- A continuous and large-scale process can be economically competitive to hexane-based operations.

Exploiting the commonality of high pressure between supercritical fluid and extrusion processing operations, a hybrid unit operation called supercritical fluid extrusion (SCFX) has been recently developed. This new process permits generation of microcellular structure at low temperature by using SC-$CO_2$ as a blowing agent instead of steam to puff the extrudate, thus decoupling the conventional dual role of water, which otherwise serves both as a blowing agent as well as a plasticizer. The use of supercritical fluid also permits deposition of solute into the extrudate matrix.

Significant progress has also been made in the analysis of food and related materials using SFE with SFC. Sample preparation for analysis often requries orders of magnitude more time than the analysis itself and the use of supercritical fluids obviates the need for hazardous organic solvents with no additional treatment prior to identification of the analyte by other techniques such as GC, GC-MS, FTIR, etc. The solubility of lipid-like materials in SC-$CO_2$ ranges from 1 to 30 wt%, depending on the density of the fluid used, and therefore, SFE has become a method of choice for rapid extraction of fats and oils from a variety of food matrices such as animal, vegetable, grain and seafood products. Other successful applications include extraction of fat-soluble vitamins, pesticides, sterols, and fatty acids. As an analytical tool, SFC has also made significant progress over the past decades but has yet to prove its superiority over the more conventional techniques.

*See also:* **II/Extraction:** Supercritical Fluid Extraction. **III/Food Technology:** Supercritical Fluid Chromatography. **On-Line Sample Preparation: Supercritical Fluid Extraction.**

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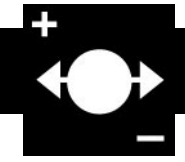
# FORENSIC SCIENCES



## Capillary Electrophoresis

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Several slab electrophoretic techniques have frequently been used to discriminate between red cell enzyme markers as a means of identification in criminal cases over many years. In 1991 capillary electrophoresis (CE) was introduced to forensic analysis. The separation of bulk heroin, heroin impurities and degradation products using micellar electrokinetic capillary chromatography (MEKC), the determination of drugs of abuse in urine and also the determination of benzodiazepines and sulfonamides in urine by CE–mass spectrometry were described in the same

year. Today, forensic applications of CE include analysis of drugs of abuse, gunshot residues, explosives, pen inks and toxins as well as polymerase chain reaction (PCR) amplified DNA.

## Drugs of Abuse

One of the major tasks for forensic laboratories is the analysis of illicit and controlled drugs, in both the seizure and biological samples.

### Seizure Samples

Seizure samples are analysed in order to identify the major compounds. In addition, the determination of trace compounds permits the samples to be allocated to the source and production procedures. Seizure samples may consist of a mixture of acidic, neutral and basic compounds that may be nonpolar and/or polar. At least two independent analytical parameters should be used to establish the identity of the drug, and infrared spectroscopy and thin-layer chromatography (TLC) are widely used for this purpose. Quantitation is usually carried out by gas chromatography (GC) and high performance liquid chromatography (HPLC). GC is a high resolution technique, but problems can arise for thermally degradable, polar and nonvolatile substances. HPLC is less suited to drug profiling, because it is a relatively low resolution technique compared with GC.

CE is a relatively new technique is forensic drug analysis. The three main separation mechanisms have been used for seizure samples: (i) low pH to analyse basic compounds; (ii) high pH to analyse acidic compounds; and (iii) MEKC to analyse neutral and/or charged compounds.

Most abused drugs are bases which are generally water-soluble and ionized as cations at low pH. The use of simple electrolyte solutions such as phosphate, citrate and formate at pH values of 2–3 gives a useful initial separation. Basic drugs can be analysed by TLC and HPLC, but interactions with the stationary phases can lead to peak tailing. This problem does not occur so frequently in CE. In addition, these simple electrolytes have low background UV absorbance and can be operated at low wavelengths of 190–200 nm, where many drugs have significantly enhanced UV absorbance coefficient. CE at low pH can be used to detect by-products in purified codeine, to investigate amphetamine derivatives in Ecstasy tablets, and for assay for various pharmaceutical formulations which contain 1,4-benzodiazepines and phenothiazines.

At high pH the migration direction of acidic compounds is against the electroosmotic flow, which maximizes mobility differences. Operation with simple electrolytes such as phosphate pH 7 or borate pH 9.5 often leads to useful initial separation for acidic compounds.

MEKC can be used when dealing with uncharged solutes or mixtures of charged and neutral species. This approach may also be considered when simple mobility differences prove insufficient in capillary zone electrophoresis (CZE). Both anionic and cationic surfactants have been used as micelle modifiers, which, furthermore, are complementary approaches. MEKC has been applied to a wide range of controlled substances, including heroin, cocaine, opium alkaloids, amphetamines, hallucinogens,

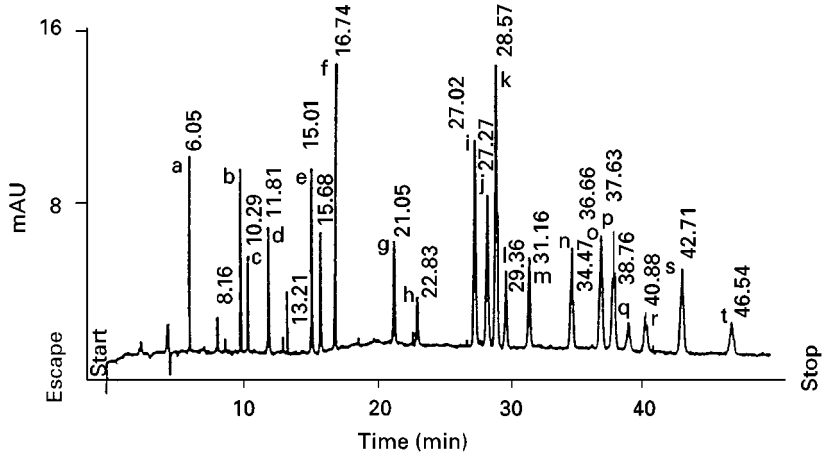


**Figure 1** Typical example of a MEKC separation of the components of a drug mixture. Buffer: 25 mmol $L^{-1}$ borate (pH 9.24)–20% methanol–100 mmol $L^{-1}$ SDS. Capillary: bare fused silica, i.d. 50 μm, total length 55 cm (35 cm to detector). Potential 20 kV. UV detection at 200 nm. Peak identification: a, caffeine; b, barbital; c, pentobarbitone; d, morphine; e, narceine; f, 6-monoacetylmorphine; g, codeine; h, nalorphine; i, lidocaine; j, procaine; k, heroin; l, flunitrazepam; m, acetylcodeine; n, thebaine; o, papaverine; p, amphetamine; q, narcotine; r, cocaine; s, diazepam; t, tetracaine. (Reprinted with kind permission of Elsevier Science from Tagliaro F *et al.* (1996) *Journal of Chromatography A* 735: 227–235.)

barbiturates, benzodiazepines and cannabinoids. An electropherogram of a complex mixture of 20 drugs (acidic, neutral and basic) is shown in **Figure 1**.

It is clear that MEKC represents an excellent technique for drug screening. In addition, photodiode-array UV, laser-induced fluorescence (LIF) and mass spectrometry (MS) detection can greatly increase specificity of the analysis. Greater specificity of screening could be obtained by using two complementary separation techniques, e.g. MEKC with either GC or HPLC. The complementary nature of MEKC and CZE for the identification of 17 illicit drugs and related compounds ionized at pH 2.35 has also been demonstrated. MEKC with sodium dodecyl sulfate (SDS) at pH 9.2 gave a highly noncorrelated separation compared to that obtained on a CZE system at pH 2.35. MEKC was found to be significantly, but inversely, correlated with a CZE system at pH 9.2. The reproducibility of migration times or relative migration times in MEKC is most important for screening applications. Migration time precision of 1% relative standard deviation (RSD) for repeated injection has been shown; this is essential to allow confirmation of the identity of each individual compounds present. Relative migration times generally give better repeatability, with RSD values of less than 1%.

The results generated by MEKC are often compared with those of HPLC and/or GC. GC affords higher resolution than MEKC; however, derivatizations are commonly required. MEKC offers significantly greater efficiency, selectivity, peak symmetry and speed compared to HPLC. In addition, the drugs that are poorly chromatographed by HPLC or not at all by GC exhibit good electrophoretic behaviour using MEKC. A recognized deficiency of MEKC is sensitivity, which is below that for HPLC-UV.

### Biological Samples

The analysis scheme of drugs of abuse in biological samples involves screening using an immunoassay test. This does not enable positive identification to be made, but permits negative samples to be detected and discarded. Subsequently, the results must be confirmed by a more specific method. Without doubt, GC-MS is the reference method to confirm positive screening tests. At present, blood and urine represent most samples analysed for abused drugs and toxicants in most laboratories. If analyte concentrations are high enough, biological fluids, even those containing high concentrations of ions and proteins, can be directly injected on to a CE system, after simple filtration/centrifugation of the sample. Urine analysis can be very fast and simple, while SDS additive must be used with plasma to solubilize protein.

The important problem faced by CE in the field of biological sample analysis is still its relatively low concentration sensitivity. Increased sensitivity can be obtained both instrumentally and by processing the sample before analysis. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) methods have been used as sample pretreatment for CE. After LLE and SPE, extracted mixtures can be dried and resolved with a small volume of solvent, thus achieving detection limits of about 10 ng $mL^{-1}$. The sensitivity can be gained from employing more sensitive fluorescence detection. When LIF can be applied, the sensitivity limit of CE can be improved by a factor of about 1000 or more over UV absorbance detection. Unfortunately, the choice of wavelength emitted by laser is limited and this is the main limitation of LIF application to drug analysis. Concentrating the sample on the capillary – 'stacking' – is a simple technique that overcomes the poor detection limits of CE. Three general stacking methods are used in CE: (i) low ionic strength buffer in the sample; (ii) stacking by including acetonitrile in the sample; and (iii) isotachophoresis (ITP).

For forensic purposes it is often more appropriate to identify the metabolite rather than the parent drug, since additional information yielded by the full metabolic profile of a drug may be important in ascertaining the route of administration. Drug metabolites are most often studied by HPLC; however, phase II metabolites, e.g. glucuronide and sulfate conjugates, are acidic and highly polar and elute with little resolution in reversed-phase HPLC systems. Phase II metabolites are ideal for direct analysis by CE without the need for previous derivatization or hydrolysis. MEKC with diode array detection has been used for the determination of morphine, morphine-3-glucuronide, morphine-6-glucuronide, normorphine, meclofenamic acid and its metabolites in equine urine. SPE procedures were developed to concentrate and purify the analytes from post-administration urines. The low concentration sensitivity of MEKC in comparison with HPLC and GC-MS can be overcome by using a suitable sample preparation procedure, in particular offline SPE.

Hair analysis is a tool to prove drug abuse in questions of drug-related fatalities, revocation and restoration of driver licences, criminal responsibility, prenatal drug exposure and offences of narcotics law. The concentration of drugs in the hair are in the ng $mL^{-1}$ range, at least in cases of chronic abuse. CE applications in hair analysis are still in an early stage of development. The few reports published until now come from Tagliaro's group. The use of CE with UV detection, at 238 nm for cocaine and 214 nm for morphine, has permitted the achievement of

moderate sensitivity 0.2 ng $mL^{-1}$ for both analytes. Later, stacking techniques were developed in order to increase sensitivity (about five times). MEKC has also been applied to hair analysis. The sensitivity was slightly worse – 0.4 ng $mL^{-1}$ – than with CZE, but selectivity was much higher. Good resolution and efficiency were obtained with both methods. The same-day RSDs of migration time were $< 1\%$ in CZE and $< 2\%$ in MEKC. Same-day precision RSD was 3–5%.

Among banned pharmaceutical substances, those which are of greatest relevance to sport medicine are anabolic agents, stimulants, diuretics, narcotics and $\beta$-blockers. In addition to several methods for screening (immunoassays, GC and HPLC), confirmation by MS, if needed, is used. Also, CE is a useful technique for the simultaneous screening of different types of drugs, e.g. $\beta$-blockers, anabolic steroids, diuretics and narcotics. LIF detection provides ultimate sensitivity while maintaining the extreme separation efficiency of CE. Both CE and MEKC have been applied. The main advantage of MEKC over CZE is that neutral and charged compounds as well as compounds which are insoluble in water can be separated in a single run.

## Chiral Separations

The chiral resolution of drugs is of forensic significance for legal and intelligence purpose. In many instances, only one enantiomer is controlled under legal status, and proper identification is therefore critical. For example, only the (+)-enantiomer of nerpseudoephedrine and the (−)-enantiomer of propoxyphene are controlled. In addition, the enantiomeric composition of seized samples can provide information on the possible different synthetic routes. The enantiomeric purity of drug detected in urine or other biological samples may exclude the possibility that the subject has taken that drug in a racemic preparation.

CE is a technique that has been shown to be ideally suited for chiral separations. When compared to other techniques, such as liquid chromatography, CE has the advantages of efficiency, resolution, selectivity, speed and direct chiral separation. Therefore, CE has become a method of choice in the chiral analysis of therapeutic drugs, but the attention paid to controlled or illicit drugs is still low. As for chromatography, it is possible to separate enantiomers directly or after a pre-derivatization step.

In the separation of amphetamine analogues, 2,3,4,4-tetra-*O*-acetyl-$\beta$-D-glucopyranosyl isothiocyanate as a derivatization reagent was used and enantiomers were separated using MEKC. However, more applications are conducted by CE with $\beta$-cyclodextrin ($\beta$-CD) as a chiral selector. Different $\beta$-CD derivatives have been used with success to separate ephedrine and analogous compounds, amphetamines, methamphetamine and methylenedioxy-derivatives of amphetamines.

The versatility of modified uncharged and charged $\beta$-CDs in the direct resolution of $\beta$-agonists, $\beta$-antagonists, phenylethylamines, alcohol stimulants and thalidomide and its metabolites by CE was shown. A total of 42 compounds were optically resolved using hydroxypropyl-$\beta$-CD and 20 with sodium sulfobutyl ether-$\beta$-CD. The preliminary analysis of ephedrine, amphetamine, methamphetamine and methylenedioxy-derivatives of amphetamine in urine (**Figure 2**) and hair (**Figure 3**) showed that after a liquid–liquid extraction, urine samples could be analysed with a sensitivity below 500 ng $mL^{-1}$. For hair analysis, it is necessary to increase sensitivity (0.1 ng $mL^{-1}$) by applying a stacking procedure.

## Forensic DNA Samples

DNA polymorphism analysis has recently been recognized as a source of identification for individuals in criminal cases and unidentified human remains. The conventional technique for DNA typing based on restriction fragment length polymorphism (RFLP) has been replaced by more accurate, sensitive and faster PCR procedures. In contrast to RFLP, the PCR procedures require less DNA and can be used on DNA which is degraded. There are several PCR-based procedures under development; however, short tandem repeat (STR) sequences are currently of major importance in the field of identification of individuals in forensic cases. STRs are DNA segments, typically found in noncoding regions, which are composed of repeating units of 2–5 base pairs (bp). Co-amplification of two or more of these loci in one PCR provides an efficient mechanism for typing multiple genetic loci simultaneously. The detection of STRs is based on the variation in the length of STR-containing PCR products. These PCR-amplified STRs must be separated to determine the size, quantity and/or sequence of each fragment.

DNA restriction fragments and PCR products have traditionally been separated by slab gel electrophoresis.

Recently, CE has emerged as a novel, high performance DNA analysis tool. Early work on DNA separation was done on cross-linked polyacrylamide gel-filled capillaries. However, gel-filled capillaries are difficult to prepare and have a short life time. The utility of CE has been greatly enhanced by using noncross-linked polymer networks instead of rigid gel media. These polymer solutions offer low-to-medium

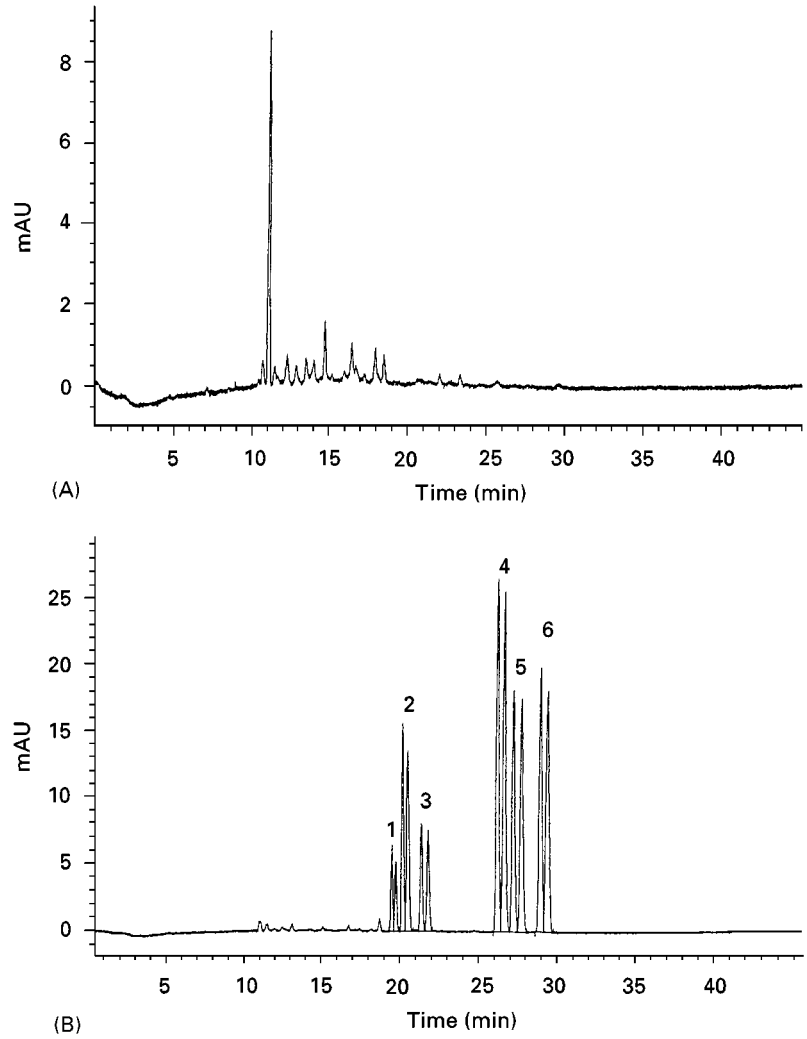


**Figure 2** Typical electropherograms of: (A) blank human urine extract; (B) extract from blank human urine spiked with: 1, racemic ephedrine; 2, amphetamine; 3, methamphetamine; 4, 3,4-methylenedioxyamphetamine; 5, 3,4-methylenedioxymethamphetamine; 6, 3,4-methylenedioxyethylamphetamine, at concentrations of 1 μg $mL^{-1}$ for every racemic analyte. Conditions: buffer, 100 mmol $L^{-1}$ phosphate, pH 2.5, containing 10 mmol $L^{-1}$ $\beta$-cyclodextrin. Capillary, uncoated fused silica, 45 cm × 50 μm i.d. Potential, 10 kV. Detection, UV absorbance at 200 nm. (Reprinted with kind permission of Wiley-VCH from Tagliaro F *et al.* (1998) *Electrophoresis* 19: 42–50.)

viscosity, which makes replacement of separation medium possible after each electrophoresis run. The polymer solution also has a broader effective DNA size range due to its flexible and larger effective pore size structure.

The CE system produced results which were comparable to those obtained on slab gel electrophoresis, with a level of precision of $\pm 0.1\%$ bp (between instruments). This comparison is very important if a comparison is to be made of results obtained by different laboratories and to standardize available procedures.

DNA fragments cannot normally be separated in free solution. However, the first clinical experimental results demonstrated that adding an uncharged molecule at the end(s) of the DNA fragments could lead to efficient separation of relatively large DNA fragments (100–900 bp) in free solution. Contrary to current electrophoretic methods, this method requires no sieving matrix, provides better results at high voltage and leads to shorter preparation time and faster separations.

Detection of PCR products has been achieved in three ways: (i) UV absorbance by the DNA fragment; (ii) LIF using intercalating dyes; and (iii) fluorescence of primers tagged with fluorescence dyes.

In automated fluorescence analysis the alleles from an STR locus are PCR-amplified from human genomic DNA using an unlabelled primer and one primer labelled at the 5′-end with a fluorescent dye.

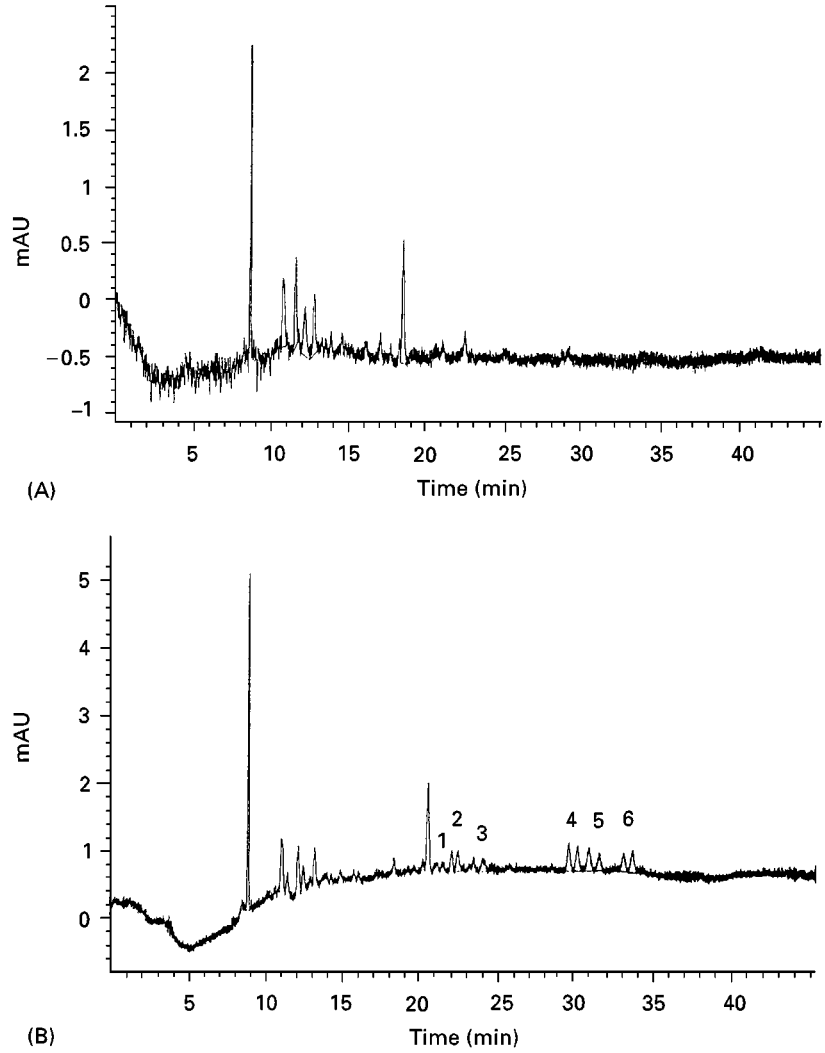


**Figure 3** Typical electropherograms of: (A) blank human hair extract; (B) extract from blank human hair spiked with: 1, racemic ephedrine; 2, amphetamine; 3, methamphetamine; 4, 3,4-methylenedioxyamphetamine; 5, 3,4-methylenedioxymethamphetamine; 6, 3,4-methylenedioxyethylamphetamine, at concentrations of 1 ng mg$^{-1}$ for every racemic analyte. Conditions: Buffer, 100 mmol L$^{-1}$ phosphate, pH 2.5, containing 15 mmol L$^{-1}$ $\beta$-cyclodextrin. Capillary, uncoated fused silica, 45 cm $\times$ 50 $\mu$m i.d. Potential, 10 kV. Detection, UV absorbance at 200 nm. (Reprinted with kind permission of Wiley-VCH from Tagliaro F *et al.* (1998) *Electrophoresis* 19: 42–50.)

Denatured PCR products are then analysed by CE with in-lane size standard (DNA-fragments of known size labelled in a different colour dye) on slab gel or CE capable of real-time multicolour fluorescence detection. The collected data are then analysed by software which automatically determine allele size based on a standard curve for the in-line size standard. STR loci which overlap in size can be distinguished using different dyes that fluoresce at different wavelengths. Results have indicated that the sizes obtained for STR alleles can differ depending on the gel and electrophoresis conditions and depending on the instrument used, however, high precision can be obtained in multiplex PCR analysis by using an in-line internal standard ($< 0.16$ nucleotide SD).

Recently, a new CE instrument capable of simultaneous multicolour detection and high resolution of DNA fragments was developed. This instrument, the ABI Prism 310 Genetic Analyser, is highly automated. Multiplex STR products are sequentially injected into a single capillary and detected by LIF. LIF is detected on a charged coupled device camera, which simultaneously detects all wavelengths from 525 to 680 nm. Ninety-six samples in a single tray can be analysed by the instrument. The polymer used on the instrument has many performance features that are critical to the success of STR analysis in forensic work: alleles differing in size by a single base (up to 250 bp in length) can be detected; sizing precision (between alleles of the same length) of less than 0.15 nucleotide SD is

possible; analysis time per sample is less than 30 min; capillary life is at least 100 injections; and the run temperature is set at 60°C to provide a highly denaturing environment for the DNA samples.

The disadvantage of CE is that it is a serial technique, making its total throughput no better than the long run times and parallel separations in convential slab gel electrophoresis. Several attempts to obtain faster and higher throughput separations have been reported. These include capillary array electrophoresis in ultra thin slab gels. These two techniques are limited by difficulty in assembling the separation system and in carrying out sample introduction. The use of CE to provide continuous automated loading of PCR products on to ultra thin slab gels shows new potential for increasing sample throughput in STR analysis, although separation resolution still needs to be improved.

Over the years CE has become widely used as a power tool in post-PCR analysis. However, it is difficult to introduce PCR to routine laboratories, because of the possibility of false-positive results. These false positives may be caused by sample-to-sample contamination or by the carry-over of previously amplified PCR product. The online coupling of fused silica capillary as the microreactor for PCR and CE for separation and detection can be recommended in order to avoid false-positive results.

## Other

The analysis of inks as part of the detection of fraudulent documents is a small but important part in the operation of a forensic laboratory. TLC and HPLC have been extensively used to separate and distinguish inks during the last decades. In comparison, CE has been applied only rarely. UV-Vis, fluorescence and particle-induced X-ray emission (PIXE) detection of electrophoretically separated diluted original inks and ink extracts (**Figure 4**) from substrate material provide sufficient information for the comparison of different inks (**Table 1**). The possibility of comparison of 50 forensic inks by MEKC has also been investigated. The separation patterns of individual dyes were compared with those obtained by HPLC and TLC, showing a much higher separation efficiency for MEKC. Some inks, which cannot be discriminated by applying the HPLC and TLC method, can definitely be distinguished using MEKC.

Nicotine, nornicotine and anabasine, the active principal components in all tobacco products, have been separated by CE and the potential for CE to

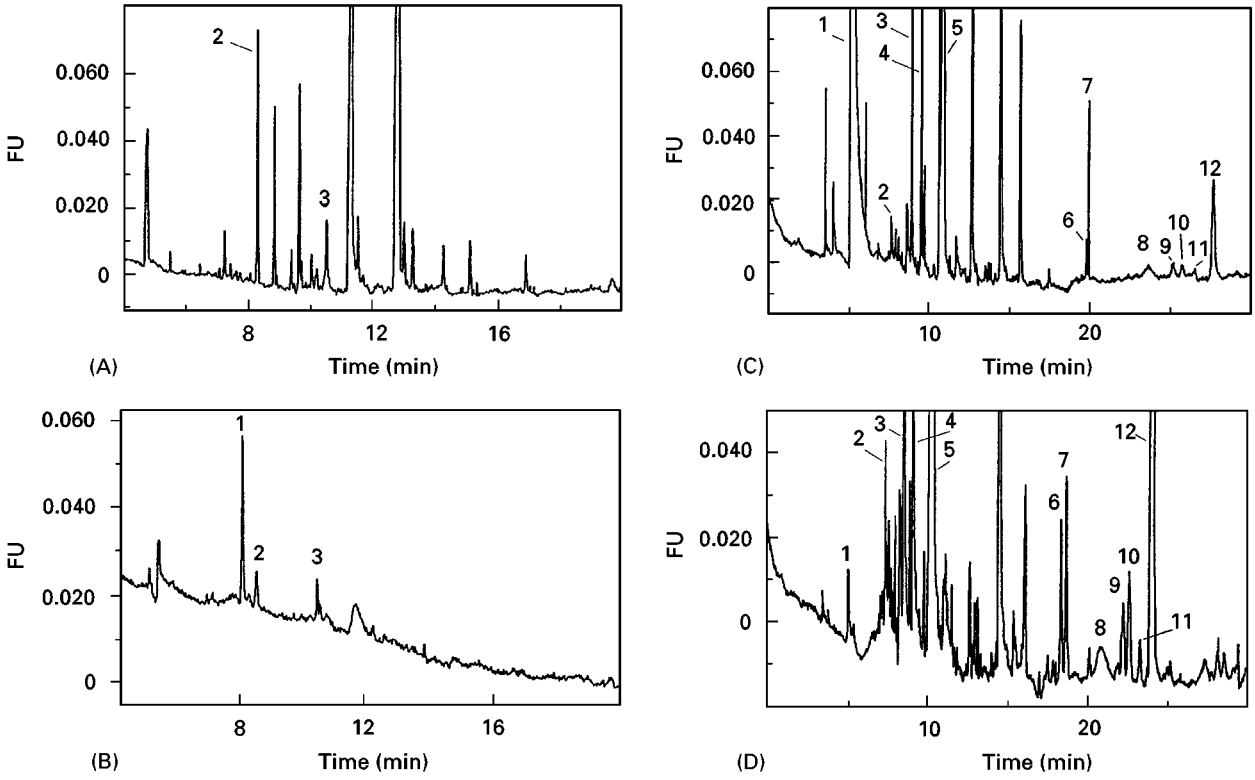


**Figure 4** Electropherograms of extracts from dried blue and black inks see (see **Table 1**) and original inks diluted 100-fold in 5 mmol $L^{-1}$ borate buffer, pH 8.25. Capillary, fused silica 45 cm × 50 μm i.d. Potential, 25 kV. Detection, laser-induced fluorescence at $\lambda_{exc/em} = 320/436$ nm. (A) Extract ink 1; (B) original ink 1 (diluted 1 : 100); (C) extract ink 2; (D) original ink 2 (diluted 1 : 100).

**Table 1** Listing of fountain pen inks investigated

| *Ink number* | *Colour* | *Manufacturer* | *Country of origin* |
|---|---|---|---|
| 1 | Blue | Cross | USA |
| 2 | Black | Cross | USA |
| 3 | Royal blue | Pelikan | Germany |
| 4 | Brilliant black | Pelikan | Germany |
| 5 | Blue | Pilot | Japan |
| 6 | Black | Pilot | Japan |
| 7 | Blue | Lamy | Germany |
| 8 | Black | Lamy | Germany |
| 9 | Royal blue | Geha | Germany |
| 10 | Brilliant black | Geha | Germany |
| 11 | Blue-black | Parker | USA |
| 12 | Washable blue | Parker | USA |
| 13 | Permanent black | Parker | USA |
| 14 | Royal blue washable | Parker | France |
| 15 | Permanent blue | Parker | France |
| 16 | Black | Waterman | France |
| 17 | Royal blue | Mont Blanc | Germany |

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characterize tobacco products on their alkaloid profiles for classification purposes has also been demonstrated.

Marine phytotoxines present a major public health problem because they can contaminate seafood. CE and MEKC enable okadaic acid, microcystins and maitotoxin to be detected in the picogram range.

Since any case of mushroom intoxication may have legal consequences, the accurate determination of mushroom toxins is of primary importance for forensic pathologists and toxicologists. The analysis of amatoxins by CE instead of radioimmunoassay has several advantages: analysis is faster, less costly and it requires smaller amounts of sample. Rapid and sensitive CE method for the separation and determination of the psilocybin and baeocystin in hallucinogenic mushrooms has also been reported.

Many types of explosives consisting of inorganic and organic components have been used in criminal cases. It has been demonstrated that the original composition of some explosive devices can be derived from the components of the post-blast residue. In short, CE offers a powerful tool which is suitable for both high and low explosive and gunshot residue analysis. CE is suitable for the determination of both inorganic and organic components, showing greater versatility than the traditional methods such as atomic absorption spectrometry and PIXE.

## Conclusion

CE is a new technique in the forensic laboratory for the separation and quantitation of a wide variety of molecules based not only on charge, but also on size, hydrophobicity and stereospecificity. CE offers certain advantages for forensic analysis:

1. higher theoretical plate number than HPLC;
2. in many instances CE is faster than GC and HPLC;
3. with regard to sample preparation, CE is easier than GC and HPLC. In many instances the sample can be injected directly with little or no preparation;
4. lower cost per analysis;
5. full automation;
6. CE is complementary to GC and HPLC;
7. two complementary techniques such as CE and MECC can be carried out with the same instrument.

Despite these features, the technique has not yet been widely accepted in the forensic community. This may be in part due to the legal system. Different countries have different standards to achieve legal defensibility of analytical results in court and forensic laboratories rely to a large extent on commercial instruments which are specially built and approved by a governmental agency for specific analysis. Even so, the US Drug Enforcement Agency is now using CE for general drug screening to quantitate heroin samples.

Two drawbacks of CE are often stated: low reproducibility and low sensitivity. However, due to several recently presented results (e.g. detection limits in the region of ng $mL^{-1}$, the precision of migration times $< 1\%$ RSD, same-day and day-to-day repeatability characterized by RSD values in the range of 1–4%, when peak area ratios were used), these drawbacks seem not to be so critical.

As stated by Kuffner *et al.*: 'The legal criteria of Daubert, as long as they are met by the scientific community, will allow CE into evidence as acceptable expert testimony'.

*See also:* **II/Electrophoresis:** Capillary Electrophoresis; Capillary Electrophoresis-Mass Spectrometry; Capillary Electrophoresis-Nuclear Magnetic Resonance. **III/Clinical Chemistry: Thin-Layer (Planar) Chromatography. Forensic Sciences:** Liquid Chromatography.

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# Liquid Chromatography

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Forensic science is the application of the sciences to the court of law. Consequently, forensic science and the legal system are intimately intertwined. Results obtained from the examination and analysis of forensic samples and the forensic samples themselves comprise evidence of a crime. It is the individualization of the sample, i.e. the singular association between the samples(s) and an illegal act, that is unique to forensic science. Because of the legal consequences, results require a high degree of certainty and the techniques used must be admissible in court. The Daubert rule, a 1993 decision upheld by the US Supreme Court, assigns to the judge the role of determining admissibility of scientific evidence. Among the factors considered by the judge are: (i) whether the technique has been tested and subjected to peer review; (ii) whether error rates have been defined; (iii) whether standards controlling the operation of a technique exist; and (iv) whether the technique has been widely accepted in the scientific community. Techniques used in a forensic laboratory may be applied to the investigation of a wide variety of crimes. Some examples are illegal drug use, counterfeiting, arson, tampering, fraud, poisoning, terrorism and environmental crimes. It is this diversity of cases, variety of sample matrices and the staggering number of potential analytes that necessitate a continuous evaluation of the testing that is needed to constitute proof in each situation.

Analytical requests in a forensics laboratory may be classified into four categories: (i) screen for the presence or absence of known compounds or class of compounds; (ii) screen for unspecified analytes; (iii) perform a comparative analysis; and (iv) verify and/or quantify substances in a sample. Many qualitative tests are used in forensic laboratories for their speed and ability to identify unknowns. In some cases qualitative results (identity) suffice, while in others quantification is important. Like gas chromatography (GC), high performance liquid chromatography (HPLC) can be used for both qualitative and quantitative analyses. However, 80–85% of all known compounds are not amenable to GC. The stationary and mobile-phase combinations available and the many detection modes possible make HPLC a universal separation scheme. Unlike GC, it is not limited by the volatility or thermal stability of an analyte. HPLC can analyse solutes encompassing a wide molecular weight range, from monatomic species to proteins. A range of solute hydrophobicities and polarities can be accommodated, from acidic and basic species that incorporate many drugs of abuse, pharmaceuticals, dyes and food colourings, to neutral and/or hydrophobic molecules such as pesticides and herbicides, hydrocarbons in petroleum products and carotenoids in foods. In situations where GC can determine certain compounds more readily with greater selectivity, resolution and sensitivity, HPLC offers a secondary, confirmatory method. In other cases, the use of HPLC avoids sample derivatization required by GC, and eliminates steps that could contribute to sample loss and increase analysis time. Another advance in HPLC in recent years involves the introduction of narrowbore (2.1 mm inner diameter) and microbore (1 mm inner diameter) columns. These smaller columns decrease the sample size required for injection and increase mass detection sensitivity versus the typical 4.6 mm inner diameter analytical columns.