), the benzodiazepines, and the explosives PETN, RDX and HMX, during GC analysis [1,2].

The term 'capillary electrophoresis' describes a family of related techniques in which separations are carried out in narrow bore capillaries under the influence of an electric field [3]. The separations obtained by capillary electrophoresis are highly efficient, rapid, and may be applied to both charged and neutral species. An on-line search of the Scifinder Scholar database revealed in excess of 30,000 references to capillary electrophoresis to date with applications in a wide range of areas, including pharmaceuticals, food and beverages, environmental and clinical analysis.

The potential of this technique for forensic analysis was first demonstrated in 1991 by Weinberger and Lurie, who applied it to the analysis of a wide range of illicit drugs in synthetic mixtures [4]. Since the first paper, the application of capillary electrophoresis to forensic analysis, as well as comprehensive textbooks and book chapters [14,15], has been the subject of a number of reviews [5–13]. The exceptional power of separation and resolution, rapid analysis time, low mass detection limits, economy of reagents, and minimum sample requirements make capillary electrophoresis an attractive methodology for forensic laboratories [9,16]. In this paper,

Abbreviations: CE, capillary electrophoresis; CZE, capillary zone electrophoresis; MEKC, micellar electrokinetic capillary chromatography; CEC, capillary electrochromatography; cITP, capillary isotachophoresis; CIE, capillary ion electrophoresis; CE–MS, CE–mass spectrometry; LIF, laser-induced fluorescence; GHB, γ-hydroxybutyric acid; GBL, γ-butyrolactone; BD, 1,4-butanediol; MA, methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDA, 3,4-methylenedioxyamphetamine; CD, cyclodextrin; ODS, octadecyl silica; NACE, non-aqueous capillary electrophoresis; NACZE, non-aqueous capillary zone electrophoresis; NACE/FS, non-aqueous capillary electrophoresis/fluorescence spectroscopy; OFM-OH, tetradecyltrimethylammonium hydroxide; CHES, 2-[*N*-cyclohexylamino]-ethane sulfonic acid; NAIP, 3-(1,8-naphthalimido)propyl-modified silica gel; LLE, liquid–liquid extraction; SPE, solid-phase extraction; CMC, critical micelle concentration

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we review the use of capillary electrophoresis for the determination of drugs in forensic samples since 2001.

2. Capillary electrophoresis

Electrophoresis can been defined as the differential migration of charged species (ions) in an electric field, and was first described as a separation technique by Tiselius in 1937 [17]. His work, involving the separation of proteins, placed between buffer solutions in a tube across which an electric field was applied, earned him the Nobel Prize for Chemistry in 1948 [18]. His approach to electrophoresis in free solution was limited by incomplete separation due to the effects of thermal convection [19]; use of anti-convection agents, such as cellulose or polyacrylamide gels, mitigated this problem. Electrophoresis using such media has become a standard technique for size-dependant separation of biomolecules. Separations carried out in this format are characterised by long analysis times and low efficiencies, when compared to other analytical separation techniques, such as HPLC [20].

Attempts to improve analysis time and efficiency in the slab or planar format by increasing the voltage applied are limited due to the effects of Joule heating [21]. This problem can be addressed by using narrow-bore tubes or capillaries, which allows rapid dispersion of any heat generated [21]. In a series of papers in the 1960s, Hjerten performed separations of a range of analytes from small molecules, such as inorganic ions and nucleotides to proteins and viruses, in open tubes with a bore of a few milimeters [19]. Thermal convection effects were minimised by rotation of the tubes along their longitudinal axis. In his work, Hjerten also decribed methods to reduce the effect of electro-osmotic flow [19]. The term ‘electro-osmotic flow’ (or electro-endoosmotic flow) describes the movement of a conducting liquid against a charged surface when an electric field is applied; this was seen as a problem to be eliminated for free-solution electrophoresis in tubes [19]. In the early 1980s, Jorgenson and Lukacs [22–24] demonstrated that high-performance analytical electrophoretic separations in capillaries were possible. In their instrumentation, electro-osmosis was utilised in order to allow analysis of negatively and positively charged species in a single run, as well as leading to extremely rapid separations. Since they described the first workable capillary electrophoresis instrument, many thousand papers have been published in this area along with numerous textbooks on theory and applications to which the reader is directed for detailed discussion of the underlying principles [14,15,25–30].

Capillary electrophoresis is an instrumental evolution of traditional slab gel electrophoretic techniques [31]. Since its introduction, capillary electrophoresis has shown great potential in a range of applications, such as the separation of small ions to drug analyis. Capillary electrophoresis instrumentation was introduced in the 1980s and early 1990s by companies, such as Microphoretics, Applied Biosystems, Beckman Coulter, Spectraphysics, Hewlett Packard and Waters [8,10]. However, the popularity of HPLC over capillary electrophoresis, as an analytical instrument, resulted in only a few companies continuing to sell capillary electrophoresis instruments. A comprehensive review on the introduction of capillary electrophoresis as an analytical technique from its beginning until the present day can be found by Weinberger [10] and Issaq [32].

2.1. Separation modes

In its simplest form, capillary electrophoresis involves the separation of charged analytes, based on the difference in their electrophoretic mobilities, resulting in different migration velocities. These separations are carried out in fused silica capillaries, typically 25–75 μm i.d. and 50–100 cm in length, filled with a background electrolyte [25]. Electro-osmotic flow can ensure that both negatively and positively charged species migrate towards the same end of the capillary, where under typical conditions, is towards the cathode end, with neutral species not being separated and migrating with the electro-osmotic flow. This mode is also termed capillary zone electrophoresis (CZE). The key factor effecting selectivity, when using CZE for separations, is charge to size ratio and pH. The later parameter will determine the degree of ionisation for moderate and weakly basic, or moderate and weakly acidic analytes. The background electrolyte requires a good buffering capacity at a chosen pH for reproducible separations, and low conductivity not to generate a high current, which leads to excessive Joule heating. The most commonly employed background electrolytes have been derived from the large body of work with gel electrophoresis, and include phosphate, borate, phosphate/borate, and citrate buffers [3].

The versatility of capillary electrophoresis is derived from its additional separation modes. The ability to perform these separations on the same capillary and instrument makes this technique attractive in the laboratory. With CE, a commonly encountered mode of capillary electrophoresis is micellar electrokinetic chromatography (MEKC). This combination of electrophoresis and chromatography allows for the separation of both neutral and charged solutes [33]. It is achieved by the addition of surfactants to the background electrolyte at concentrations greater than the critical micelle concentration (CMC). Separation is based upon interaction of the analytes with the micelles, which can be considered as a “pseudostationary phase”[15]. The nature of the interaction between the solute and micelle can be altered by using different types of surfactants. The most commonly employed surfactants are sodium dodecyl sulphate (SDS), bile salts, and quaternary ammonium salts [3]. The presence of organic solvents, such as methanol and acetonitrile may be used as organic modifiers to alter the selectivity of a run, similar to reversed-phase chromatography [15].

Capillary electrochromatography (CEC) is another hybrid of capillary electrophoresis and HPLC, where analytes are separated by partitioning between the mobile and stationary phases while moving with the electro-osmotic flow through

a column [11,34]. The electric field is used to displace the mobile phase, and solvent transport is achieved by the electro-osmotic flow, resulting in an absence of column back pressure. However, this technique has not reached the stage of maturity of other capillary electrophoresis modes, and its practical applications are still limited.

Capillary isotachophoresis (cITP) is a 'moving boundary' electrophoresis technique, where a combination of two background electrolytes are used where these zones move at the same velocity [35]. These zones remain sandwiched between leading and terminating electrolytes, and a separation is established by the aid of the background electrolyte. In cITP, samples of only one charge are separated in the same run.

2.2. Detection

Small sample volumes make detection a significant challenge in capillary electrophoresis. The most widely used detection method in capillary electrophoresis is on-column UV detection, which involves burning off a section of the polyimide coating of the capillary to form an optical window. This type of detector is standard on commercial instruments, which can also be fitted with a diode array detector for the simultaneous acquisition of spectra [25]. However, the short internal diameter of the capillary (detection path length) limits the sensitivity of this detection system, which is 1–2 orders of magnitudes less than that found in HPLC [36], and consequently, detection limits for UV–vis absorption are usually in the micromolar range. One way to improve the sensitivity in capillary electrophoresis is to increase the path length or inner diameter of the capillary by using a Z-shaped capillary, in which detection is by axial illumination [3]. Alternatively, a bubble cell can also be employed. Here, the path length is also increased, improving the sensitivity of the system, 3–5 fold [25].

Other modes of detection include fluorescence and laser-induced fluorescence. On-column fluorescence detection encounters sensitivity limitations, due to the small path lengths provided by the capillaries [3]. Thus, this detection mode is advantageous if selectivity is required, as only few molecules produce significant fluorescence. Laser-induced fluorescence (LIF) is a very sensitive detection system, but is instrumentally complex, requiring the appropriate laser sources.

Indirect detection has been applied to a variety of analytes that do not absorb UV radiation or do not possess a chromophore. Indirect detection involves the employment of a UV absorbing species or fluorescent species in the background electrolyte [37]. The solute ions displace the additive, and a decrease in absorbance or fluorescence occurs when the zones pass the detector region, resulting in a negative peak at the detector. Buffering capacity and mass-sample overloading is a concern when using this detection technique.

Capillary electrophoresis–mass spectrometry (CE–MS) provides an orthogonal approach to analysis in a single analytical run. CE–MS combines the advantage of both techniques, so that quantitative and migration time information, in combination with molecular masses and/or fragmentation patterns can be obtained in one analysis. Currently, electrospray ionization (ESI) serves as the most common interface between capillary electrophoresis and MS, as it can produce ions directly from liquids at atmospheric pressure, and with high sensitivity and selectivity for a wide range of analytes of clinical and forensic significance [38,39].

In addition to the above detection techniques, a variety of other approaches have been taken, including chemiluminescence [40–45], conductivity [46], and a number of electrochemical methods [47]. Electrochemical techniques, such as amperometry, are useful for species which are redox-active, yielding in some cases exceptionally low-detection limits [47]. In a similar fashion, chemiluminescence detection has the potential to give low-detection limits with the added advantage of simpler electropherograms [41,43]. Conductivity can act as a "universal" detector; however, this can also be a disadvantage due to the lack of selectivity [47]. In each of these cases, the detection method requires modification to the basic capillary electrophoresis instrument, and are currently still research tools rather than routine detection techniques.

3. Applications

The application of capillary electrophoresis to the forensic analysis of drugs can be divided into two main areas: the analysis of drug seizures and toxicology.

3.1. Forensic analysis of drug seizures and non-biological samples

Capillary electrophoresis has been successfully applied to the determination of various analytes in drug seizure samples using UV, fluorescence, and laser-induced fluorescence (LIF) methods of detection, which have been summarised in Table 1.

A variety of approaches to the routine analysis of drug seizures have been reported, including MEKC [48–50], CZE [51–54], chiral separations [55–60], capillary electrophoresis with dynamic coatings [61–65], and capillary electrochromatography [66]. Each of these approaches are discussed in detail below.

A MEKC method for the simultaneous analysis of coca alkaloids polyhydric alcohols and sugars in illicit cocaine using indirect UV detection, was developed by Ishii et al. [48]. A 75 μm × 85 cm capillary was used, with a run buffer consisting of 15% (v/v) acetonitrile in 8 mM Na_2HPO_4/5 mM phthalate/10 mM CTAB, pH 12.5. Separations were carried out within 25 min, with an applied voltage of −27 kV at 15 °C, with UV detection at 310 nm, and the reference wavelength set at 200 nm. Good linearity was obtained with excellent correlation coefficients for all analytes. Fair migration

Table 1
Forensic analysis of non-biological samples

Analyte(s)	Matrix	Sample preparation	Method	Detection	Calibration range	Detection limit	Reference assay	Reference
Cocaalkaloids, sugars	Illicit cocaine	Direct injection	MEKC	UV (indirect)	0.5–10 mg/ml (0.1–18 mg/ml for cocaine)	0.03% w/w (for cocaine)	Not reported	[48]
GHB, BGL, BD	GHB seizure samples	Dilution	MEKC	UV-DAD	0–200 mg/l (GHB)	5.1 mg/l (GHB)	FTIR	[49]
Heroin and impurities	Illicit heroin	Dilution	MEKC	UV-DAD	0.02–1.0 mg/ml	0.02 mg/ml	GC	[50]
Heroin, impurities and additives	Illicit heroin	LLE	CZE	UV	0.62–4.39 mg/ml	500 ng/ml (heroin)	GC	[51]
Amphetamines	Illicit amphetamine tablets	Direct injection	CZE	UV-DAD	0.2–1.0 mg/ml	Not reported	HPLC	[52]
Amphetamines	Illicit amphetamine tablets	Dilution	CZE	UV-DAD	0.42–0.78 μg/ml	Not reported	Not reported	[53]
Opium alkaloids	Gum opium	LLE	CZE	UV	2–20 μg/ml	450 ng/ml (thebaine)	Not reported	[54]
Methamphetamine, ephidrine, pseudoephidrine	Methampheta-mine seizure samples	Direct injection	Chiral CE	UV-DAD	5–250 μg/ml	5 μg/ml	GC–MS, FTIR	[55]
Propoxyphene	Reference standards	Dilution	Chiral CE	UV	Not reported	Not reported	Not reported	[56]
Amphetamines	Reference standards	Direct injection	Chiral CE	UV-DAD	Not reported	12 pg (dnorephedrine)	Not reported	[57]
Methamphetamine, methcathinone, ephedrine, pseudoephedrine	Clandestine tablets, urine	LLE	Chiral CE	UV-DAD	Not reported	Not reported	GC–MS	[58]
MDMA and MDA	Clandestine tablets, urine	LLE	Chiral CE	Fluorescence	Not reported	Not reported	GC–MS	[59]
Cocaine stereoisomers	Reference standards	Dilution	Chiral CE	UV	Not reported	Not reported	Not reported	[60]
Amphetamines and cocaine	Seizure samples	Direct injection	CZE (coated capillary)	UV-DAD	0.003–0.10 mg/ml (0.003–0.40 mg/ml for cocaine)	Not reported	NMR, CZE (uncoated capillary)	[61]
Opium alkaloids	Opium gum and latex	Dilution	CZE (coated capillary)	UV-DAD	0.001–0.07 mg/ml	Not reported	HPLC	[62]
Heroin, basic impurities, adulterants	Synthetic heroin samples	Dilution	CZE, MEKC (coated capillaries)	UV-DAD	0.01–0.80 mg/ml (heroin)	Not reported	HPLC	[63]
Phenethylamines, cocaine, oxycodone, heroin, LSD, opium, hallucinogenic mushrooms, GHB-GBL	Synthetic samples and seized samples (methampheta-mine tablets, heroin, LSD, hallucinogenic mushroom)	Dilution	CZE, MEKC (coated capillaries)	UV-DAD	0.0317–0.50 mg/ml (oxycodone), 0.025–0.802 mg/ml (heroin), 0.0007–0.025 mg/ml (LSD), 0.304–9.73 mg/ml (GHB), 0.606–9.69 mg/ml (GBL)	Not reported	Not reported	[64]
73 Basic pharmaceuticals	Synthetic samples	Dilution	CZE	UV	Not reported	Not reported	HPLC, GC	[65]
Acidic and neutral impurities	Crude heroin samples	LLE	CEC	LIF	Not reported	66 pg/ml (acetylthebaol)	HPLC	[66]

time and area-ratio repeatability were obtained (R.S.D. of ≤1.1% and ≤3.5%, respectively; $n = 6$). Recoveries from synthetic mixtures obtained for this method were in the range of 92.8–108.1%. The quantities of sugars and polyhydric alcohols were determined in cocaine seizures using this method, yet, comparisons with a reference method were not reported.

A MEKC method was optimised for the analysis of γ-hydroxybutyric acid (GHB), γ-butyrolactone (GBL) and 1,4-butanediol (BD) in GHB seizure samples by Dahlen and Vriesman [49]. Baseline separations within 14 min were attained, utilising a run buffer, consisting of 30 mM sodium barbital and 150 mM SDS, pH 10.2. Linearity was demonstrated in the range of 0–100 g/l for GBL and BD, and 0–200 mg/l for GHB. Limits of detection for GHB, GBL, and BD were 5.1, 0.34 and 0.25 g/l, respectively. Excellent migration time and peak area were also obtained (R.S.D. of ≤0.41% and ≤3.05%, respectively, $n = 9$). This method was also applied to two GHB seizures which were diluted 4000 times to achieve a reasonable peak size.

A simple and rapid method for the analysis of heroin seizures by micellar electrokinetic chromatography with short-end injection was described by Anastos et al. [50] Separations were performed using an uncoated fused-silica capillary, 50 cm × 50 μm i.d. × 360 μm o.d., with an effective separation length of 8 cm. The system was run at 25 °C with an applied negative voltage of −25 kV. The background electrolyte consisted of 85:15 (water:acetonitrile, v/v) containing final concentrations of 25 mM SDS and 15 mM sodium borate, pH 9.5. Samples and standards were prepared in 0.1% v/v acetic acid, and diluted in the run buffer containing 1 mg/ml of *N*,*N*-dimethyl-5-methoxytryptamine as an internal standard. Under these conditions, a text mixture containing caffeine, paracetamol, morphine, codeine, heroin and acetylcodeine was resolved within 1.5 min. The method was used to determine the concentration of heroin in heroin seizure samples, and the results were in good agreement with those obtained by a validated gas chromatographic method.

CZE methodological optimisation and validation for components of clandestine heroin samples were reported by Macchia et al. [51]. These included monoacetylmorphine, acetylcodeine, heroin, papaverine, narcotine and phenylethylamine (internal standard). Separations were performed using a 50 μm × 37 cm capillary (30 cm effective length), with a run buffer composed of 20 mM β-cyclodextrin in 0.1 M KH_2PO_4 and 0.1 M H_3PO_4, pH 3.23. CZE runs were carried out in less than 10 min using an applied voltage of 15 kV at 24 °C, with UV detection at 200 nm. In the preliminary stage of method development, a plain phosphate buffer at pH 6.38 was used with an applied voltage of 7 kV. As co-migration of various analytes was observed, cyclodextrins were incorporated into the running buffer. While β-cyclodextrin was effective in providing resolution, α- and γ-cyclodextrin failed to do so. Calibration curves were linear in the range of 0.62–4.39 mg/ml, with correlation coefficients >0.9997. Intra-day R.S.D. values ($n = 6$) for relative migration time and peak area were in the range of 0.13–0.17% and 1.11–3.13%, respectively. Day-to-day R.S.D. ($n = 6$) relative migration time and peak area were in the range of 0.33–0.71% and 1.20–3.20%, respectively. Analysis of illicit heroin seizure samples gave good agreement with results obtained, using a validated GC method. Furthermore, simultaneous separation of morphine, codeine, methadone, amphetamine, its methylenedioxy derivatives and cocaine are reported. Consequently, narcotine and an enantiomer of amphetamine, and cocaine and an enantiomer of 3,4-methylenedioxyethylamphetamine comigrated. However, each one is distinguishable, based on their UV spectra, and unlikely to be present together in a seizure sample.

Di Pietra et al. [52] developed a CZE method for the identification of illicit amphetamines in 9 min, using an uncoated capillary with a low-pH run buffer. The R.S.D. ($n = 5$), evaluated for the migration times of the analytes, ranged between 0.6 and 0.74%. A capillary electrophoresis method for the achiral separation of amphetamines was also described with the addition of hydroxypropyl-β-cyclodextrin to the previously described running buffer. Enantiomers of methamphetamine and 3-amino-1-phenylbutane were not resolved using the described chiral system. In this same study, a HPLC procedure was developed; thus, capillary electrophoresis provided greater peak symmetry and shorter run times.

Piette and Parmentier [53] used CZE for the determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethamphetamine, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butamine, and ephedrine, in illicit amphetamine seizures. A 50 μm × 47 cm capillary (40 cm effective length) was used with a running buffer consisting of 0.1 M phosphoric acid adjusted to pH 3.0 with triethanolamine. At this pH, triethanolamine is adsorbed to the wall, resulting in a reversal of the EOF. Separations were performed in under 8 min, using an applied voltage of 25 kV at 25 °C with diode array UV detection from 190 to 350 nm. No interferences from the adulterants, caffeine and paracetamol, were observed under these conditions. Excellent migration time R.S.D. values ($n = 10$) of 0.26% and 0.23% were obtained for amphetamine and MDMA, respectively. The MDMA content in one 'Ecstasy' tablet was determined using normalised peak areas with phenylephrine as an internal standard. The amount of MDMA present in the tablet was calculated to be 23.5%, yet, a secondary method to validate this result was not performed. A correlation coefficient of 0.9984 for MDMA using this method was obtained, as was an R.S.D. of <2.0% by means of duplicate preparation of the sample.

Reddy et al. [54] used a CZE method for the qualitative and quantitative determination of morphine, codeine, thebaine, papaverine and narcotine in gum opium. A 50 μm × 70 cm capillary (55 cm effective length) was used with a running buffer consisting of 100 mM sodium acetate, pH 3.1, 70%, v/v methanol. Separations were performed for 25 min, using an applied voltage of 15 kV at 25 °C. Quantification of the samples were carried out using the external standard method at

224 nm. The extraction method of the alkaloids was adopted from Ayyangar et al. [67]. Peak identification was done by sample spiking; in addition, spectral scanning was performed between 200 and 300 nm. Calibration curves were linear in the range of 2–20 μg/ml, with correlation coefficients ≥0.996 for all standard alkaloids. The limit of detection for each alkaloid was 850 ng/ml for morphine, 450 ng/ml for thebaine, 500 ng/ml for codeine and narcotine, and 550 ng/ml for papaverine. The peak area R.S.D. ($n = 5$) ranged between 1.03 and 3.56%, and the migration time R.S.D. ($n = 5$) ranged between 0.34 and 0.69%. Recoveries ranged from 98 to 102% for spiked samples.

The addition of a chiral selector to a capillary electrophoresis buffer is an effective approach for the separation and analyis of enantiomers. A simple CZE method, using a low-pH buffer with β-cyclodextrin as a chiral selector for the enantiomeric separations of (±)-ephedrine, (±)-pseudoephedrine, and (±)-methamphetamine was reported by Cheng et al. [55] in less than 30 min. The robustness of this method was determined by analysing 138 illicit samples containing methamphetamine, and was found to be satisfactory. Calibration curves were linear in the range of 5–250 mg/ml, with correlation coefficients greater than 0.9993. Optical purity down to 2% e.e. could be detected by running a standard solution of 99:1 (+)-MA:(−)-MA. The intra-day R.S.D. of relative migration times ($n = 9$) was <0.1%. The inter-day average R.S.D. of relative migration times (10 days; $n = 20$) was <0.6%.

The use of a neutral cyclodextrin, as an additive for the enantiomeric separation of propoxyphene, was described by Magoon et al. [56]. Using TM-β-CD provided a resolution of 1.94 and a baseline separation within 7 min.

The use of highly sulfated cyclodextrins for the chiral separation of nine amphetamine-type stimulants was investigated by Iwata et al. [57]. An enantiomeric separation was achieved within 32 min using a highly sulfated anionic γ-cyclodextrin (SU(XIII)-γ-CD) in the reversed-polarity mode. Separation involving reversed-phase polarity resulted in improved peak shapes and overall resolution compared to separation in the normal-polarity mode.

The use of β-cyclodextrins in the running buffer for the separation of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine, (±)-pseudoephedrine and (±)-amphetamine in clandestine tablets and urine samples was demonstrated by Liau et al. [58]. The addition of 5% acetonitrile was necessary for the separation of the methamphetamine-related compounds which was observed within 20 min.

The *R*- and *S*-isomers of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) were prepared, identified using GC/MS, and used as standards by capillary electrophoresis/fluorescence spectroscopy by Huang et al. [59]. Separation of the isomers of MDMA and MDA were achieved in 30 min with the use of a low-pH buffer containing β-CD. Optimum conditions for the chiral separation of a model mixture of MDMA and four of its metabolites resulted in a separation within 100 min.

Cabovska et al. [60] investigated the effect of organic modifier on the separation of cocaine stereoisomers by capillary electrophoresis using sulfated β-cyclodextrins. The separation selectivity was affected by varying the methanol content. Good resolution and analysis time of four cocaine stereoisomers were obtained when 10% methanol as the organic modifier was used.

Lurie et al. [61] used CZE with dynamically coated capillaries for the routine analysis of methamphetamine, amphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA) and cocaine in seized drugs.

Dynamic coating of a capillary consists of a two-step process, whereby, the capillary (after flushing with base) is first coated with a proprietary polycation (an initiator), then with a proprietary polyanion (an accelerator). The buffer is the latter coating reagent. This methodology used a 50 μm × 32 cm capillary (23.5 cm effective length) with a commercially available buffer kit. CE runs were accomplished within 9 min, with an applied voltage of 10 kV at 15 °C and diode-array detection at 195 nm. Dynamic coating of a capillary, which is performed every injection, consists of a 1-min flush with 1 M NaOH, followed with a proprietary polycation (CElixir Reagent A, phosphate buffer, pH 2.5) for 1 min, and a proprietary polyanion (CElixir Reagent B, phosphate buffer, pH 2.5) for 2 min. In contrast to CZE, doubly dynamic coating gives rise to a stable and enhanced electro-osmotic flow over a wide pH. With dynamically coated capillaries, it was possible to separate neutral acidic substances and non-ionic solutes in the presence of basic drugs in less than 13 min. The time for separation of a standard mixture of amphetamine and related compounds was about 50% shorter using coated capillaries compared to uncoated capillaries. A comparison of a separation of cocaine and related compounds also exhibited a three-fold decrease in migration time when coated capillaries were used. Excellent precision, reproducibility and correlation coefficients for each drug were obtained using dynamically coated capillaries. Quantitative results for seizure samples were also in good agreement with results obtained using GC and NMR.

Lurie et al. [62] used the above coating conditions for the separation of morphine, papaverine, codeine, noscapine and thebaine in opium alkaloids; however, this approach gave poor resolution. The addition of dual cyclodextrins (hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin) to the run buffer, imparted excellent selectivity for the opium alkaloids. Excellent migration time and peak area (R.S.D. ≤ 0.12% and ≤1.2%, respectively) were obtained. Good agreement for the determination of opium alkaloids in opium gum and opium latex samples were obtained using CE and HPLC. CE afforded better resolution with significantly faster analysis time (12 min versus 29 min). The CE conditions reported were also applicable to the analysis of LSD exhibits. Excellent linearity and precision were obtained, with runs carried out within 8 min. Capillary perfor-

Table 2
Forensic analysis of biological samples

Drug	Sample matrix	Sample preparation	CE method	Detection	Calibration range	Detection limit	Reference assay	Reference
Acidic and basic	Urine serum	SPE, LLE	CZE MEKC NACE	DAD	Not reported	Not reported	Not reported	[69]
Amphetamines	Blood	Acid hydrolysis, protein percipitation	CZE	DAD	2.5–250 μg/ml (range varied for each drug)	10–30 ng/ml (80 ng/ml for MDMA)	Not reported	[70]
Heroin metabolites	Urine	SPE	CZE	UV	100–500 ng/ml	30–40 ng/ml	Not reported	[71]
GHB	Urine serum	Dilution	CZE	UV (indirect)	25–500 μg/ml	3.0 μg/ml	Not reported	[72]
Nitrite	Urine	Dilution	CZE	UV	40–8000 mg/ml NO_2^-	20 mg/ml NO_2^-	Not reported (method evaluated using the National Committee for Clinical Laboratory Standards protocol)	[73]
Morphine	Plasma	Direct injection	CZE MEKC	UV	50–500 mg/ml	50 ng/ml	Not reported	[74]
Endogenous anions	Urine	Dilution	CIE	UV (direct and indirect)	1–20 μg/ml	0.1 μg/ml	Colorimetric analysis, spectrophoto-metry	[75]
Potassium	Vitreous humour	Dilution	CIE	UV (indirect)	Not reported	Not reported	Not reported	[76]
3,4-MDMA	Urine	LLE	CZE	Fluorescence	Not reported	4.7×10^{-6} M (NACZE), 2.6×10^{-8} M (NACZE stacking), 5.0×10^{-9} M (LT-NACZE)	Not reported	[77]
MDMA	Urine	LLE	NACE	Fluorescence spectroscopy	5–100 ppm	50 ppb	GC–MS	[78]
Normorphine, morphine, 6-acetylmorphine, codeine	Urine	SPE	CE	Fluorescence, LIF	500–1000 ng/ml	200–300 mg/ml (fluorescence), 50–100 pg/ml (LIF)	Not reported	[79]
Opioids	Urine	Dilution, SPE, LLE	CZE	UV, EIMS	Not reported	10 ng/ml	Not reported	[80]
Morphine and related opioids	Urine	Direct injection, dilution, LLE, SPE	CZE	UV, tandem MS, triple MS	Not reported	5 mg/ml (direct injection), 100–200 ng/ml (extraction)	MS	[81]
GHB	Urine	LLE	CZE	UV (indirect)	5–100 μg/ml	2 μg/ml	ESI–MS	[82]
2,5-Methylenedioxy derivatives of amphetamine and phenylethylam-ine	Urine	SPE	CZE	ESI–MS	5–1000 ng/ml	0.31–4.29 ng/ml	Not reported	[83]
Barbiturates	Serum	LLE	CEC	UV-PDA	2.90–43.29 μg/ml	0.83 μg/ml (phenobarbital)	Not reported	[84]
Methamphetamine, methcathinone, ephedrine, pseudoephedrine	Urine	LLE	Chiral CE	UV-DAD	Not reported	Not reported	GC–MS	[58]
MDMA and MDA	Urine	LLE	Chiral CE	Fluorescence	Not reported	Not reported	GC–MS	[59]

mance was also examined with over 500 samples analysed, using the same capillary. Migration times increased over time; however, resolution remained constant.

The dynamically coated approach, used for the analysis of amphetamines and related compounds by Lurie et al. [61], resulted in poor resolution, when applied to the separation of heroin and basic impurities [63]. The addition of 100 mM dimethyl-β-cyclodextrin to Reagent B, doubling the length of the capillary and increasing the temperature led to the baseline separation of heroin, morphine, O^6-monoacetylmorphine, O^3-monoacetylmorphine, acetylcodeine, noscapine and papaverine within 15 min. Excellent linearity was obtained. Excellent intra-day migration time and corrected area precision were obtained (R.S.D. of ≤0.071% and ≤2.00%, respectively; $n = 5$). The analysis of acidic, weakly basic and neutral adulterants in heroin was accomplished using a modification of the commercially available doubly coating kit. The polyanion process was replaced with a negative coating, using an SDS phosphate–borate buffer, pH 6.5, with a separation achieved in 8 min. Excellent linearity was obtained. Good intra-day migration time and corrected area precision were obtained (R.S.D. of ≤0.45% and ≤0.88%, respectively; $n = 5$). Considerable improvement in migration time precision was also obtained by partially recoating between injections for 2 min with buffer as opposed to 10 min with base, water and buffer. Good agreement for the analysis of heroin samples by capillary electrophoresis and HPLC were also observed.

A wide variety of seized drugs were also analysed by Lurie et al. [64] on a single capillary, using capillary electrophoresis with dynamic coatings and multiple run buffers. These short capillaries gave rapid, precise and reproducible separations.

Two capillary-zone electrophoretic methods for the mobility determination of 73 basic pharmaceutical compounds were developed by Boone et al. [65]. A method with and without the use of dynamically coated capillaries for analysis was compared. CZE using the CElixir coating was superior to normal CZE for identification purposes, and run times for each method were 8 and 18 min, respectively. The reproducibility of the run was also a factor of 2 better when the coating was employed. Limits of detection and calibration ranges were not reported.

Capillary electrochromatography (CEC), a hybrid of capillary HPLC and CE, has shown to be efficient in the analysis of seized drugs [68]. The use of CEC with laser-induced fluorescence (LIF) detection, using a doubled argon-ion laser at 257 nm, was used for the analysis of acidic and neutral impurities in heroin by Lurie et al. [66]. A column, packed with 1.5 μm non-porous octadecyl silica (ODS), provided similar resolving power to that of a sulfonic acid C_{12} polymer monolithic column. However, a higher organic content in the mobile phase was required for the monolithic verses the packed column to obtain comparable retention times. MEKC with fluorescence detection was also employed to analyse heroin seizure samples; thus, the resolving power of CEC was 2.5 times greater than that of MEKC.

3.2. Forensic toxicology and biological samples

Capillary electrophoresis has been used to identify many drugs in a variety of biological samples. Blood and urine serve most frequently as sources of biological specimens for analysis, although analysis can be extended to other specimens, such as saliva, vitreous humor, hair etc. The measurement of drugs in body fluids and tissues is necessary for the determination of specific drugs and/or metabolites, and for the confirmation of illicit drugs for forensic interest. Capillary electrophoresis has been successfully applied to the determination of various analytes in biological samples, using UV and fluorescence spectroscopy methods of detection, which have been summarised in Table 2.

A capillary electrophoresis-based assay for the presence of acidic and basic drugs in serum and urine, using CZE, MEKC and NACE, was described by Boone et al. [69]. Each method exhibited a different selectivity due to the composition and properties of the run buffer.

Boatto et al. [70] reported a CZE method for the analysis of amphetamines in blood using UV detection. A 50 μm × 50 cm capillary (41 cm effective length) was used with a 100 mM phosphate run buffer, pH 2.5. Separations were performed within 7 min, using an applied voltage of 10 kV at 25 °C, with UV detection at 200 nm. Good linearity was obtained with excellent correlation coefficients. The detection limit was between 10 and 30 ng/ml for most amphetamines, except for MDMA for which it was 80 ng/ml.

Alnajjar and McCord [71] investigated the determination of heroin metabolites in urine, utilising urinary 6-acetyl morphine as a diagnostic indicator of heroin abuse. CZE with β-CD and UV detection provided low detection limits (30–40 ng/ml) which may have been attributed to the combination of electrokinetic injection and stacking.

Bortolotti et al. [72] were able to develop a CZE method with indirect detection for the analysis of GHB in untreated urine and serum at potentially toxic concentrations for application in emergency toxicology and GHB overdoses.

Using CZE for the chiral separation of methamphetamine and related compounds, Liau et al. [58] were able to identify the distribution of enantiomers in urine. Additionally, the distribution of (RS)-MDA and (RS)-MDMA stereoisomers in urine samples were identified by Huang et al. [59].

CZE was employed by Kinkennon et al. [73] for the analysis of nitrite in adulterated urine samples. Separation was performed, using a 25 mM phosphate background electrolyte, pH 7.5, with a limit of detection of 20 mg/ml. Ten anions were tested for potential interference with the assay. Interferences with quantitation were observed for CrO_4^{2-} and $S_2O_8^{2-}$, whereas high concentrations of chloride interfered with chromatography.

The capability of SDS to solubilise proteins signifies that plasma samples can be directly injected into an untreated fused-silica capillary containing a buffer with SDS, as demonstrated by Emara et al. [74]. MEKC was used for the determination of morphine in human plasma without

the need of sample pre-treatment. Endogenous components present in plasma were also shown not to co-migrate with morphine.

Capillary ion electrophoresis (CIE), a form of capillary electrophoresis, uses the differential electrophoretic mobility of ions to perform a separation of ionic mixtures. Applications of CIE for the direct detection of endogenous anions and anionic adulterants in human urine was performed by Ferslew et al. [75]. Separations were performed within 5 min on a 60 cm × 75 μm capillary, with an applied voltage of 16.5 kV, with UV detection at 254 nm. The run buffer consisted of 4.7 mM sodium chromate, 4 mM OFM-OH, 10 mM CHES and 0.1 M calcium gluconate, pH 9.1. Excellent migration data coefficients of variation ($n = 20$) between 0.16 and 0.90% for each anion were obtained. Correlation coefficients >0.999 were obtained for calibrations in the range of 1–20 μg/ml. A comparison of results obtained by CIE and colorimetric analysis were acceptable.

Tagliaro et al. [76] also demonstrated the use of CIE into the potassium concentration differences in vitreous humour between two eyes at the identical post-mortem interval. No significant differences in potassium concentrations were observed.

Low-temperature and ambient temperature non-aqueous stacking techniques in capillary electrophoresis was described for the first time by Tsai et al. [77]. 3,4-methylenedioxymethamphetamine (3,4-MDMA) was determined at a concentration of 4.7×10^{-6} M by normal non-aqueous capillary zone electrophoresis. These detection limits were improved when stacking and low-temperature NACZE stacking techniques were applied to afford detection limits of 2.6×10^{-8} and 5.0×10^{-9} M, respectively. The separation of isomers of 3,4-MDMA were also successful when low-temperature non-aqueous MEKC was applied.

Fang et al. [78] demonstrated that non-aqueous capillary electrophoresis/fluorescence spectrometry (NACE/FS) was a sensitive analytical technique for the identification of 3,4-MDMA in urine. This method provided results within 5 min with a detection limit of 0.5 ppm without sample pre-treatment. A liquid–liquid extraction method was also developed with improved detection limits of ∼50 ppb. A 75 μm × 35 cm capillary (30 cm effective length) was used with a buffer composed of 100 mM sodium cholate and 20 mM ammonium acetate in a formamide–methanol solution (30:70, v/v). Separations were performed with an applied voltage of 15 kV at 77 K, with an excitation and emission wavelength of 285 and 320 nm, respectively. Poor intra- and inter-day peak area R.S.D. values ($n = 6$) of 7.47% and 9.88% for 3,4-MDA were obtained, respectively. However, fair intra- and inter-day migration time R.S.D. values of 1.48% and 3.86% were attained, respectively.

Methods for the separation and determination of a variety of drugs of abuse in biological fluids, using capillary electrophoresis with native fluorescence and LIF detection, were described by Alnajjar et al. [79]. Normorphine, morphine, 6-acetylmorphine and codeine were detected using fluorescence detection. Detection was performed at an excitation wavelength of 245 nm and a cut-off emission filter of 320 nm, with detection limits of approximately 200 ng/ml. LIF detection was used employing a two-step precolumn-derivatization procedure. Detection was performed with an excitation wavelength of 488 nm and an emission wavelength of 520 nm. Detection limits for the derivatized analytes were in the range of 50–100 pg/ml. Additionally, few endogenous compounds from the urine samples were extracted using the CE-LIF extraction procedure.

CE with head-column field-amplified sample stacking was applied to the determination of opioids in urine by Wey and Thormann [80]. This method is based upon electro-injection of analytes from sample extracts of low conductivity, resulting in a sensitivity enhancement 1000-fold, using UV detection. Electro-injection, applied to CE-ion trap MS–MS and MS–MS–MS of two-fold diluted urines, urinary solid-phase and liquid–liquid extracts provided greater sensitivity compared to hydrodynamic injection of these samples. Unambiguous confirmation of free opioids and their glucuronic acid conjugates from solid-phase extractions with electroinjections was also observed.

Wey and Thormann [81] used CE-tandem MS and CE-triple MS with atmospheric pressure electrospray ionization for the analysis of morphine and related opioids in urine. Commencing with 2 ml urine and reconstitution in 0.2 ml sample solvent, detection limits for free opioids of 100–200 ng/ml were obtained with hydrodynamic sample injection.

Baldacci et al. [82] described the analysis of γ-hydroxybutyric acid (GHB) in urine by CE, with indirect UV detection and detection limits of 2 μg/ml. Furthermore, electrospray ionization ion-trap mass spectrometry was used to confirm the presence of GHB in urinary extracts.

Boatto et al. [83] described a method for screening and quantification of ten 2,5-methylenedioxy derivatives of amphetamine and phenylethylamine in urine samples using CE–ESI–MS. The sensitivity obtained using this method was comparable to that observed by CE–MS for similar amphetamines.

A CEC method for the separation of barbiturates in serum using short-end injection was employed by Ohyama et al. [84]. Separations were performed using a 9 cm effective length capillary, packed with 3-(1,8-naphthalimido)propyl-modified silica gel (NAIP) and a run time of 4.5 min.

The analysis of illicit drugs in biological specimens, such as blood and urine, are the main specimen for analysis. Alternatively, hair specimens can also be tested for drugs of abuse. This is a relatively non-invasive method of sample collection, and can provide long-term exposure of drug misuse. The analysis of several opiates and metabolites in hair was illustrated by Tavares et al. [85], resulting in a separation within 14 min. Unfortunately, codeine and tramal, and nalorphine and 6-acetylcodeine, were not baseline-resolved. Capillary dimensions, as well as statistical data in respect to method performance, were not reported.

4. Conclusions

It is clear from the body of work reviewed above that capillary electrophoresis offers significant operational advantages in terms of resolving power and analysis time. It should also be noted that as the basis for separation is fundamentally different to that in GC and HPLC, it is useful in situations where an orthogonal approach to analysis of forensic samples is required [86].

A major future direction for capillary electrophoresis for forensic analysis is miniaturisation. Capillary electrophoretic separations have been carried out on micro-fluidic devices and the potential for portable instrumentation for forensic science based on this technology has already been recognised [87,88]. Such instrumentation would allow multi-component analysis of illicit substances at the crime scene, providing the investigator with useful intelligence at an early stage in a criminal investigation.

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