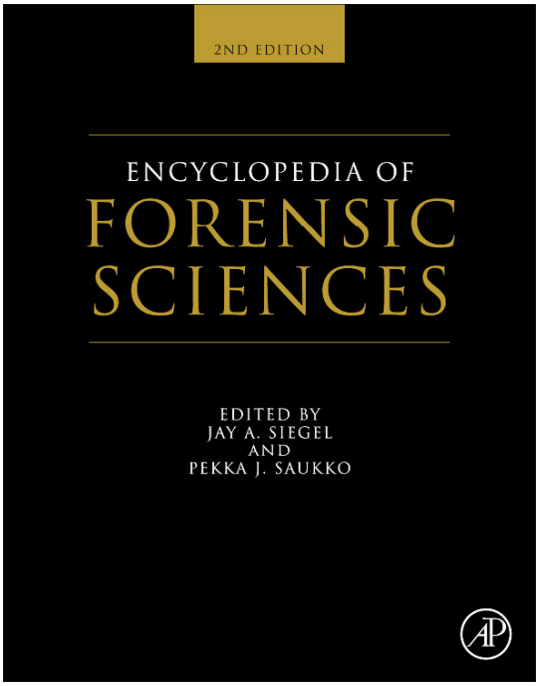

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Capillary Electrophoresis in Forensic Chemistry

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Abbreviations

BGE	Background electrolyte
CDT	Carbohydrate-deficient transferrin
CE	Capillary electrophoresis
CE-ESI-MS	Capillary electrophoresis-electrospray ionization-mass spectrometry
CZE	Capillary zone electrophoresis
GC	Gas chromatography
GHB	Gamma-hydroxybutyric acid
HPLC	High-performance liquid chromatography
MEKC	Micellar electrokinetic chromatography

Introduction

The separation and quantification of chemical substances in a wide variety of complex matrices is a significant analytical challenge for the forensic chemist. While there is no doubt that capillary electrophoresis (CE) has had its largest impact in the area of forensic biology, specifically in the field of DNA analysis, it has also found application in forensic chemical analysis in general. Since the potential of CE for forensic analysis was first demonstrated in the early 1990s, it has been applied to a wide range of analytes of forensic interest including illicit drugs, toxicology samples, explosives residues, and pen ink.

CE is attractive for forensic applications because of its exceptional separating power (up to millions of theoretical plates), rapid analysis times, and high mass sensitivity (from femtomoles – 10^{-15} moles – down to yoctomoles – 10^{-21} moles). It is an economical technique in terms of reagents and consumables, requiring only minimal amounts of sample. Additionally, owing to the variety of separation modes possible (electrophoretic, electrokinetic, chromatography-like, etc.) and the detection systems available (UV–visible absorbance, luminescence, and mass spectrometry are all available commercially), it is applicable in the determination of a wide variety of chemical substances including inorganic ions, small organic molecules, chiral compounds, macromolecules, and intact viruses and cells. The Achilles heel of CE in the past has been its relatively poor detection limits when compared with liquid chromatography and gas chromatography (GC). However, improved detection optics and electronics for the most commonly encountered detector in CE, namely UV–visible absorbance, and the introduction of new detection techniques such as contactless conductivity detection have led to improved detection limits that are compatible with many forensic applications. Significant improvements in terms of sensitivity and specificity have been achieved in recent years by the hyphenation of CE with mass spectrometry (MS). CE–MS has become useful in clinical and forensic toxicology, doping control, and workplace drug testing, gaining attractiveness in respect to GC–MS and LC–MS.

The purpose of this article is to provide an overview of the application of CE to forensic analysis, illustrated with recent examples. For more comprehensive treatments of specific areas, the reader is directed to the textbooks, monographs, and review articles listed in Further Reading.

Applications

Illicit Drug Analysis

CE has been used widely for the analysis of drugs and related compounds in pharmaceutical research and development since its introduction as a commercial analytical technique in the late 1980s. The potential of CE for forensic analytical problems was first demonstrated in the early 1990s with its application to the separation of illicit drugs in synthetic mixtures. Since that time, the determination of illicit drug seizures and clandestine lab materials has become a major application area for this group of techniques. Some examples of the application of CE techniques to such samples are presented on Table 1.

In many instances, the choice of CE as an alternative technique to the more traditional GC and high-performance liquid chromatography (HPLC) methods is justified by the minimal amount of sample required and by the shorter and easier sample pretreatment. CE often allows for very short separation times, particularly when using techniques such as short-end injection are being used (see Figure 1). These features are beneficial to forensic analysis, particularly to screening for illicit drugs in clandestine preparations. In addition, CE lends itself to the difficult task of separations of enantiomers by the addition of a chiral selector to the background electrolyte (BGE). In addition to determining active illicit drugs, CE is also useful in separating cutting agents and other compounds that might be present, thus making it highly suitable for profiling type analyses.

Toxicology

CE has been successfully applied to a wide range of drugs, both therapeutic and illicit, and their metabolites in a wide range of biological matrices including urine, blood, and serum for forensic toxicological purposes.

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Table 1 Applications of capillary electrophoresis to illicit drugs in seizures and toxicology samples

Analytes	*Matrix*	*Method*
Coca alkaloids and sugars	Illicit cocaine	MEKC with indirect UV detection
D-methamphetamine, L-methamphetamine, D-MDMA, L-MDMA, D-ketamine, L-ketamine, and heroin	Surfaces of banknotes, plastic bags, and silver paper	Liquid-phase microextraction followed by CZE using beta-cyclodextrin as a chiral selector, detection by UV absorbance
Heroin, morphine, acetylcodeine, caffeine, paracetamol	Heroin seizures	MEKC with short-end injection, detection by UV absorbance
Glucose, sucrose, lactose, mannitol	Heroin seizures	CZE with borate complexation and short-end injection, detection by UV absorbance
Codeine, ketamine, methamphetamine, morphine, alprazolam, and oxazepam	Urine	MEKC with UV absorbance detection
Methamphetamine, amphetamine, dimethylamphetamine, and *p*-hydroxymethamphetamine	Urine from subjects using methamphetamine and dimethylamphetamine	CZE using cyclodextrins for separation of enantiomers with MS detection
LSD	Blood	CZE with LIF detection
MDMA	Hair	CZE with UV absorbance detection

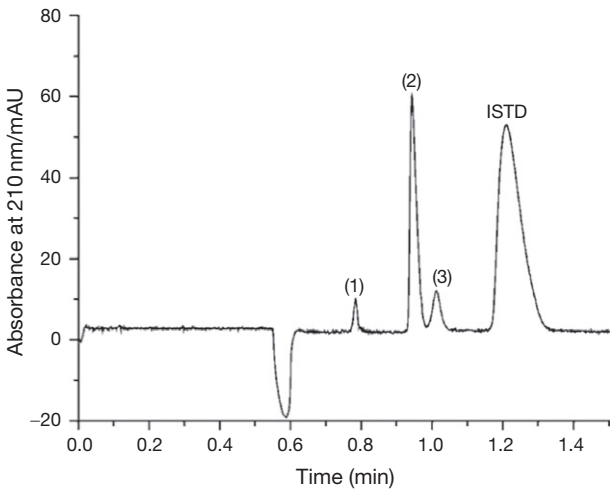

Figure 1 Electropherogram showing the separation of a seized heroin sample by using MEKC with short end injection (1) morphine, (2) heroin, (3) acetylcodeine, ISTD = internal standard (*N*, *N*-dimethyl-5-methoxytryptamine). UV absorbance at 210 nm, uncoated fused silica capillary 50 cm × 50 μm I.D. ×360 μm O.D., effective separation length 8 cm, back- ground electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15% (v/v) acetonitrile, pH 9.5, 25 °C, –25 kV, hydrodynamic injection: 2 s at −50 mbar. Reprinted from Anastos N, Lewis SW, Barnett NW, Pearson JR, and Kirkbride KP (2005) The rapid analysis of heroin drug seizures using micellar electrokinetic chromatography with short-end injection. *Journal of Forensic Sciences* 50: 37–42, with permission.

CE has been applied to therapeutical drug monitoring, which has both clinical and forensic implications. Lithium salts are one of the most popular therapeutic approaches to the treatment of bipolar disorders, notwithstanding the introduction of modern, less toxic drugs. Because of its narrow therapeutic range, lithium serum concentration must be strictly monitored during the treatment to avoid life-threatening neurotoxicity. Capillary zone electrophoresis (CZE) was successfully applied to the routine determination of lithium in serum samples after a simple sample pretreatment of dilution with water and indirect detection using UV absorbance.

Small organic molecules, such as the central nervous system depressant and hypnotic gamma-hydroxybutyric acid (GHB), feature heavily as analytes in toxicological samples. GHB has been increasingly used as a recreational drug (owing to its euphoric effects and ability to reduce inhibitions) and as a rape facilitation drug. GHB has also been used as a doping agent for enhancing muscle growth. Analogs of GHB, namely gamma-butyrolactone and 1,4-butanediol, share its biological activity and are rapidly converted in vivo into GHB. CZE, in combination with UV detection, capillary electrophoresis-electrospray ionization-mass spectrometry (CE-ESI-MS), or conductivity detection, has been shown to be a rapid and accurate approach to the determination of GHB and its metabolites in urine and blood serum. Additional examples of illicit drugs determined in toxicological samples by CE are provided in Table 1 with an example of analysis of hair for 3,4-methylenedioxymetham-phetamine (MDMA) shown in Figure 2.

CE can also be used for the determination of large biopolymers. It has been applied in the field of antidoping analysis to identify macromolecules. Recombinant human erythropoietin (rHuEPO) and the novel erythropoiesis-stimulating protein were analyzed by CE-ESI-MS using an ion trap mass spectrometry instrument as the analyzer. Additionally, affinity probe capillary electrophoresis/laser-induced fluorescence was developed for the detection of rHuEPO-alpha using a specific single-stranded DNA as aptamer probe. After optimization, the method was successfully applied for the quantification of rHuEPO-alpha in buffer, artificial urine, and human serum.

Toxins and venoms are products of living organisms, which in most cases have complex and unstable polypeptide structures. For these reasons, they are in general not suitable for GC analysis and are often 'difficult' to analyze with HPLC. CE provides an alternative approach for analyses of these challenging analytes. For example, the CE-ESI-MS method, using an ion-trap mass spectrometer, has been used for the identification of the biologically active oligopeptides of Amanita fungi, namely, alpha-, beta-, gamma-amanitin, phalloidin, and phallacidin. These complex analytes were also determined in real samples using a simple CZE separation with UV

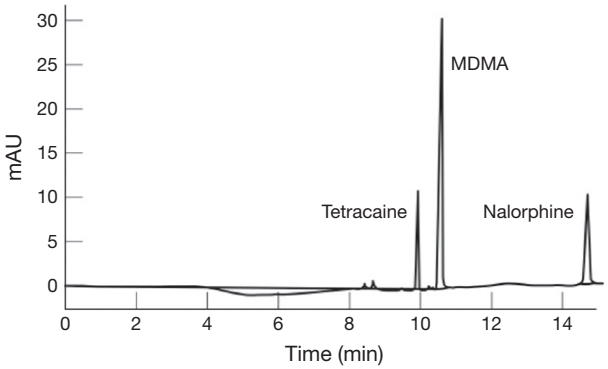

Figure 2 Electropherogram of an extract from a sample of hair from a user of 'ecstasy' containing 3,4-methylenedioxymetham-phetamine (MDMA) at a concentration of 4.0 ng mg^{-1}. Tetracaine and nalorphine were added as internal standards. Injection was by electromigration under field-amplified sample stacking conditions. Separation was by CZE using 100 mM phosphate pH 2.5 as the running buffer. Detection was by UV absorbance at 200 nm. Reprinted from Tagliaro F, Manetto G, Crivellente F, Scarcerlla D, and Marigo M (1998) Hair analysis for abused drugs by capillary zone electrophoresis with field-amplified sample stacking. *Forensic Science International* 92: 20–211, with permission.

absorbance detection. Alpha- and beta-amanitin were determined in urine in less than 7 min with a simple sample treatment of dilution in BGE. Urine samples from suspected cases of intoxication with amanitins were analyzed, with beta-amanitin being identified in two samples.

Forensic Medicine

Recently, a number of protein molecules have become attractive as biological markers of chronic alcohol abuse. In particular, carbohydrate-deficient transferrin (CDT) has gained universal acceptance in this context, as reported in recent research papers and reviews. CDT is the collective name of a group of minor glycoforms of transferrin (the main iron transporting protein in human serum) with a low degree of glycosylation. CDT includes asialo-, monosialo-, and disialo-transferrin (Tf). CDT concentrations increase after sustained alcohol intake ($\geq$50–80 g day^{-1}), lasting for at least 7–10 days; there is a decrease after cessation of drinking with a half life of about 14 days.

CE was first applied to CDT analysis in the late 1990s and has since then rapidly gained acceptance. Today, together with HPLC, it is considered one of the most reliable analytical methods in the international literature. After the introduction of multicapillary instrumentation and ready-to-use commercial reagents, CE has become by far the most productive instrumental technique for CDT determination. Most CE methods are based on CZE separations using borate buffers added with organic amines (e.g., with diaminobutane, spermine, or diethylenetriamine) to hinder protein interactions with the capillary wall. Detection is based on absorption of UV radiation at 200–214-nm wavelengths. Examples of its application include CDT determination in blood microsamples collected from newborns using microhaematocrit tubes and analysis of cadaveric blood to study the postmortem stability and possible redistribution of CDT. Notwithstanding a relevant haemolysis, CE separation allowed for CDT determination in 41% of the blood samples collected after death, showing an acceptable stability of the protein and the absence of an appreciable postmortem redistribution.

In a similar manner, changes in hemoglobin (HbA) induced by reaction with acetaldehyde, which is the first and most reactive metabolite of ethanol, has been proposed as a possible biomarker of alcohol abuse. A CE-ESI-MS method has been developed to study these adducts and applied to studies *in vitro*. When applied to real samples, this technique led to the identification of stable modifications of hemoglobin even in moderate alcohol drinkers.

Inks

Inks are complex mixtures of colorants, vehicles, and additives that are adjusted in composition to produce the desired writing characteristics. From a forensic point of view, ink analysis is mainly requested for ink-source comparisons, commonly conducted in casework involving such crimes as tax evasion, insurance fraud, and currency counterfeiting. The ability to distinguish different inks can be useful in criminal cases of document alteration, where two or more inks of the same apparent color, but with different dye compositions, were used in a document.

CE in common with other instrumental separation techniques, such as HPLC and GC, has been applied to the forensic analysis of pen ink. There are a number of reports in the literature, for example, of separations of water-soluble inks by CZE, using UV absorbance and laser-induced fluorescence detection. CE has also been used to analyze roller ball inks as part of ink aging investigation where the age of various ink samples was determined based on the relative quantities of components separated by CE.

The possibility of comparing inkjet printing inks by using micellar electrokinetic chromatography (MEKC) with diode array detection has also recently been proposed. The analytical procedure discriminated between the electrophoretic profiles of inks (extracted from paper) that were produced by five different manufacturers. The effective differentiation of individual inks was possible in terms of migration time, order, and specific shapes of characteristic peaks. The comparison of the recorded UV–Vis spectra also allowed for the identification of the main dyes.

Explosives and Gunshot Residues

Explosives have a long history of criminal use, and have become of increasing concern with their widespread use for international terrorism. The provision of information concerning the identity of an explosive at an early stage in an investigation is critical. Explosives and explosive residues can include a wide range of organic and inorganic components, thus necessitating an equally wide range of analytical techniques to identify them in the often complex matrix of postblast debris. Instrumental techniques, such as SEM-EDX, MS coupled with liquid or GC, x-ray spectrometry, infrared spectrometry, and ion chromatography, are used in combination with wet chemical tests (presumptive tests) to provide information on the identity and quantity of explosive residues.

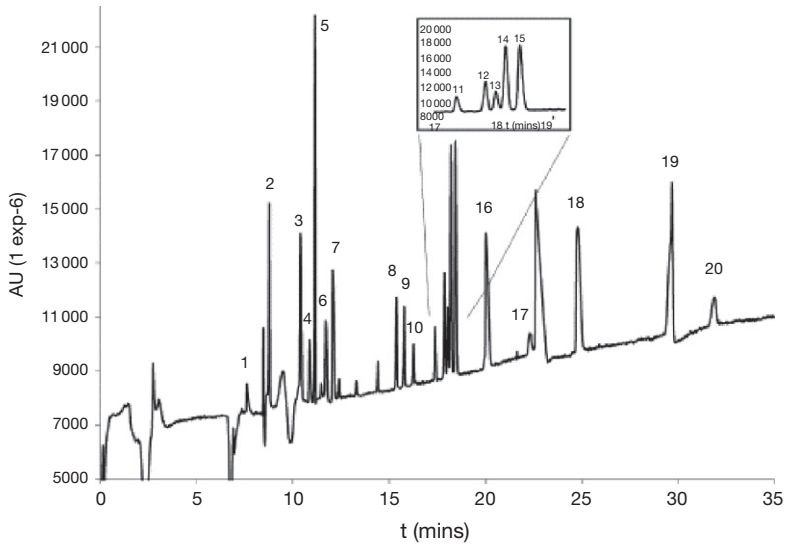

Figure 3 Simultaneous separation of organic and inorganic gunshot residues under optimal conditions. Electrolyte: 40 mM borate buffer, 16 mM SDS, 0.5 mM CDTA, capillary: 79.2 cm (69.2 cm detection length) ×75 μm i.d. Hydrodynamic injection: 0.5 p.s.i. for 5 s at 25 °C, UV detection at 200 nm. (1) Sb (30), (2) resorcinol (11.1), (3) 24-DNT (10.93), (4) 26-DNT (14.57), (5) Fe (10), (6) 23-DNT (18.2), (7) MF (6.0), (8) Ba (30), (9) Ca (20), (10) Mg (20), (11) Al (20), (12) Ni (20), (13) Zn (10), (14) Pb (10), (15) Cu (20), (16) EF (17.8), (17) DPA (16.9), (18) MC (14.4), (19) EC (22.6), (20) BF (10). Standard concentrations in parentheses in milligrams per liter. Reprinted from Morales EB and Vázquez AL (2004) Simultaneous determination of inorganic and organic gunshot residues by capillary electrophoresis. *Journal of Chromatography A* 1061: 225–233, with permission.

Many organic and inorganic explosives and their residues are amenable to analysis by CE with acceptable limits of detection. CE is particularly well suited to the determination of inorganic ions that might be present in the postblast residue from improvized explosive devices (IEDs). In order to achieve the required level of confidence in identification of an explosive residue, corroboration through analysis by two independent techniques is required. In addition to the advantages of CE referred to earlier, the separation mechanisms in CE are substantially different from those of ion chromatography, thus providing a suitable orthogonal approach.

CE has also been shown to be useful in the separation and identification of components in gunshot residues (GSR). These residues are typically analyzed to identify persons who fired weapons and to investigate the sources and supply chains of ammunition. GSR analysis is traditionally based on the determination of heavy metals (usually lead, barium, and antimony) originating from the primer, but the production of 'metal-free' ammunition requires robust analytical methods for the identification of the organic components in the gunshot and explosive residues. Both the inorganic and organic components of GSR can be separated by CE simultaneously through the use of complexation for the metal ions and MEKC for the uncharged organic compounds (see **Figure 3** and **Table 2**). The method was tested on real samples collected from weapons and from hands after firing.

Table 2 Characteristic organic and inorganic gunshot residue components listed with abbreviations used in **Figure 3**

Compound	*Abbreviation*	*Usage*
Nitroglycerin	NG	Propellent
Resorcinol	Rs	Stabilizer
2,4-Dinitrotoluene	24-DNT	Flash inhibitor
2,6-Dinitrotoluene	26-DNT	Flash inhibitor
2,3-Dinitrotoluene	23-DNT	Flash inhibitor
Dimethyl phthalate	MF	Plasticizer
Diethyl phthalate	EF	Plasticizer
Dibutyl phthalate	BF	Plasticizer
Diphenylamine	DPA	Stabilizer
Methyl centralite	MC	Stabilizer
Ethyl centralite	EC	Stabilizer
Antimony	Sb	Fuel
Iron	Fe	Bullet material
Barium	Ba	Oxidizing agent
Calcium	Ca	Fuel
Magnesium	Mg	Fuel
Aluminum	Al	Fuel
Nickel	Ni	Bullet material
Zinc	Zn	Bullet material
Lead	Pb	Explosive (lead styphnate)
Copper	Cu	Bullet material

Reprinted from Morales EB and Vázquez AL (2004) Simultaneous determination of inorganic and organic gunshot residues by capillary electrophoresis. *Journal of Chromatogratography A* 1061: 225–233, with permission.

Miniaturization

An area of significant recent research has been the move to develop portable instrumentation for at- or near-scene analysis. This is of particular importance for investigations involving clandestine drug laboratories and explosions. The National Institute of Forensic Sciences in Australia identified the rapid at-scene identification of illicit drugs and explosives to be of paramount importance in combating organized crime and terrorism in its Forensic Science Innovation Strategy reported in 2001. The provision of chemical information at an early

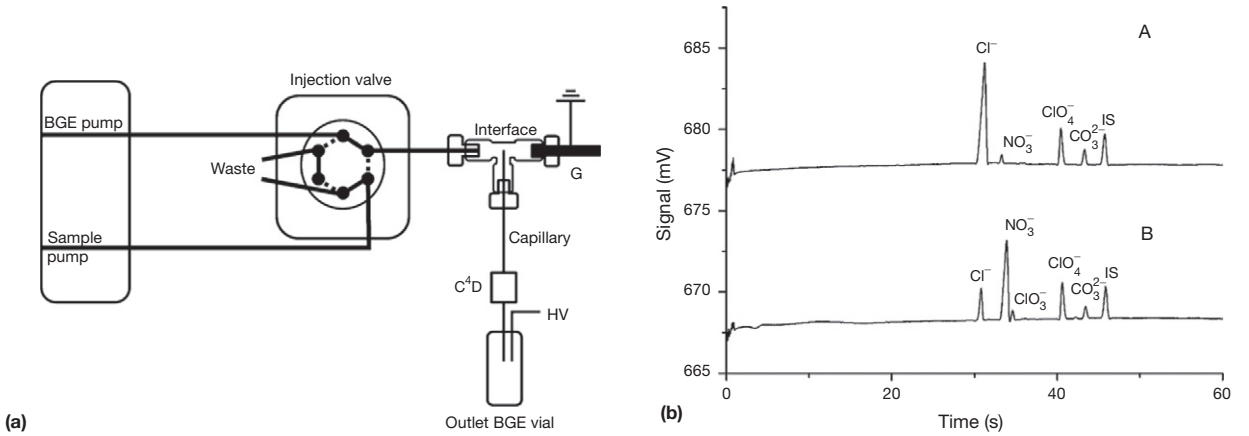

Figure 4 (a) Schematic diagram of the SI-CE system: G, ground; HV, high-voltage electrode; C4D, capacitively coupled contactless conductivity detector. (b) Electropherogram obtained from the aqueous extracts of a soil sample after the detonation of a (A) perchlorate/sugar IED; (B) chlorate/perchlorate/nitrate/sulfur/charcoal IED. IS, propanesulfonic acid 5 mg l^{-1}. BGE composition, 50 mM Tris, 50 mM CHES, 0.05% (w/v) PEI. Capillary, 25 μm i.d. 35 cm (25 cm effective length); separation, −25 kV; injection, −1 kV × 1 s. Reprinted from Blanco GA, Nai YH, Hilder EF, et al. (2011) Identification of inorganic improvised explosive devices using sequential injection capillary electrophoresis and contactless conductivity detection. *Analytical Chemistry* 83: 9068–9075, with permission.

stage of an investigation is extremely useful in rapid identification of potential suspects, in addition to being important for occupational health and safety reasons.

As described above, CE is well suited to the analysis of illicit drugs and explosives residues. However, commercially available benchtop instrumentation is too bulky to be used in the field. An area that shows great promise for forensic analysis is microfluidics, the so-called 'lab-on-a-chip.' Joseph Wang, one of the world's leaders in microfluidics, stated in a 2004 review that "microfluidic devices offer great promise for transporting the forensic laboratory to the sample source." Many microfluidic devices rely upon electrophoresis to separate analytes, with the advantage that miniaturization also leads to associated reductions in analysis times. For example, micro-chip capillary electrophoresis has been applied to the analysis of nitrate ester explosives, with ethylene glycol dinitrate, pentaerythritol tetranitrate, propylene glycol dinitrate, and nitroglycerin being separated in less than 3 min. Amphetamine, methamphetamine, and pseudoephedrine derivatized with a fluorescent label were separated by MEKC and quantified using a commercially available lab on a chip device intended for bioassays. The derivatization was achieved using a dry heating block procedure, which allowed for labeling of the drug analytes in 3 min and could feasibly be used in the field.

In addition to these lab-on-a-chip devices, other workers have focused on using shorter capillaries in combination with higher voltages to achieve rapid separations in portable instrumentation. These have required the development of novel flow-based sample introduction techniques. For example, a simple sequential injection capillary electrophoresis (SI-CE) instrument with contactless conductivity detection has been developed and applied to the determination of anions relevant to the identification of inorganic IEDs. The portable device was able to separate mixtures of key explosive tracer ions (nitrate, perchlorate, chlorate, and azide) in combination with common background ions (chloride, sulfate, thiocyanate, fluoride, phosphate, and carbonate) within 55 sections (see Figure 4).

Software control enabled a high analytical frequency of 60 samples per hour with high repeatability of separation times and detection limits in the 25–50 μg l^{-1} range. National Institute of Justice Guide for the Selection of Commercial Explosives Detection Systems for Law Enforcement Applications states that the nominal capability for explosive screening technology is that it "Can detect 100 μg of each target explosive in the swipe collection mode at least 95 percent of the time." For a typical ammonium nitrate-fuel oil explosive, 100 μg translates to approximately 73 μg of nitrate, which if dissolved in 1 ml of water, gives a concentration well above that of the detection limits for the SI-CE instrument.

See also: **Chemistry/Trace/Fire Investigation:** Analysis of Fire Debris; **Chemistry/Trace/Paint and Coating:** Forensic Paint Analysis; **Documents:** Ink Analysis; **Forensic Medicine/Causes of Death:** Gunshot Wounds; **Forensic Medicine/Pathology:** Estimation of the Time Since Death; **Toxicology:** Drug Screening in Sport; Herbal Psychoactive Substances; Volatile Substances and Inhalants; **Toxicology/Alcohol:** Blood; Urine and Other Body Fluids; **Toxicology/Drugs of Abuse:** Drug-Impaired Driving; Drugs in Hair; Urine; Validation of Twelve Chemical Spot Tests for the Detection of Drugs of Abuse.

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