Electromigrative separation techniques in forensic science: Combining selectivity, sensitivity, and robustness

ARTICLE *in* ANALYTICAL AND BIOANALYTICAL CHEMISTRY · NOVEMBER 2014

Impact Factor: 3.44 · DOI: 10.1007/s00216-014-8271-0 · Source: PubMed

4 AUTHORS, INCLUDING:

Tjorben Nils Posch
Institute of Energy and Environmental Tech…
11 PUBLICATIONS **83** CITATIONS
SEE PROFILE

Michael Pütz
Bundeskriminalamt
49 PUBLICATIONS **922** CITATIONS
SEE PROFILE

Anal Bioanal Chem
DOI 10.1007/s00216-014-8271-0

REVIEW

Electromigrative separation techniques in forensic science: combining selectivity, sensitivity, and robustness

Tjorben Nils Posch · Michael Pütz · Nathalie Martin · Carolin Huhn

Received: 9 June 2014 / Revised: 12 October 2014 / Accepted: 14 October 2014

Abstract In this review we introduce the advantages and limitations of electromigrative separation techniques in forensic toxicology. We thus present a summary of illustrative studies and our own experience in the field together with established methods from the German Federal Criminal Police Office rather than a complete survey. We focus on the analytical aspects of analytes' physicochemical characteristics (e.g. polarity, stereoisomers) and analytical challenges including matrix tolerance, separation from compounds present in large excess, sample volumes, and orthogonality. For these aspects we want to reveal the specific advantages over more traditional methods. Both detailed studies and profiling and screening studies are taken into account. Care was taken to nearly exclusively document well-validated methods outstanding for the analytical challenge discussed. Special attention was paid to aspects exclusive to electromigrative separation techniques, including the use of the mobility axis, the potential for on-site instrumentation, and the capillary format for immunoassays. The review concludes with an introductory guide to method development for different separation modes, presenting typical buffer systems as starting points for different analyte classes. The objective of this review is to provide an orientation for users in separation science considering using capillary electrophoresis in their laboratory in the future.

ABC Highlights: authored by *Rising Stars and Top Experts.*

T. N. Posch · C. Huhn
Forschungszentrum Jülich GmbH, Central Institute for Engineering, Electronics and Analytics, Analytics ZEA-3, 52425 Jülich, Germany

M. Pütz · N. Martin
Bundeskriminalamt – Federal Criminal Police Office, Forensic Science Institute, KT-34 – Toxicology, 65173 Wiesbaden, Germany

C. Huhn (✉)
Institute of Physical and Theoretical Chemistry, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany
e-mail: carolin.huhn@uni-tuebingen.de

Published online: 09 November 2014

Keywords Chiral separation · Electrophoretic immunoassay · MEKC · CEC · Drugs of abuse · Capillary electrophoresis

Introduction

A forensic-toxicological scientist is faced with multiple problems: unambiguous identification and quantification of chemical substances is needed with greatly varying matrices (tablets, raw materials, biofluids, wipe samples, etc.) and concentrations (from main components to traces), sometimes with only a small amount of sample available, and of course in a short time frame. Nowadays, additional challenges arise from the new psychoactive substances, sold as "research chemicals," "bath salts," or "legal highs" [1], originating from the rapid development of designer drugs and the ongoing interplay of reconfiguration and prohibition by federal laws [2]. However, the "common" drugs are also subject to changes and developments, e.g. via new synthetic routes including changes of the matrices. This demands a permanent update of established methods and the fast development of new methods with the inclusion of new compounds used as adulterants or to cover side-effects (e.g. nausea) by medicinal drugs. Medicinal drugs must also not be neglected in forensic science, because they are distributed via the grey market and have a high potential of abuse, and because they may illegally be introduced into the country. All this demands a deep knowledge of multiple separation techniques and detection systems by the forensic scientist. Also, the techniques used have to fulfill the requirements for high robustness and reliability.

Especially with regard to the new psychoactive substances, it is desirable to have a separation technique which enables fast method development, which is basically unlimited in terms of polarity of the analytes, which does not need a lot of sample preparation because of its high matrix tolerance, and which is technically applicable to any possible analyte. For

this reason, this article will cover literature without special regard to a specific time frame, instead selecting excellent and/or illustrative examples. To enable an easy reproduction and overview of the discussed methods, a table summarizing all relevant information on the experimental conditions is provided in Table 1, sorted in alphabetical order by analyte class. For information on the advances in capillary electrophoresis in forensic sciences in a regular, updating format, the authors recommend to the interested reader the reviews of the Tagliaro group [32–35]. This article is intended for readers who want to get a first glimpse into the possibilities of electromigrative separation techniques, and may thus be ideal for analytical chemists and forensic toxicologists who are currently more or only familiar with chromatographic techniques.

Advantages and limitations

Flexibility in separation and detection modes

Similar to chromatographic techniques, a wide variety of separation and detection modes may be used with electromigrative separation techniques with standard, commercial equipment. Common capillary electrophoresis (CE) equipment can be very flexibly used with all modes. Except for capillary electrochromatography (CEC), only a change in the buffer composition is necessary to switch modes. This is in contrast to HPLC, where a large set of different stationary phases is required to address specific separation problems and different equipment may even be required, e.g. for ion chromatography. However, in this section we will reveal that the flexibility of capillary electrophoresis (CE) instrumentation is higher because most separation modes do not require special or further equipment, unlike HPLC vs. ion chromatography. All that is needed for a change of the separation mode is—in most cases—just a few milligrams of inexpensive chemicals.

Separation modes

One of the most striking arguments for using electromigrative separation techniques is their ability to operate in many different modes without the need for new hardware or larger amounts of expensive chemicals. An overview of modes and terms is given in the IUPAC recommendations by Riekkola et al. [36]. For those readers without basic prior knowledge of electromigrative separation techniques, we recommend the "Brief introduction to capillary electrophoresis" [37] or the Primer "High performance capillary electrophoresis" [38] for a brief overview. In addition, there are many good text books on the basics of electromigrative separation techniques (e.g. [37, 39]), some of them with dedicated chapters about the use of CE in forensic science, and reviews on this topic [32–35, 37, 40–62]. Capillary electrophoresis with aqueous (CE) and non-aqueous (NACE) [63–70] buffers and with additives for chiral separation [71, 72] are the most common modes used and are well suited for the analysis of small molecules up to the size of proteins, with the only requirement being that a charge (mostly depending on pH) is present on the analytes to enable electrophoretic migration upon application of an external electric field. Most of this review deals with CE, NACE, and chiral separations, so in this section we focus more on less widely used electromigrative separation techniques.

The limitation of the necessity of charge on the analyte can be overcome when charge is introduced by so-called separation carriers, either complexing agents (e.g. sulfated cyclodextrins, also suitable for chiral analysis) or charged detergents, to also target neutral analytes [73]. In micellar electrokinetic chromatography (MEKC) a surfactant, usually sodium dodecyl sulfate (SDS), is used with a concentration above its critical micelle concentration (CMC). The micelles create a micro phase which may be charged and thus migrates through the separation column, serving as a pseudo-stationary phase to induce chromatographic separation. As in standard chromatography, the separation is based on the equilibrium distribution of the analytes in the free buffer solution as mobile phase and within the micelles as pseudostationary phase. These effects can freely be combined with additives (e.g. organic solvents or urea) changing the relative polarities of the two phases and their interaction, evoking secondary equilibria (e.g. cyclodextrins) or modifying the electroosmotic flow velocity and thus the relative velocity of the mobile phase (e.g. calcium ions, coatings). A fully optimized MEKC method is theoretically capable of separating any pairs of analytes, but limits the applicable detection systems because the surfactants are usually not MS-compatible, although some solutions have been presented [74] (see also the section "Universal separation modes: MEKC and MEEKC").

A further development is microemulsion electrokinetic chromatography (MEEKC) in which a microemulsion is established, serving as a pseudostationary phase [75–77]. The applicability of MEKC is thus broadened towards highly unpolar compounds, though comparatively polar compounds may also be analyzed. MEEKC has an increased solubilization capacity and can achieve a highly efficient mass transfer of solutes between the pseudostationary phase and the organic and/or aqueous phase [78, 79]. It also has an even higher potential for fine tuning and flexibility than MEKC, enabling an enlargement of the separation window and faster analysis, originating from the more complex buffer composition, while being easy and cheap to use [12, 80]. The

Table 1 Experimental details to selected literature covered in this article, sorted alphabetically with regard to the analyte (class). (LLE=liquid−liquid extraction, SPE=solid-phase extraction, AM=amphetamine, MA=methamphetamine)

Analyte	Matrix	Sample preparation	CE method	BGE	Capillary	Linear range	Detection limit	Ref.
(2-hydroxy-4-iodo-5-methodxyphenyl)acetic acid	Rat urine	SPE	CE-MS	20 mmol L^{-1} NH_4Form +25 % isopropanol (pH 10.0)	90 cm, 75 μm	Not given	Not given	[3]
17 drugs of different classes	Standards, urine, plasma	Extraction with chloroform–isopropanol (9:1), evaporation, reconstitution in BGE	CE–UV	50 mmol L^{-1} NaH_2PO_4 (pH 2.35)	60(52.5) cm, 75 μm	Not given	Not given	[4]
19 cathinone derivatives	Standards	Dissolution	CE–UV	50 mmol L^{-1} NH_4Ac, 20 mg mL^{-1} sulfated-β-CD +10 % ACN (pH 4.5)	78.5(70) cm, 50 μm	Not given	Not given	[5]
34 drugs, heroin+ impurities, cocaine+ impurities	Seizure	Dissolution	MEKC	85 mmol L^{-1} SDS, 8.5 mmol L^{-1} phosphate, 8.5 mmol L^{-1} borate +15 % ACN (pH 8.5)	122(100) cm, 50 μm, 72(50) cm, 50 μm, 72(50) cm, 25 μm, 47(25) cm, 50 μm	Not given	Impurities 0.2 % rel. to heroin	[6]
AM, MA	Urine	LLE, 0.45 μm filtration	CE–UV	75 mmol L^{-1} Tris +10 mmol L^{-1} DM-β-CD, 5 mmol L^{-1} β-CD, (pH 2.5, phosphoric acid)	64.5(56) cm 50 μm, diol-modified	Not given	Not given	[7]
AM, MA	Urine	LLE, SPE	CE-MS	1 mol L^{-1} formic acid, 3 mmol L^{-1} β-CD, 10 mmol L^{-1} DM-β-CD, (pH 2.2)	100 cm, 50 μm	0.2–10 μg mL^{-1}	0.03–0.05 μg mL^{-1}	[8]
AM, MA	Urine	LLE	CE-MS	1 mol L^{-1} formic acid, 0.85 mmol L^{-1} heptakis(2,6-diacetyl-6-sulfato)-β-CD, (pH 1.7)	100 cm, 50 μm	0.05–10 μg mL^{-1}	0.02 μg mL^{-1}	[9]
AM, MA, amphetamine-type stimulants	Standards, seizures	Dissolution, filtration	CE-MS	2.5 mmol L^{-1} highly sulfated-γ-CDs (pH 3.1)	56 cm, 50 μm	Not given	2 pg on column	[10]
AM, MA	Urine	Filtration	CE-MS	1 mol L^{-1} formic acid–1 mol L^{-1} NH_4Ac (10:0.2 mixture), 1.5 mmol L^{-1} heptakis(2,6-diacetyl-6-sulfato)-β-CD, (pH 2)	100 cm, 50 μm	0.05–10 μg mL^{-1}	0.02 μg mL^{-1}	[11]
Caffeine	Beverages, smart drugs	Dilution, methanolic extraction	MEEKC	3.3 % SDS, 1.5 % *n*-hexane, 6.6 % *1*-butanol in 8.85 mmol L^{-1} sodium tetraborate solution (pH 9.5)	50(40) cm, 50 μm	5–100 μg mL^{-1}	LOD 2 μg mL^{-1}, LOQ 5 μg mL^{-1}	[12]
Cannabinoids, hashish, marijuana	Seizure	Extraction in MeOH: chloroform (9:1)	CEC-UV	25 mmol L^{-1} phosphate buffer (pH 2.57) in 75 % acetonitrile	49 cm (40 cm) 100 μm, 40 cm, 3 μm, Hypersil C18	Not given	0.5 μg mL^{-1} for d9-THC	[13]
Codeine, MA, morphine, ketamine, benzodiazepines	Urine	LLE with EtOAc	Large volume sample stacking sweeping MEKC	50 mmol L^{-1} NaH_2PO_4, 150 mmol L^{-1} SDS +10 % MeOH (pH 2.3)	50(40) cm, 50 μm	0.025–1.5 μg mL^{-1}, 0.05–3.0 μg mL^{-1}	0.0075–0.030 μg mL^{-1}	[14]
Fentanyl derivatives	Standards, seizures, heroin, garment	Dissolution, MeOH extraction	NACE-MS		80 cm, 50 μm	0.002–6.33 μmol L^{-1}	0.001 μmol L^{-1}	[15]

Table 1 (continued)

Analyte	Matrix	Sample preparation	CE method	BGE	Capillary	Linear range	Detection limit	Ref.
				200 mmol L^{-1} NH_4Ac in glacial acetic acid +90 % ACN				
Food colorants	Seizures, tablets	Extraction with BGE	CE-MS	200 mmol L^{-1} formic acid (pH 2.2)	82 cm, 75 μm	Not given	Not given	[16]
GHB, GBL	Standards, Gatorade	–	MEKC	50 mmol L^{-1} phosphate +3 % SDS (pH 6.5)	32(23.5) cm, 50 μm, dynamic cationic coating	300–9700 μg mL^{-1} (GHB), 600–9700 μg mL^{-1} (GBL)	Not given	[17]
Heroin	Standards, seizures	–	CE–UV	Celixir B, 13.3 % DM-β-CD (pH 2.5)	64(55.5) cm, 50 μm, dynamic anionic coating	250–800 μg mL^{-1}	Not given	[17, 18]
Heroin, amphetamines, impurities	Seizures	Not given	MEEKC	3.3 % SDS, 6.0 % *1*-butanol, 0.9 % octane, 89.8 % in a 5 mmol L^{-1} sodium tetraborate solution (pH 9.5)	40(30) cm, 75 μm	1–600 μg mL^{-1}	LOD 1.0 μg mL^{-1}, LOQ 3.0 μg mL^{-1}	[19]
Heroin, basic impurities, basic adulterants	Standards, seizures	Dissolution	CE–UV	100 mmol L^{-1} HP and DM-β-CD in CElixir Reagent B (pH 2.5)	64(55.5) cm, 50 μm, dynamic, polyanionic double layer	10–800 μg mL^{-1}; 0.6–80 μg mL^{-1}	Not given	[18]
Indole alkaloids, Kratom alkaloids	Standards, plant material	Extraction with MeOH, centrifugation	NACE-MS	60 mmol L^{-1} NH_4Form in a mixture of ACN–glacial acetic acid (100:6)	67.5 cm, 50 μm	1–500,000 μmol L^{-1}	0.025–0.075 μmol L^{-1}	[20]
LSD, opium	Standards, seizures	–	CE–UV	Celixir B, 3.94 % HP-β-CD, 9.98 % DM-β-CD (pH 2.5)	32(23.5) cm, 50 μm, dynamic anionic coating	0.8–26 μg mL^{-1} (LSD),	Not given	[17, 21]
MA, MDMA, heroin, ketamine	Banknotes, plastic bag, kraft paper, silver paper	Dispersive LL microextraction	CE–UV	100 mmol L^{-1} KH_2PO_4+20 mmol L^{-1} β-CD	37(30) cm, 50 μm	0.15–6000 μg L^{-1}	0.05–0.2 μg L^{-1}	[22]
Neutral, acidic, and weakly basic adulterants	Standards	–	MEKC	50 mmol L^{-1} phosphate–borate +3 % SDS (pH 6.5)	32(23.5) cm, dynamic cationic coating	8–900 μg mL^{-1} (caffeine)	Not given	[17, 18]
Neutral, acidic, weakly basic heroin impurities	Seizures	Dissolution	MEKC	103.2 mmol L^{-1} SDS, 50 mmol L^{-1} phosphate–borate (pH 6.5)	32(23.5) cm, 50 μm, polycationic coating (CElixir A)	7–900 μg mL^{-1} (caffeine), 3–50 μg mL^{-1} (phenacetine)	Not given	[18]
Opiates	Standards	Dissolution in MeOH	CEC-UV	10 mmol L^{-1} Tris, 50 mmol L^{-1} SDS buffer (pH 8.3) in 20 % acetonitrile	28 cm (unknown), 75 μm, 15 cm 1.5 μm C18 packing	Not given	Not given	[23]
Opiates amphetamines	Standards, urine	SPE	CEC-MS	25 mmol L^{-1} NH_4Form (pH 3) in 30 % acetonitrile	36 cm (27.5 cm), 100 μm, 26 cm, 3 μm CN-modified packing	0.0025–0.1 ng mL^{-1}	LOD 0.0008 μg mL^{-1}, LOQ 0.00025 μg mL^{-1}	[24]
Opiates, amphetamines	Standards, urine	SPE	CEC-UV	20 mmol L^{-1} sodium phosphate (pH 2.5) in 20 % acetonitrile	33 cm (24.5 cm), 75 μm, 23 cm, 3 μm CN-modified packing	0.1–1.2 μg mL^{-1}	LOD 0.005 μg mL^{-1}, LOQ 0.01 μg mL^{-1}	[25]
Opiates, amphetamines, cocaine+metabolites	Urine	Centrifugation, filtration	CE-MS	1 mol L^{-1} formic acid (pH 1.8)	80 cm, 50 μm, double layer, dynamic polyanionic coating	0.01–1.0 μg mL^{-1} (cocaine), 0.021–1.0 μg mL^{-1} (methadone)	0.002 μg mL^{-1}	[26]
Opioids	Serum, plasma, urine	SPE, LLE			41(22) cm, 50 μm			[27]

Table 1 (continued)

Analyte	Matrix	Sample preparation	CE method	BGE	Capillary	Linear range	Detection limit	Ref.
			Head-column FASS CE–UV	75 mmol L^{-1} Na_2HPO4 and 25 mmol L^{-1} NaH_2PO_4 in ethylene glycol–water mixture (6:4 *v*/*v*) (pH 7.9)		0.02–0.5 μg mL^{-1} (dihydrocodeine) in plasma	0.001 μg mL^{-1} (dihydrocodeine) in plasma	
Phenethylamines, cocaine, oxycodone	Standards, seizures	–	CE–UV	CElixir B (pH 2.5)	33(24.5) cm, 50 μm, dynamic, anionic coating	3–100 μg mL^{-1}	Not given	[17, 28]
Piperazine derivatives	Seizures, tablets	Extraction with H_2O–isopropanol (1:1)	CE-MS	100 mmol L^{-1} formic acid, 10 mmol L^{-1} 2-HP-β-CD +10 % MeOH (pH 2.4)	82 cm, 75 μm	–	Not given	[16]
Plant alkaloids	Standards	–	CE–UV	100 mmol L^{-1} NH_4Ac (pH 3.1) +50 % ACN	55(50) cm, 50 μm	Not given	0.25 μg mL^{-1} (for gramine)	[29]
Plant alkaloids	Standards	–	CE–UV-MS	100 mmol L^{-1} NH_4Ac (pH 3.1) +50 % ACN	75(20) cm, 50 μm	Not given	Not given	[29]
Psychoactive mushrooms	Standards	–	CE–UV	CElixir B (pH 1.8)	32(23.5) cm, 50 μm, dynamic anionic coating	Not given	Not given	[17]
Psychoactive plant materials, preparations	Plant material, aq. Solutions	Extraction with MeOH, centrifugation	NACE-MS	58 mmol L^{-1} NH_4Form in a mixture of ACN–glacial acetic acid (975:60)	67.5 cm, 50 μm	1–500,000 μmol L^{-1}	0.025–0.075 μmol L^{-1}	[30]
Weakly–strongly acidic or basic drugs of forensic interest	Standards	Dissolution in mobile phase	CEC-UV	25 mmol L^{-1} phosphate buffer (pH 2.5) in 75 % acetonitrile	34 cm (25 cm), 100 μm, 25 cm, 3 μm Hypersil C8	Not given	Not given	[31]

first MEEKC method and buffer system was introduced by Watarai in 1991 [75], consisting of water–SDS–1-butanol–heptane (89.3:3.3:6.6:0.8). This system is still most frequently used, but has rarely been used in forensic toxicology.

Use of real stationary phases is possible, either by filling the capillary with chromatographic material or by synthesizing monolithic stationary phase material directly inside the column, giving rise to capillary electrochromatography (CEC) [81–83]. Here the eluent is transported via the electroosmotic flow induced on the charged stationary-phase surface (e.g. via sulfate groups). The opinion of CEC among experts is diverse: some regard this technique as combining the advantages of CE, with its high separation efficiency, and of HPLC, with its stable retention times, high sample capacity, and ability to handle thermally labile and neutral substances [13]; whereas others regard it as combining all the disadvantages of both techniques (low reproducibility, poor limits of detection, possibility of cross contamination, sensibility to matrix compounds because of the stationary phase). Use of CEC has mostly been in model applications, most probably because of the limited commercial availability of column hardware and necessary instrumental extras, for example an LC pump. However, an undeniable advantage of CEC is the high compatibility of the mobile phases with MS-detection, and promising studies with remarkable separation efficiency are available [13, 23] and will be discussed in the section "Not yet in routine use: capillary electrochromatography".

Isotachophoresis (ITP), in which charged analytes are separated on the basis of differences in their effective electrophoretic mobility as in CE, although using a discontinuous buffer system [84–86], is the oldest electromigrative separation technique. Today it is mostly used as a sample preconcentration technique, but can advantageously also be used in a column-coupling format for very high sensitivity and increased matrix tolerance [87]. Further modes of electroseparation, for example gel electrophoresis and isoelectric focusing, are well known for DNA sequencing and protein analysis, but have not been used for drug analysis as far as we are aware. During recent years some publications dealt with the transfer of immunoassays into capillary formats to enhance identification and thus selectivity. An example is shown in the section "Immunoassays and immunoaffinity capillary electrophoresis".

Some modes or methods require the fused silica capillary to be coated to reduce adsorption phenomena (of analytes and/or matrix components) or to change the magnitude of the electroosmotic flow. The latter can be used to increase the speed of analysis or to enable the analysis of anions with high effective electrophoretic mobility. Coatings may also help to increase the repeatability of a specific method, e.g. when working with background electrolytes at intermediate pH value close to the pK_a-value of the fused-silica surface or when working with high matrix loads, where a renewal of the capillary surface is frequently required [17].

For switching between modes or methods, only the background electrolyte (BGE) and most probably the separation capillary (cost ca. 10 € m^{-1}, length typically 20–80 cm) have to be exchanged. This can be achieved within minutes, because conditioning procedures for new capillaries are typically 30 min long (rinsing with 0.1 mol L^{-1} NaOH and BGE). This very fast method-switching option has been emphasized in several previous reviews [35, 58, 88]. An excellent example of a very fast and efficient transfer of methods, even using a single capillary, is the work of Lurie et al. [17], who used different aqueous buffers and dynamic capillary coatings for the analysis of β-phenylethylamines, cocaine, oxycodone, heroin, lysergic acid diethylamide, opium alkaloids, ingredients of psychoactive mushrooms, γ-hydrodxybutyrate, and γ-butyrolactone with their respective, individual side compounds. Using commercial buffer solutions with or without addition of different cyclodextrins or detergents enabled baseline separation for all analytes, usually within a 10 min total run time; all separation procedures were validated for routine forensic drug analysis.

Manifold detection possibilities

Most current commercial CE instruments are fabricated with an integrated UV-detection or a diode-array-detection system. They are suitable for standard photometric detection, where the absorbance of the analyte molecule is measured, or for indirect detection, where an absorbing compound, the probe ion, is added to the background electrolyte. In this case the reduced concentration of the probe ion is recorded, when it is displaced by the analytes in the peak for reasons of electroneutrality. Direct detection is suitable for any molecule with π-bonds, whereas the indirect method is usually used for small organic or inorganic ions or carbohydrates without a suitable chromophore. Examples are given in the section "Presence of compounds in excess". Laser-induced fluorescence (LIF) detection is also widely available and is known for its extremely high sensitivity and selectivity, and has been used in combination with CE as a direct [89, 90] or indirect [91–94] detection system, on-column or post-column [95]. In the past, capillary electrophoresis has been conducted with further detection techniques [96] based, e.g., on Raman scattering or refractive-index changes, although these are barely used today and were gradually superseded by the previously mentioned systems [96]. Non-optical post-column detection techniques are potentiometric detection, conductivity detection, and amperometric detection. Of these three, only capacitively-coupled contactless conductivity detection (C4D) has become a universal detection technique [97]. All detection modes can be freely combined; on-column formats are mostly used. This is especially useful when the detection systems are

complementary, for example if one delivers quantitative information and the other is used for identification, as in ITP-C4D-ICP-MS [98] or CE-LIF-MS hyphenation [90].

Like LC, capillary electrophoresis is coupled to mass spectrometry [99] mostly via electrospray ionization (ESI), especially in proteomics and metabolomics and increasingly also in forensic studies. The crucial point in coupling capillary electrophoresis with mass spectrometry is the interface: although many, sometimes very specialized interfaces and ion sources were developed for CE-MS coupling (summarized in [100]), including fast atom bombardment [101], laser vaporization [102], sonic spray [103], or offline coupling with matrix-assisted laser-desorption ionization [104] up to "pseudo"-online MALDI using a rotating ball interface [105, 106], only electrospray ionization has become established and is used regularly with a commercial interface, the triple-tube sheath-liquid interface. However, because capillary electrophoresis typically delivers only some nanoliters per minute as effluent or may even have no solvent net flux, the stability of the ionizing electrospray is problematic. There are basically two approaches to solve this problem: the sheathless and the sheath-liquid-assisted interfaces [107]. In the sheathless approach, the end of the capillary is usually etched until it becomes porous and analyte ions can directly evaporate from the surface and thus be introduced into the mass spectrometer [108]. The sheath-liquid-assisted interfaces use a make-up liquid (typically a water–isopropanol mixture containing 1 % formic or acetic acid) to yield a robust electrospray that is independent from the efflux from the CE capillary. The commercially available interface uses a pneumatically assisted electrospray, thus having a sheath-liquid flow of approximately 4 μL min^{-1}. Although it is only the case for some applications, this additional flow rate is widely perceived as a dilution of the analyte solution, impairing detection limits. However, this additional liquid can be used to directly enhance the ionization efficiency of the analytes, e.g. adapting pH values and surface tension. Likewise, buffer systems unfavorable for ionization can be diluted. It is even possible to obtain a sensitivity enhancement compared with sheathless interfaces. Mass calibrants for the mass spectrometer can be added to the sheath liquid, and chemical reactions within the ion spray (e.g. in-source decay) can be introduced or suppressed. However, to circumvent or to reduce the dilution, new developments in the sprayer design make it possible to greatly reduce the flow (<0.1 μL min^{-1}) of the sheath liquid [107, 109]. These new interfaces do not need pneumatic assistance, and actually use a real electrospray for ionization.

As well as ESI, atmospheric-pressure chemical ionization (APCI) and atmospheric-pressure photoionization (APPI) sources have been used for coupling CE with mass spectrometry, increasing the number of detectable analytes in terms of polarity and ionization efficiency. For a more detailed overview of capillary electrophoresis mass spectrometry, some excellent reviews are recommended [99, 107, 110–112].

Separation efficiency, broad applicability, and profiling

High separation efficiency is desired for all analytical separation techniques, especially in combination with high separation selectivity and thus high peak capacity and resolution. In electromigrative separation techniques, these characteristics are further combined with short analysis times of mostly below 15 min for CE and 20 min for MEKC. A high sample throughput can thus easily be achieved, with no or only few overlapping signals for most analytical applications using simple photometric detection. This has led to several publications on profiling strategies where a large number of structurally related analytes at trace or minor-component level have been separated within only one analytical procedure to yield a characteristic signature of the analyzed materials. Likewise, many different electromigrative separation techniques have been optimized for use as universal screening methods for an impressive range of analytes.

Broad applicability of capillary electrophoresis

In 1993 Chee and Wan [4] investigated the implementation of capillary electrophoresis with UV-detection for a forensic-toxicological application, namely the analysis of 17 psychotropic alkaloids of a variety of drug classes including a.o. amphetamine, methamphetamine, procaine, lidocaine, codeine, and methaqualone. Impressively, baseline separation (permitting undisturbed quantification with UV detection) was achieved for all analytes within 12 min by simply using 5 mmol L^{-1} sodium dihydrogenphosphate, titrated to pH 2.35 with phosphoric acid, as BGE in an uncoated bare-fused-silica capillary. The analysis of the drugs in spiked human urine and serum was performed after liquid–liquid extraction (LLE) (chloroform–isopropanol, pH 10.5) and redissolution in BGE after drying for sample preparation. This publication reveals the ease of using CE, despite different sample matrices, and reveals that the method is fast and highly efficient. However, the method had only limited robustness, with a strong dependence of resolution on small pH variation (0.05 pH units) in the BGE.

Today, more and more groups use CE-MS for drug-profiling and screening purposes. In 1997 Unger et al. [29] presented a generic approach for the analysis of plant alkaloids. Indole alkaloids, benzophenanthridine alkaloids, β-carboline alkaloids and isoquinolines were separated in a 1:1 mixture of 100 mmol L^{-1} ammonium acetate in water (pH 3.1) and acetonitrile. In total, 37 different analytes could be separated using one generic buffer composition, revealing

the general applicability of this hyphenated technique for the separation and identification of alkaloids from plant extracts. This method was improved by Unger and Stöckigt [113], who added a field-amplified sample-stacking procedure for preconcentration (BGE: 200 mmol L^{-1} ammonium acetate in a 1:1 mixture of water and methanol). A similar but non-aqueous BGE of 60 mmol L^{-1} ammonium formate in a mixture of acetonitrile and glacial acetic acid (100:6) was used by Posch et al. [20, 30] for a large variety of indole alkaloids and plant samples (see also the section "Structurally closely related analytes"). From this and further publications it is obvious that screening of cationic analytes, especially protonated indole alkaloids in complex sample matrices, can be very well achieved using NACE. Also, opium alkaloids and amphetamines were separated very quickly and precisely in a series of excellent publications by Bjørnsdottir and Hansen [114–116], in which the underlying separation mechanisms of NACE were also investigated. They compared an MEKC procedure [117] for the separation of five opium alkaloids to the results obtained with NACE [114], and cross-validated both procedures with a validated HPLC procedure [115]. All three methods performed at a similar level. In 1999, they developed a very fast NACE procedure for the separation of 16 drugs of forensic interest (amphetamine, cocaine, heroin, etc.) in less than 2 min using a short-end injection (injection from the outlet end) with only 8.5 cm effective capillary length (total length: 32.5 cm, i.d.: 25 μm) [116]. The BGE was composed of 25 mmol L^{-1} ammonium acetate and 1 mol L^{-1} acetic acid in a mixture of acetonitrile, methanol, glycerol, and water (75:15:8.5:1.5). Retrospectively, regarding the short effective capillary length used, this article clearly reveals the power of a miniaturized separation system, which is reflected in recent intensive studies on microfluidic devices.

Because of the high separation efficiency of capillary electrophoresis in general and the complementary high sensitivity and selectivity of MS detection, CE-MS is also well suited to the screening of drugs of abuse together with their (structurally related) Phase I metabolites. An illustrative example was presented in [26] for drug screening in diluted urine samples with CE-MS after preconcentration using pH-mediated sample stacking. The developed procedure, using a simple 1 mol L^{-1} formic-acid solution (pH 1.8) as BGE, is remarkable: a very fast screening approach was obtained, with full to partial separation of 33 drugs of abuse in a small time window of 4–7 min (in total 15 min including rinsing procedures). Unambiguous identification and quantification via selected reaction monitoring in a triple-quadrupole mass spectrometer was possible with the same method as verification of positive results from the screening approach. Reduced analysis times for screening were thus obtained compared with corresponding chromatographic techniques, including recently developed multidrug-screening approaches using UPLC–TOF-MS [118] (52 common pharmaceuticals in 17 min chromatographic run time) or UPLC–MS–MS [119] approaches (29 drugs of abuse in 20 min chromatographic run time). For their CE-MS procedure, Kohler et al. [26] also performed a full validation according to the guidance of the Food and Drug Administration. Their results disprove many prejudices against electromigrative separation techniques, revealing that well-optimized methods with good performance can be obtained: very good robustness, linearity over a wide range (10–1000 ng mL^{-1} for cocaine, 21–1000 mg L^{-1} for methadone), and accuracy were reported. In the authors' opinion, such methods could be powerful tools for establishing new libraries for automated CE-MS analysis interpretation, similar to those for LC–MS or GC–MS, for which commercial and self-built libraries [120] are widely distributed and used.

Universal separation modes: MEKC and MEEKC

In MEKC, the range of polarities encompassed can be optimized by means of the type and concentration of the pseudostationary phase (the type and concentration of the micellar detergent) and the relative polarity of the mobile phase, by addition of organic solvents and other additives (see also section on "Analytes with high polarity or charge"). Relatively polar and medium-hydrophobic analytes can be covered by MEKC, whereas MEEKC can be used for very hydrophobic analytes. For a detailed explanation of MEKC and MEEKC, the interested reader is referred to the book by Pyell [121] or the publication from Terabe explaining how to affect selectivity in MEKC [122].

In 1991, Weinberger and Lurie developed a simple MEKC-UV method for the forensic screening of illicit drug substances [6]. Their screening system is composed of an 85 mmol L^{-1} SDS, 8.5 mmol L^{-1} phosphate, and 8.5 mmol L^{-1} borate buffer (pH 8.5) with 15 % acetonitrile. The analysis of heroin and cocaine, including products of decomposition in samples from police seizures, was presented. In this method most of the common illegal drugs of abuse (heroin, cocaine, psilocybin, cannabis ingredients, LSD, methamphetamine, etc.) were included within one analytical run showing impressive resolution. The authors compared their method with standard HPLC methods while explaining the separation principle, making this publication an easy entry for an experienced HPLC operator who wants to quickly obtain some information on MEKC. In 2004, Lurie et al. presented two very robust and reliable methods for the forensic screening of heroin [18]: a CE–UV method for heroin's basic adulterants and impurities, and an MEKC-UV method for the determination of neutral, acidic, and weakly basic adulterants, revealing the broad coverage of MEKC with regards to analyte characteristics. Both methods are in routine use in heroin signature programs of DEA laboratories [18]. The authors also reveal that CE and state-of-the-art LC have comparable performance. Excellent run-to-run precision

(RSD <0.9 %) and a wide linear range (0.0035–0.454 mg mL^{-1}) were obtained by MEKC using a 32 cm, 50 μm capillary, dynamically coated with a double-layer coating (using the polycationic coating (CElixir) followed by dodecylsulfate in the run buffer as dynamic coating). The migration-time precision was improved significantly by reducing the flushing and recoating steps between runs to a partial-recoating step only. The total analysis time was below 15 min.

MEEKC methods used in forensic science are scarce. However, the technique has proved its potential in the analysis of heroin, as revealed in [19]. Using a standard MEEKC system (see the section "Separation modes") as buffer, 17 analytes encompassing heroin, amphetamine, and impurities therein were separated within 12 min using a 40 cm (i.d.: 75 μm) bare-fused-silica capillary. The method is characterized by low RSDs (0.11–1.45 %) for relative retention times, linearity from 1–600 μg L^{-1}, and limits of detection suitable for routine control of drugs of abuse (1.0–1.5 μg L^{-1}). One of the few other applications of MEEKC was presented by the Tagliaro group for the analysis of caffeine in different beverages and in smart drugs [12].

Not yet in routine use: capillary electrochromatography

CEC was investigated as a potential alternative to GC–MS or HPLC–MS for the fingerprinting of cannabinoids in 1998, by Lurie et al. [13]. The study includes a short investigation of the effect of pH, acetonitrile concentration, phosphate concentration, column length, and the type of stationary phase on the separation, giving a good introduction to CEC and method optimization. A 25 mmol L^{-1} phosphate buffer (pH 2.57) with 75 % acetonitrile was finally used in a commercially available 49 cm (i.d.: 100 μm) 3 μm Hypersil C18 column. After correction of retention times and peak areas using cannabinol as internal standard, high precision in relative retention times (RSD <0.1 %) and relative peak areas (RSD <4.6 %) was obtained, while achieving low limits of detection (500 ng mL^{-1} for d9-tetrahydrocannabinol, d9-THC) using a high-sensitivity cell with extended detection path length. Also, separation efficiency was high (200,000 plates m^{-1}) and the linear detection range was significantly improved by the use of the high-sensitivity cell. The method was applied to the separation of concentrated hashish and concentrated marijuana extracts. The final procedure resolved approximately 50 % more peaks than a gradient-HPLC procedure with similar run time. In the same year, Lurie et al. presented an additional study, comparing CEC with CE, MEKC, and HPLC for the analysis of 15 organic compounds of forensic interest [31]. For CEC a 3 μm Hypersil C8 column (Hewlett–Packard) (i.d.: 100 μm, length: 34 cm, 25 cm bed length) was used with a 25 mmol L^{-1} phosphate buffer (pH 2.5) in 75 % acetonitrile containing 2 μL mL^{-1} hexylamine to suppress silanophilic interactions. The specialty of this CEC method was its capability to separate strongly basic, weakly basic, neutral, weakly acidic, and strongly acidic compounds within 33 min in a single run using a step gradient. Although CE achieved higher plate numbers than CEC, the overall resolution was better in CEC, which was explained by the authors as the consequence of a mixed-mode separation with the combination of a free-zone separation mechanism and chromatography via the packed bed. In comparison to MEKC, CEC was less prone to injection artifacts. When compared with HPLC, CEC had only small advantages: impurities from the buffer system (hexylamine) created extra peaks in chromatography because a full gradient had to be used, whereas they were absent in the CEC system because a step gradient was sufficient for the analysis. However, a clearly different selectivity, especially for weakly and strongly basic analytes, was observed. In conclusion, the authors pointed out that each technique has its distinct advantages and that a combination of multiple techniques can achieve a more comprehensive perspective for drug screening.

CEC has been optimized to enable the extremely fast analysis of six opiates in self-packed octadecyl-silica-packed capillaries (length: 28 cm, i.d.: 75 μm, particle size: 1.5 μm), with plate numbers up to 444,000 m^{-1} (for oxycodone) [23] using a 10 mmol L^{-1} Tris, 50 mmol L^{-1} SDS buffer (pH 8.3) in 20 % acetonitrile. A very detailed and well-executed study can be found in the work of Aturki et al. [25], who used a self-packed cyano stationary phase in CEC to establish a validated procedure for the analysis of ten drugs of abuse in urine, yielding a very robust separation system which sustained more than 300 runs. Their buffer system, consisting of 20 mmol L^{-1} sodium phosphate in a 4:1 mixture of water and acetonitrile (pH 2.5), separated the analytes in spiked urine samples within 18 min in a 33 cm-long capillary (i.d.: 75 μm, effective length: 24.5 cm, packed length 23 cm) packed with CN-modified 3 μm particles. Good reproducibility of retention times (RSD <5 %), even in column-to-column experiments (RSD <7 %) was obtained. The detection was sensitive (LOD 5–15 ng mL^{-1}; LOQ 10–30 ng L^{-1}), and a linear working range of 100–1200 ng mL^{-1} was obtained using field-amplified sample stacking as an online preconcentration technique. The publication also included detailed investigations on the effect of different packing materials, of the acetonitrile content, of buffer pH values and concentration, and of sample injection techniques on sensitivity. In 2010, the same group adjusted the procedure for MS compatibility [24] by switching to 25 mmol L^{-1} ammonium formate (pH 3) in a water–acetonitrile mixture (7:3) as mobile phase. The use of MS as detection system lowered the LODs to 0.8–3 ng mL^{-1} but, as often observed with MS detection, repeatability of peak areas was reduced (RSD <16.3 %) and the calibration range was shifted toward lower concentrations (2.5–100 ng mL^{-1}).

Column-coupling isotachophoresis for pre-concentration and matrix removal

With the exception of its use before the development of CE, the older method of ITP is hardly used as a stand-alone method in forensic science. Transient ITP may be used as an on-line preconcentration technique [123, 124] with a high matrix tolerance [123], leading to improved detection limits. Additionally the column-coupling modes ITP–ITP or ITP–CE (commercial instrumentation is available) were used with impressive preconcentration capabilities and matrix removal in a variety of applications. A non-forensic example is the chiral analysis of tryptophan in urine, with only dilution as a sample preparation step [87].

However, these coupling techniques have a very high potential as high-sensitivity techniques [125]. Figure 1 shows an electropherogram from the analysis of harmala alkaloids using column-coupling ITP–CE-LIF. With the possibility of injecting 30 μL of a sample into the first ITP column of the two-dimensional setup, unprecedented detection limits of 20 pmol L^{-1} for norharman and harman were obtained. These two examples, however, can only reveal the capabilities of the method; its real use in forensic science has not yet occurred. A conceivable forensic application of this technique could be the trace detection of common drugs of abuse and their decomposition products in wipe and suction samples from surfaces (e.g. from hollows in vehicles that are suspected to have been used for drug smuggling).

Analytes with special properties

CE and other electromigrative separation techniques have the reputation of being "specialized" techniques for analytes which are difficult to analyze with routine HPLC or GC methods. And indeed, there are several applications where only CE has succeeded in analyzing samples where analytes had a high charge, were highly polar or highly hydrophobic, or were a mixture of enantiomers.

Analytes with high polarity or charge

Extremely hydrophobic or highly polar or even charged analytes are a challenge for routine chromatographic applications, and different liquid chromatographic stationary phases including reverse-phase vs. hydrophilic-interaction chromatography are used. For non-polar analytes, GC is used or analytes are derivatized to meet the requirements of the method. In contrast, with CE polarity is not critical for separation as long as the analyte is sufficiently soluble in the BGE. Using MEKC, the polarity range can easily be broadened via the concentration and type of the surfactant and by use of BGE additives.

Highly charged compounds, for example sulfonic acids and sulfates, are ideal analytes for CE, providing high charge and having a very low pH. An example is the colorant analysis

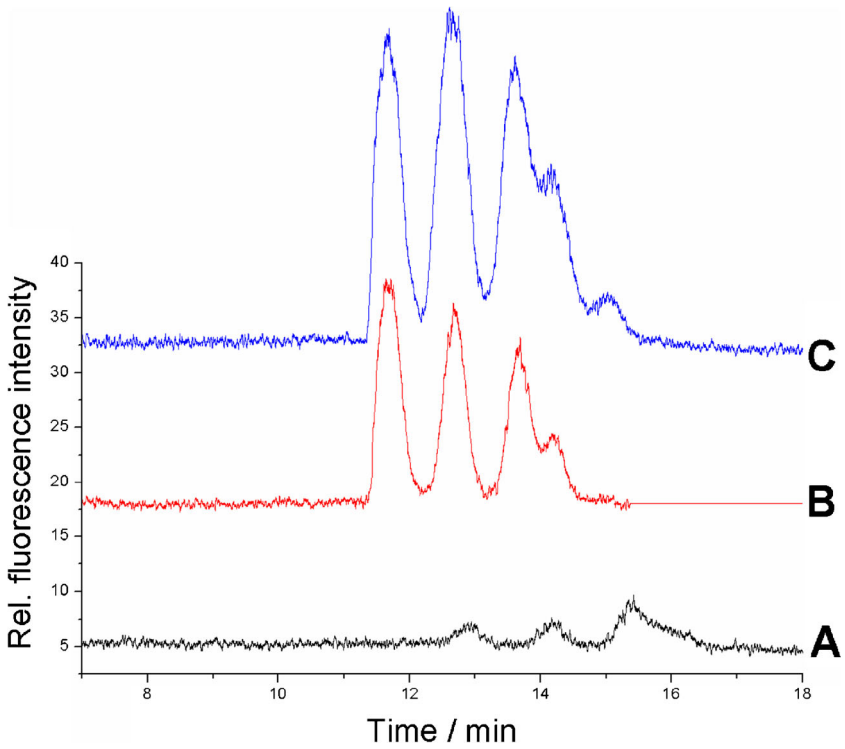

Fig. 1 High-sensitivity analysis of harmala alkaloids using ITP–CE-LIF: analyte concentration (*a*) 0.0001 μmol L^{-1}, (*b*) 0.001 μmol L^{-1}, (*c*) 0.01 μmol L^{-1}. LE: 10 mmol L^{-1} ammonia solution (titrated to pH 4.7 with acetic acid), TE: 20 mmol L^{-1} acetic acid, BGE: 20 mmol L^{-1} NaH_2PO_4, pH 2.4 (titrated with HCl) (with permission from the author [125])

within drug profiling of heroin and ecstasy pills (typically containing amphetamine derivatives). The food colorants used are usually azo or triarylmethane compounds with at least one sulfonate group [16]. Chromatographic analysis with MS detection is possible, but requires specialized stationary phases with a higher polarity to ensure sufficient retention [126, 127]. The advantage of CE for these analytes is that the low pK_a of the sulfonate moiety can be used to achieve extremely high selectivity [16] without the need for further sample clean-up: the food colorants in heroin samples and in ecstasy tablets were separated at very low pH (pH 2.2 in 200 mmol L^{-1} formic acid buffer) using negative polarity for the CE separation. Only the target analytes with a negative charge at this low pH migrate towards the detector under these conditions, giving rise to very clean electropherograms even for matrix-loaded samples including direct heroin extracts (all component opium alkaloids and heroin itself are protonated, migrate to the cathode, and thus are removed from the capillary by migration into the inlet buffer vial). In a chromatographic separation system all of the excess compounds (main active substance, side alkaloids, and adulterants typically forming more than 90 % of the sample weight) are injected into the separation column and have to be completely eluted which can lead to increased analysis time and deterioration of analyte separation. Thus, typically an additional sample preparation step to remove the excess compounds before chromatographic separation will be required. For the described CE-MS procedure the drug samples are simply dissolved in running buffer, filtered (0.22 μm syringe filter), and directly injected into the separation capillary. Selective detection is possible either with UV detection in the visible range or with MS detection for unambiguous identification. The method was used for the differentiation of ecstasy and heroin seizures via the colorants. Typical cutting agents for heroin consist of caffeine, paracetamol, and a mixture of several food colorants to create a brown color (comparable to the color of uncut heroin) to conceal the extent of cutting. A combination of different concentrations of the azo dyes E 102, E 110, and E 151 is often used for that purpose, enabling establishment of links between different seizures of heroin on the basis of the colorant composition. Ecstasy tablets are also frequently colored with a range of different food colorants to increase the value of brand recognition together with the tablet logo, and to shade unfavorable color effects caused by synthesis impurities. It is interesting to note that the separation of the constitutional isomers of quinoline yellow (E 104) was successfully performed also in real samples (yellow-colored ecstasy tablets), using MS–MS spectra to differentiate between the isomers (Fig. 2). Thus, ecstasy pills can be linked on the level of the tableting process on the basis of colorant composition, even if only one colorant is present: in this case by the relation of the proportions of the two constitutional isomers present in commercially available E 104 food colorant.

Another good example of the suitability of CE for the analysis of highly polar compounds in forensic toxicology is the quantification of γ-hydroxybutyric acid (GHB) in samples of so-called "liquid ecstasy". Although structurally not related to the amphetamine derivatives typically contained in ecstasy pills, GHB and its corresponding internal ester γ-butyrolactone (GBL) have been frequently abused as recreational drugs. Furthermore, GHB unfortunately has gained a substantial level of awareness because of its misuse as a "date-rape drug", meaning use for drug-facilitated sexual assaults, by pouring the drug into the beverage of the victim. GHB is typically consumed in the form of aqueous solutions of its sodium or potassium salt and, consequently, it is reasonable to also analyze GHB in its anionic form at high pH to prevent conversion to GBL. Quantification of γ-hydroxybutyrate can easily be achieved by a very rapid CE procedure using a simple sodium tetraborate buffer (20 mmol L^{-1}) at pH 10 and UV detection at 206 nm. To attain high reproducibility, potassium sorbate was used by Pütz et al. (unpublished results) as an internal standard for normalization. For eight consecutive runs the precision of the migration time was 0.08 % and that of the peak area 0.8 % for GHB after normalization (Fig. 3). This procedure is routinely used for GHB quantification in the forensic laboratory of the BKA (German Federal Criminal Police Office) and has been successfully cross validated by participation in round-robin tests.

As well as the separation of permanently charged and highly polar analytes, very hydrophobic compounds, even those insoluble in water, e.g. the hydrophobic steroidal alkaloids present in plants including *Solanum elaeagnifolium* or *Splanum sodomaeum*, are also analyzed in buffer systems containing high amounts of organic solvents or being purely non-aqueous. Cherkaoui et al. [128] analyzed extracts from these plants using NACE-MS, achieving a high resolution, and thus very pure spectra for unambiguous identification and reliable quantification, using 25 mmol L^{-1} ammonium acetate and 1 mol L^{-1} acetic acid in a mixture of MeOH:MeCN (1:4).

MEKC together with MEEKC can cover an impressive range of polarity, with only a small set of buffer combinations to be tested. The standard MEKC buffer with borate pH 9.2 and 60 mmol L^{-1} SDS is well suited for relatively polar analytes. The polarity range can be extended to medium-polarity analytes by addition of organic solvents, mainly methanol and acetonitrile, or with a combination of urea and acetonitrile for aromatic analytes in sassafras oils with octanol–water partition coefficients of up to 5 [129]. Selectivity changes are possible via changing the micellar pseudostationary phase: just using another detergent. For the choice of a suitable detergent, the linear-solvation free-energy relationship (LSFER) model as applied to MEKC by Poole's

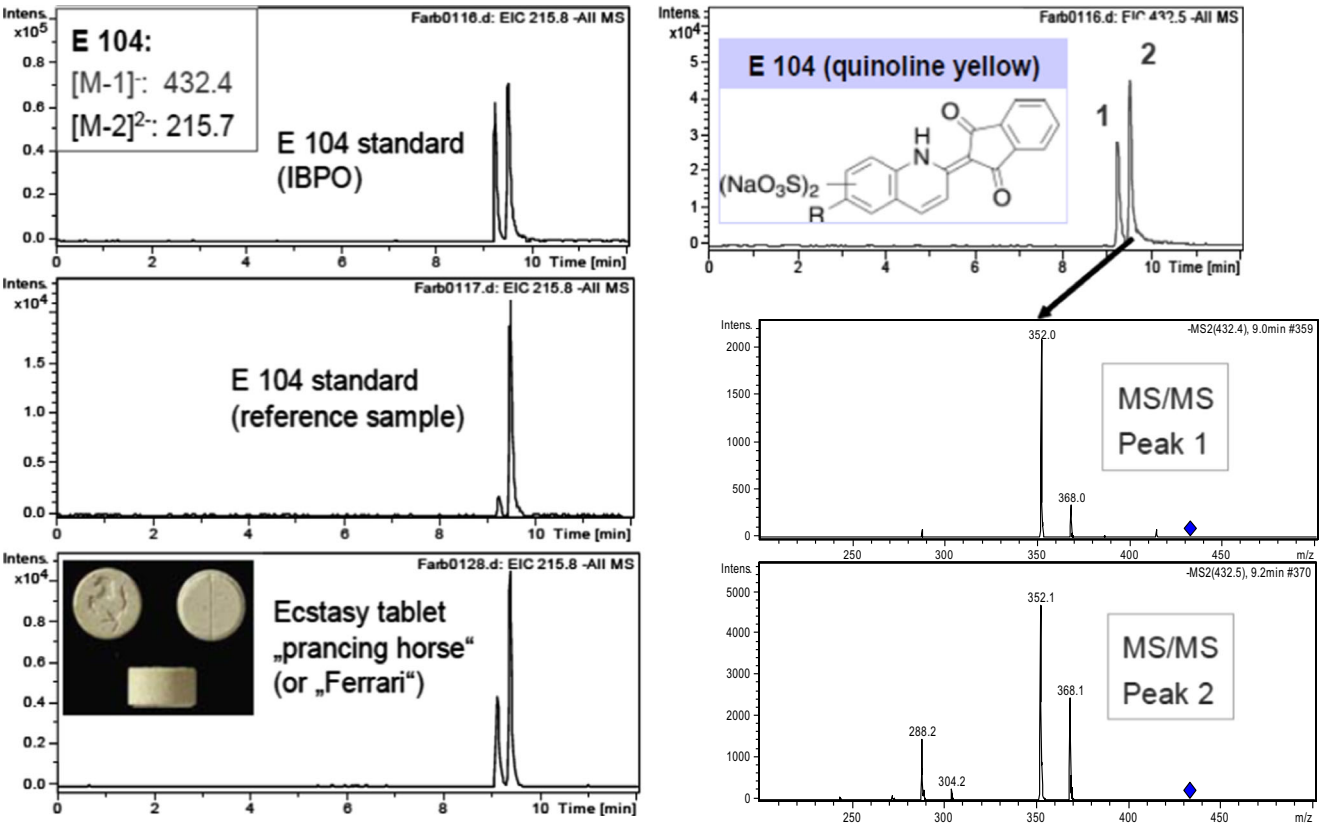

Fig. 2 CE-MS analysis of two constitutional isomers of quinoline yellow (E 104) in two different reference materials and in a yellow-colored ecstasy tablet. BGE: 200 mmol L^{-1} formic acid (pH 2.2), capillary: length: 82 cm, i.d.: 75 µm

group [130–132] can assist because it is based on possible interactions and thus physicochemical properties of the analytes with the mobile vs. pseudostationary micellar phase. The characteristics of most micellar phases are well described. The main underlying interactions are hydrophilic or hydrophobic in nature, or stem from hydrogen bonds. Electrostatic interactions or Van-der-Waals interactions also occur. Ionic interactions can be found mainly at the outer sphere of the micelle, at the charged head groups (see also Fig. 5). In the core of the micelle steric hindrance can also affect and determine the allocation of a compound. For a general discussion on MEKC see the section "Universal separation modes: MEKC and MEEKC".

Structurally closely related analytes

Diastereomers Especially with regard to mass spectrometric detection, diastereomers and positional isomers have to be baseline-resolved by the separation technique because, being isobars, their individual mass spectra and usually also their fragment spectra cannot be differentiated. This task is not trivial, because the near-identical hydrodynamic radii and

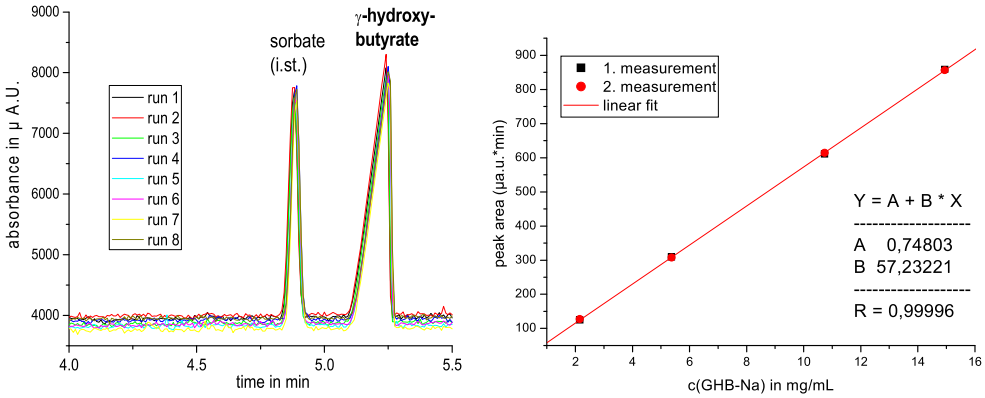

Fig. 3 Eight consecutive CE runs for the quantification of GHB using sorbate as i.s. (*left*) and calibration plot with duplicate determination of four weighed portions of sodium γ-hydroxybutyrate (*right*); tetraborate buffer (20 mmol L^{-1}) at pH 10 and UV detection at 206 nm

charge of the molecules relevant for electromigrative separation techniques lead to very similar migration behavior in CE. Posch et al. [20, 30] used an experimental design for the analysis of the biogenic drug "Kratom" (made from the leaves of *Mitragyna speciosa*) in a combined approach to (i) assess the robustness, (ii) optimize an NACE-MS set-up, and (iii) gain insight into the separation principles and interacting variables in NACE [20]. The optimization of the method led to the following conditions: 60 mmol L^{-1} ammonium formate in a mixture of acetonitrile and glacial acetic acid (100:6) was used as background electrolyte, and a mixture of isopropanol, water, and acetic acid (62:33:5) was used as sheath liquid at a flow of 7 μL min^{-1}. Although 167 analytical runs had to be conducted for the method development, this experimental setup can still be regarded as a fast method development, because the results enabled insight into the separation principle and the relevance of all important experimental conditions. The basic separation procedure, with its relevant tuning conditions, is now known, so adaption to new forensic applications can easily be performed. The broad applicability of this NACE-MS procedure was further investigated for the analysis of a variety of psychoactive plant materials, and proved to be nearly generic for indole alkaloids [30]. A possible explanation for the good separation efficiency for diastereomers in NACE is the absence of a large hydration shell in the non-aqueous environment, with reduced analyte–solvent interactions, emphasizing small differences in the Stokes radii of the diastereomers which would otherwise be masked by the attached water molecules in aqueous solution. The high selectivity is further explained by a different separation mechanism in NACE, where ion-pairing and homo- and heteroconjugation of the analyte or of the analyte–ion-pair complex with the ammonium ion from the BGE is the dominant separation principle [30]. Resolution optimization is thus possible via the ammonium-formate concentration. More work regarding the separation principles in NACE is still required to further broaden its applicability.

Constitutional isomers A very good visualization of an easy and fast method development and optimization of the separation of positional isomers of 1-(chlorophenyl)piperazine (CPP) was presented by Pütz and Martin [16], which is of forensic relevance, because only the 1-(3-chlorophenyl)piperazine is scheduled in most controlled substance acts. As shown in Fig. 4, baseline separation of the three constitutional isomers (1-(2, 3, 4-chlorophenyl)piperazine) in CE-MS was achieved simply by adding 10 mmol L^{-1} hydroxypropyl-β-cyclodextrin to the run buffer. Cyclodextrins are complexing agents with a bowl-like shape to incorporate small organic compounds. Isomers clearly differing in their three-dimensional structure fit into the relatively hydrophobic cyclodextrin cavity to a different extent, and also have different numbers and strengths of hydrogen bonds with the cyclodextrins OH group at the rim of the cavity. However, ideally near-equal signal intensity would be obtained for each isomer when injected at like concentration. As visible from Fig. 4, this is clearly not the case. With an increase in the migration time, which is correlated with a stronger interaction with the neutral cyclodextrin, a distinct quenching effect of the analyte CE-MS signal is visible, and the ionization efficiency of the analyte-CD complex is clearly reduced compared with the free, uncomplexed analyte. Additionally, the presence of the cyclodextrin in the running buffer leads to a general loss of ionization efficiency for all compounds in the electrospray, irrespective of the individual interaction of the cyclodextrin with the analytes. Exemplarily, the peak area for the ortho isomer of CPP is reduced by a factor of five in the running buffer containing the cyclodextrin compared with the buffer without cyclodextrin. However, the signals of the background compounds are also significantly suppressed when the cyclodextrin is present in the running buffer, so the overall reduction of the sensitivity caused by the use of a cyclodextrin in the described procedure is only marginal.

With the knowledge of separation principles of several electromigrative separation techniques, it seems logical that the high separation potential of NACE was also used for the separation of constitutional isomers no longer necessitating the addition of chiral selectors. This was reported by Cherkaoui et al. [133, 134], in the manipulation of the separation of the constitutional isomers littorine and hyoscyamine for the analysis of a plant extract. They were able to inverse the migration order of both isomers upon addition of the strong ion-pairing reagent trifluororacetic acid to the BGE, revealing that the counter ion has an important function in NACE and enables impressive tuning possibilities. They used 25 mmol L^{-1} ammonium acetate and 1 mol L^{-1} acetic acid (or 1 mol L^{-1} TFA) in acetonitrile in a 64.5 cm (effective length: 56 cm), 50 μm bare-fused-silica capillary for the separation of extracts made from *Datura candida* and *Datura aurea*.

Positional and cis-trans isomers Positional and *cis-trans* isomer separation with MEKC or NACE is possible: good separation of stereoisomers including, e.g., *cis-trans* isomers of internal double bonds can be achieved with MEKC. Selectivity of MEKC stems from different interactions of analyte and micelle, with different dominating interactions at the surface of the micelle (mostly ionic interactions), the inner core (mostly hydrophobic interaction including London forces, dipoles and induced dipoles), and hydrogen bonding in the interim of the shell, where water is present to a high extent as visualized in Fig. 5.

Amphiphilic molecules may be incorporated between the detergent molecules of the micelle, taking advantage of both hydrophilic and hydrophobic interactions. The high selectivity for structural isomers stems from steric effects on these interactions and differences in dipole moment and polarizability. The good separation is further aided by MEKC's high separation efficiency, caused by a negligible resistance in mass transfer occurring because the micelles are dynamic entities

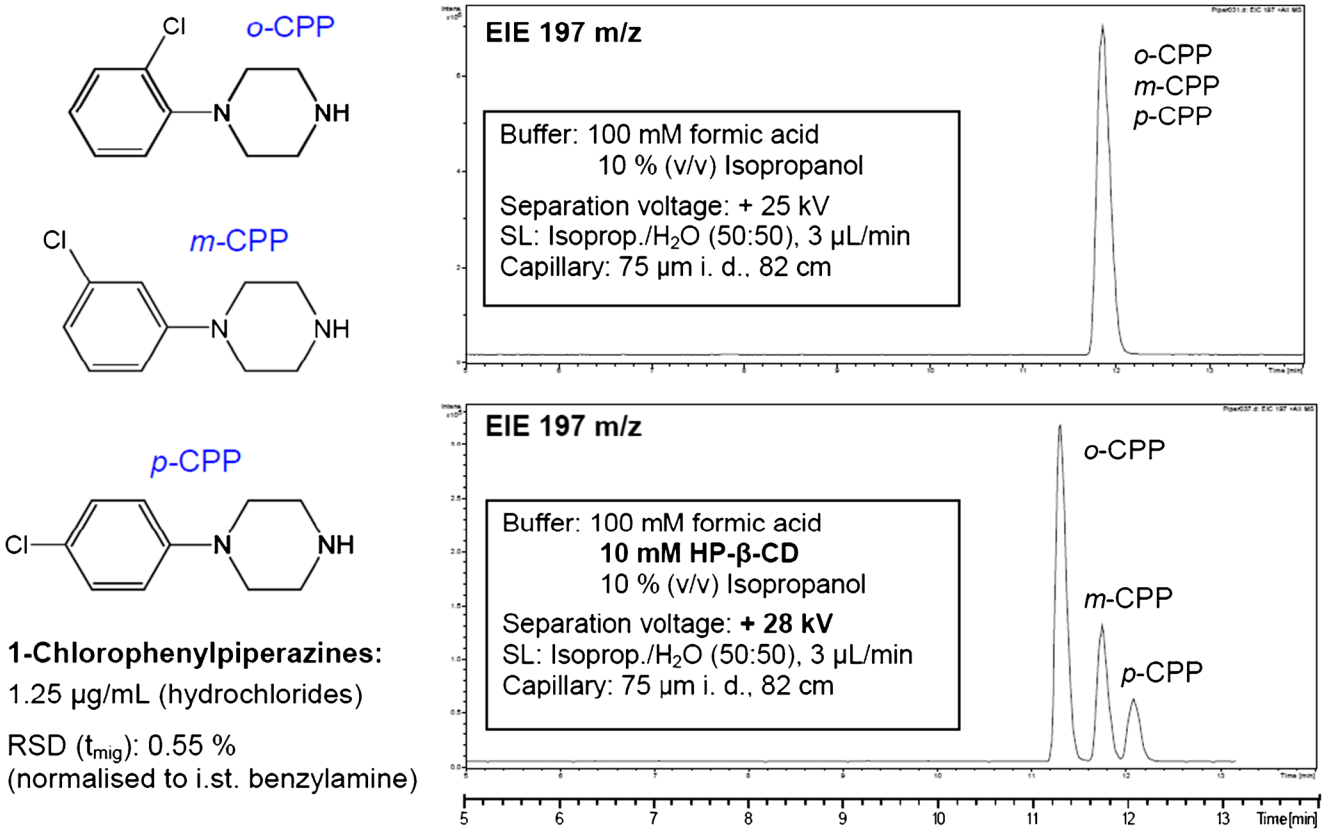

Fig. 4 Baseline separation of the three constitutional isomers of 1-(chlorophenyl)piperazine by CE-ESI-MS, using the chiral selector 2-hydroxypropyl-β-cyclodextrin (with permission from the authors [16])

with lifetimes of 100–1000 ms [135] and leading to high plate numbers, with longitudinal diffusion as the major source of band broadening. Resolution and peak capacity may be improved by optimization of the micellar vs. the electroosmotic mobility, increasing the migration time window and thus resolution [135]. Good examples of the separation of *cis–trans* isomers with MEKC can be found in the work of Lucangioli et al. [136] and Huhn et al. [137]. Lucangioli et al. analyzed sertraline in a 20 mmol L^{-1} sodium borate, 50 mmol L^{-1} sodium cholate, 15 mmol L^{-1} sulfated-β-cyclodextrin, and 5 mmol L^{-1} hydroxypropyl-β-cyclodextrin buffer (pH 9) in a 60 cm (effective length: 53 cm, i.d.: 50 µm.) bare-fused-silica capillary. They achieved a low limit of detection (0.2 µg mL^{-1}) and good repeatability. The method was also applied to the analysis of the bulk drug containing 2 mg mL^{-1} sertraline hydrochloride, and was able to determine small (0.1 %) impurities including *R*-(-)-mandelic acid. Comparison of their procedure to an HPLC procedure revealed good agreement. The separation of *cis* and *trans*-isoeugenol, with several other analytes present in sassafras oils, containing safrole as an important precursor chemical for methylenedioxyamphetamine derivatives, was part of the work of Huhn et al. [137]. Separation was performed in 1.875 mmol L^{-1} sodium tetraborate, 60 mmol L^{-1} SDS buffer with 20 % acetonitrile, 4 mol L^{-1} urea and 0.5 mmol/L $CaCl_2$ in a 27 cm (20 cm effective length, 50 µm i.d.) bare-fused-silica capillary.

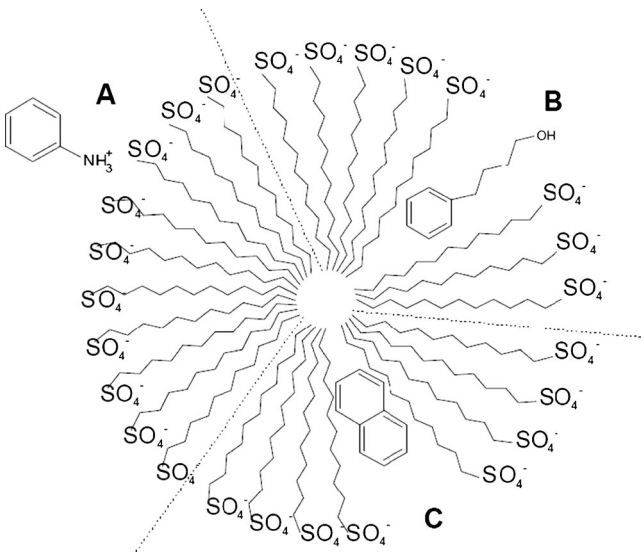

Fig. 5 Different allocations of charged, polar, and hydrophobic compounds in a dodecyl sulfate micelle. *A*: ionic interactions at the micelle's outer sphere, *B*: bridging via hydrogen bonds between the compound and the Stern-Layer, *C*: hydrophobic interactions in the core of the micelle (with permission from the author [125])

In NACE, separation of cationic *cis–trans* isomers results from different formation equilibrium constants with anionic

chiral counterions, as investigated by Bjørnsdottir et al. [138]. This publication also featured a theoretical model for the separation principle, and is highly recommended as an introduction. Hansen et al. [139] also used NACE for the separation of *cis–trans* isomers without using any surfactants, cyclodextrins, or complexing agents in the analysis of several drug substances with isomerism. This publication is also an excellent example of the analysis of minor compounds in the presence of excessive amounts of similar compounds (see the section "Presence of compounds in excess"). The separation is presumably based on ion-pair formation, which is probably present in the non-aqueous buffer system of acetonitrile, methanol, and ammonium acetate.

Chiral analytes Chiral separation is an important tool for forensic drug profiling, because the relative concentration of the enantiomers can provide information on the synthesis pathway used, the precursor substance, and the origin of a sample, which is especially relevant for profiling clandestinely produced methamphetamine ("crystal meth") [140]. Therefore chiral analysis links drug batches, production sites, precursor-chemical supply chains, and distribution networks among others [141, 142]. In addition, chiral separation is vital, e.g., if one enantiomer has properties beneficial to health, whereas the other might have severe negative effects on health (e.g. Thalidomide), or if the potency of enantiomers differs significantly (as e.g. for (levo-)methadone and many amphetamine derivatives). Because the identification and differentiation of enantiomers cannot be performed by mass spectrometry or UV spectra alone, comparison of the migration time with a standard in combination with standard addition is usually used. This requires highly reproducible and stable migration times of the analytes, an aspect where CE is mistakenly still believed to perform badly. Several excellent reviews with a focus on enantioselective capillary electrophoresis can be found [143–146] and are recommended to the interested reader. A large number of chiral selectors have been developed, although cyclodextrins are still the most popular chiral additives [147]. The great advantage of chiral CE over other chiral-separation methods, especially chromatography, is the ease of method development. This is facilitated because, in contrast with chromatography, the chiral selector and the analyte are in the same phase, that is, both are dissolved in the BGE. As can be deduced from Eq. 1:

$$\mu_{\text{eff}} = \frac{\mu_f + \mu_c K[CD]}{1 + K[CD]} \quad (1)$$

where μ_{eff} is the overall effective electrophoretic mobility, μ_f the effective electrophoretic mobility of the free (uncomplexed) analytes, μ_c the effective electrophoretic mobility of the cyclodextrin–analyte complex, K the complexation constant, and [CD] the molar concentration of the cyclodextrins (from Ref. [148]). The separation is based both on the different complexation constants and on the different effective electrophoretic mobility of the evolving diastereomeric complexes [148] (whereas the velocity (and thus the velocity difference) of the diastereomeric complexes in LC is always zero). For a very detailed study of the effect of the nature, structure, and pH-dependence of complexation equilibria of analytes with cyclodextrins, the interested reader is referred to the work of the Scriba group [148–154].

From Eq. 1 it is possible to understand the optimization possibilities for chiral CE: as well as the type of the chiral selector, and thus the complexation constant and the mobility of the diastereomeric complexes, the concentration of the chiral selector can be optimized to improve chiral resolution. Especially for oppositely charged ligands and analytes, very high resolution can be obtained in cases where the two stereoisomers migrate in opposite direction, as was revealed for methadone, venlafaxine, tramadol, and fluoxetine separations with highly sulfated cyclodextrins [155], achieving infinite resolution. In many cases mixtures of chiral selectors have also been used, especially when the analytes greatly differed in size and in the number of possible hydrogen bonds to be established with the chiral selector.

Another, often underestimated advantage of chiral CE is the ease of changing the migration order of enantiomers to facilitate quantification of the enantiomer present in traces, for example by reversal of the electroosmotic flow (EOF), or changing the type and concentration of the chiral selector, pH value, temperature, etc. In chiral LC, a different chiral column has to be used because the optical antipodes of the stationary phase are mostly not available.

The work by Iwamuro et al. [7] is a powerful example of how to create robust analytical CE–UV methods for the chiral analysis of methamphetamine and its related compounds by using a capillary with a modified surface, with diol groups on the inner surface of the capillary. They separated 18 enantiomers within 9 min, using a 125 mmol L^{-1} Tris–125 mmol L^{-1} sodium dihydrogen phosphate buffer (pH 6.15) containing 6 mmol L^{-1} dimethyl-β-CD and 12 mmol L^{-1} β-CD as chiral selectors. RSDs of migration times for standards were 0.09 %, but 0.14 % for crude urine samples because of matrix effects.

Comparable stability for migration times (RSD <0.04 % after correction with internal standards) was achieved for a CE-MS method for the analysis of amphetamine, methamphetamine, *p*-hydroxymethamphetamine, and dimethylamphetamine [8], using a mixture of 3 mmol L^{-1} β-cyclodextrin and 10 mmol L^{-1} dimethyl-β-cyclodextrin at acidic pH (pH 2.2) for the separation. With this low pH, only a very slow EOF is induced and the authors thus largely prevented the neutral cyclodextrins entering the mass spectrometer, where this would have led to severe ion suppression and thus increased detection limits (compare Fig. 4). In a later modification of the method [9] the authors switched to the

negatively charged heptakis(2,6-diacetyl-6-sulfato)-β-cyclodextrins in 1 mol L^{-1} formic acid (pH 1.8) to further reduce ion-source contamination caused by the cyclodextrins migrating to the inlet. A major further improvement in ease and applicability enabled the direct injection of urine samples after a simple 0.45 μm filtration step, while maintaining high repeatability (RSDs between runs: 0.3 % (migration time) and 5.3 % (peak area)) [11].

Although the work of Meng et al. [22] is more focused on the development and evaluation of a liquid–liquid microextraction technique for sample preparation, the achieved chiral baseline separation of methamphetamine, 3,4-methylenedioxymethamphetamine, and ketamine is worth mentioning here, especially because the achieved limits of detection (0.05 μg L^{-1} for diacetylmorphine and 0.2 μg L^{-1} for methamphetamine, based on S/N =3) are exceptionally low and linearity was achieved from 0.15–6 500 μg L^{-1} using standard UV detection. The authors used a simple potassium dihydrogen phosphate–phosphoric acid buffer (pH 3.2) with 20 mmol L^{-1} β-cyclodextrin as chiral selector. In terms of fast chiral separations, Lurie et al.'s work [17] has to be cited here again (see the section "Separation modes"), because they achieved baseline separation of six racemic beta-phenethylamines and *d*,*l*-propoxyphene within 4.5 min using a 32 cm-long capillary in a CE equipped with a diode-array-detection system (Fig. 6).

With regard to new psychoactive substances (NPS) present in so-called "legal highs", for example "bath salt" products marketed via internet shops, [5] reports the separation of 19 cathinone derivatives used as substitutes for the banned mephedrone, using a 50 mmol L^{-1} ammonium acetate buffer (pH 4.5) with 10 % acetonitrile and 20 mmol L^{-1} sulfated-β-cyclodextrins in a CE-DAD system. Separations of enantiomers up to 3 min from peak-to-peak within a total run time of 20 min were achieved. Iwata et al. [10] combined a chiral capillary-electrophoresis method with mass-spectrometric detection for the analysis of amphetamine-type stimulants, using 2.5 mmol L^{-1} highly sulfated cyclodextrins as chiral selectors and electrolyte; although it is very probable that formate ions from the sheath liquid entered the capillary and acted as additional BGE ions. The achieved resolution, with more than 20 min between d-ephedrine and l-ephedrine and more than 10 min between d and l-amphetamine, makes the method worth being cited here, although the high resolution was at the expense of a very high total analysis time of 55 min.

It has to be noted, however, that chiral identification and identification only by migration time and spiking experiments is usually risky, as [156] reveals. To increase the reliability, they combined chiral CE with MS, using different mixtures of several semi to non-volatile chiral selectors, either charged negatively (1 mmol L^{-1} 2,3-diacetyl-6-sulfato-β-cyclodextrins) or neutrally (10 mmol L^{-1} 2-hydroxypropyl-β-cyclodextrins), while reducing the electroosmotic flow to nearly zero (BGE was 1 mol L^{-1} formic acid, pH ~1.9). With this experimental setup, they revealed that false-positive results may be obtained with UV detection only (Fig. 7): one peak, labeled as (+)-amphetamine in a seizure of an illicit methamphetamine sample, had perfect comigration with reference (+)-amphetamine, but the absence of (+)-amphetamine was proved by the implementation of the CE-MS method, and instead the peak was identified and verified by MS–MS as procaine, a local anesthetic.

Tolerance towards sample components

Matrix tolerance

A striking argument for the implementation of CE in the forensic laboratory is its tolerance towards high matrix load. As can be seen in Table 1, which summarizes the methods in this review, often only a dilution step is necessary as sample preparation for liquid samples or methanolic extracts. In some cases additional solid-phase extraction or liquid–liquid extraction is necessary, most often because the sample has a high salt

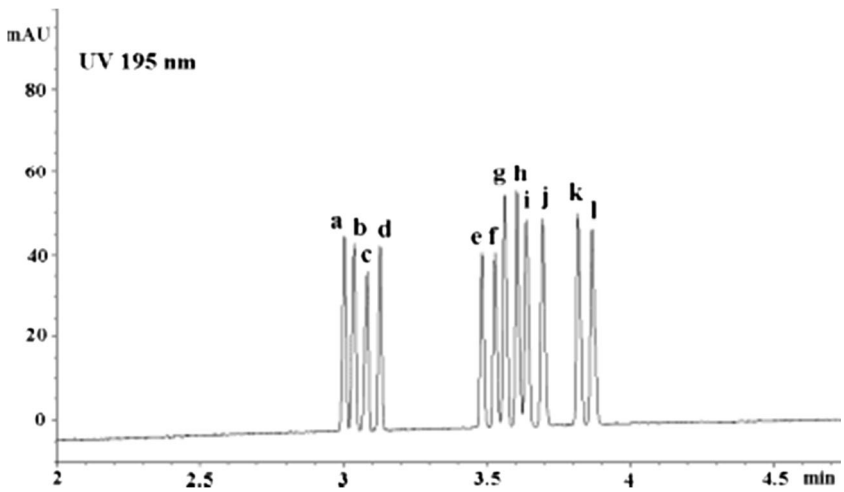

Fig. 6 Electropherogram of a standard mixture of (*a*) l-amphetamine, (*b*) d-amphetamine, (*c*) l-methamphetamine, (*d*) d-methamphetamine, (*e*) l or d-*n*-butylamphetamine, (*f*) l or d-*n*-butylamphetamine, (*g*) l or d-MDA, (*h*) l or d-MDA, (*i*) l or d-MDMA, (*j*) l or d-MDMA, (*k*) l or d-MDEA, (*l*) l or d-MDEA (with permission from WILEY-VCH [17])

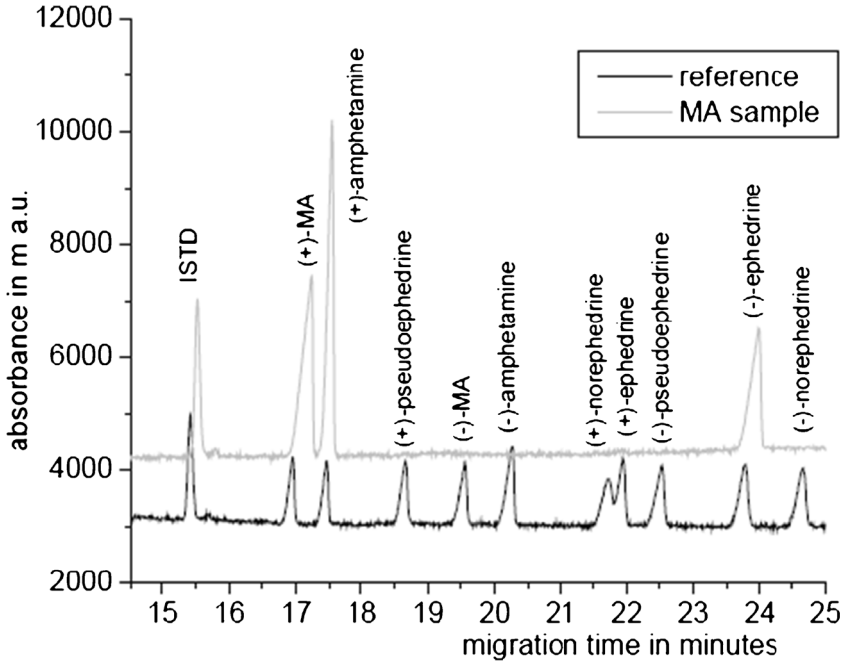

Fig. 7 Chiral CE analysis of an illicit methamphetamine sample (MA sample) using CE with UV detection in comparison to a standard mixture. The peaks are labeled according to the reference standard. Note the perfect co-migration of the peak above the (+)-amphetamine, which was first misinterpreted as (+)-amphetamine, but later identified as procaine by CE-MS. (Figure from [156] with permission from the authors)

content, e.g. urine samples. The high tolerance towards matrix effects is a result of the very small injected sample volume (a few nanoliters) and of the robustness towards aggressive chemicals of the fused-silica capillary as separation column. Also, only charged matrix compounds with a similar effective electrophoretic mobility in the BGE can interfere with the analyte; neutral compounds remain in the sample plug and are carried towards the outlet without disturbing the separation window, and ions with opposite charge are well separated. However, matrix compounds which interact with the capillary surface are problematic for CE because they consistently affect the electroosmotic flow velocity and may accumulate on the capillary wall if no coatings are applied nor adequate flushing procedures after the run are performed. Here, it is of advantage that the fused-silica capillaries can withstand very harsh conditions, enabling flushing with concentrated NaOH or strong acids to remove adsorbed material between runs. Even if, in rare cases, a capillary is irreversibly contaminated, the exchange for a new capillary takes less than half an hour and is by far less expensive (less than 10 € m^{-1} for fused-silica capillary replaced manually) than the replacement of a separation column in HPLC or UHPLC systems.

A high level of selectivity can be achieved by the choice of the separation mode, e.g. by excluding all non-charged compounds when using CE instead of MEKC. Additional selectivity can be introduced by the type of the BGE and its pH, which largely determine the degree of dissociation of the analytes vs. matrix components, e.g. having only a few charged at a specific pH. The work of Pütz and Martin [16] on the analysis of sulfonated food colorants at very low pH (see the section "Analytes with high polarity or charge") is a good example of this approach. Capillary coatings can further be used for the alteration of the EOF, making it possible to exclude slowly migrating analytes in a counter-EOF separation. However, the high tolerance is not without costs: high matrix load usually necessitates more intensive and longer rinsing steps for the capillary, coatings might be damaged and require re-coating steps, and buffer vials must be exchanged or replenished more often. Usually, an experienced operator can reduce such effects to a minimum. A good example of a chiral CE method with high matrix tolerance using mass spectrometry for identification was presented by Cherkaoui et al. [157]: they investigated the feasibility of using the partial filling technique (PFT) in combination with mixed charged cyclodextrins to achieve chiral separation and mass spectrometry detection without interference from the chiral selectors. In PFT, a solution of a chiral selector is injected as a plug into the capillary before the sample plug itself is injected. When the electric field is applied, the chiral selector countermigrates to the inlet, away from the mass spectrometry detection system. The chiral separation occurs when the analytes pass the chiral-selector solution; upon depletion of the selector in the capillary, only zone electrophoresis is present. This leads to an undisturbed electrospray with good ionization properties while maintaining the chiral resolution. Serum after a simple liquid–liquid extraction was the matrix for the injection solution in the analysis of methadone, amphetamines, venlafaxine and metabolites, and tropane alkaloids. The method was only validated for (−) and (+)-hyoscyamine, but had excellent precision in relative migration times and relative peak areas, which is not trivial for the partial filling technique. Another impressive approach, with high matrix tolerance and the ability to cope with excessive amounts of sample components, was presented in [140].

Here, the analytes had absolutely no interaction with the capillary wall. The absence of tailing proved that no overlapping with closely migrating analytes was present. Impurities in methamphetamine seizures (concentration: 20 mg mL^{-1} methamphetamine hydrochloride) were tentatively identified and quantified without sample pretreatment using a CE–UV method after dissolution of the tablet in water. This enabled the rough classification of methamphetamine seizures in Japan into three groups on the basis of the ratio of (−)-ephedrine to (+)-pseudoephedrine, which are the most important precursor chemicals for the clandestine production of (+)-methamphetamine.

Presence of compounds in excess

A typical challenge, and a good argument for the use of separation techniques prior to high-resolution mass spectrometry, is the detection and quantification of trace compounds or compounds present in a very low ratio to an undesired signal (e.g. 1:1000). Undesired signals might be part of the matrix or of the sample itself (Fig. 12) and are typically also increased in sample-preparation techniques including solid-phase extraction. A good example of where such techniques are needed is the analysis of all forms of tablets where strongly varying amounts of colorant, active ingredient, or cutting agent are encountered. However, they need to be separated from the analyte signal before detection to prevent effects including ion suppression etc.

One of the easiest approaches to determine a minor compound in the presence of excessive amounts of a closely migrating compound is to detect the minor one first. This is especially true for enantiomeric separations or impurity detection. However, this demands control of the migration order, which needs in-depth knowledge of the separation principle and how to affect complexation equilibria in case chiral selectors are used. In 1995 Schmitt and Engelhardt [159] named three different approaches to obtain a reversal of migration order in CE experiments (see also the section "Separation modes"): 1) reversal of the EOF, 2) using cyclodextrins, and 3) using charged cyclodextrins, whose charge depends on the pH of the BGE. They determined impurities from (+)-ephedrine in (−)-ephedrine, with a BGE containing 2 % (*w*/*v*) carboxymethylated cyclodextrins in a 20 mmol L^{-1} citric acid buffer at pH 2.5: in their conclusion, they stated that the use of differently charged cyclodextrins was the most elegant method for the reversal of the migration order. This list of migration time reversal techniques can further be extended to: 4) changing the concentration of the chiral selector, 5) changing the effective electrophoretic mobility of the chiral selector (e.g. via pH, organic solvents), 6) changing the binding strength between chiral selector and enantiomer (e.g. via organic solvent additives), and 7) induction of additional separation mechanisms (e.g. cyclodextrin-modified MEKC).

Examples for excess compounds in non-chiral analysis are manifold. Jung et al. [158] developed and validated a CE method using indirect UV detection for the analysis of ethyl glucuronide (EtG), a biomarker for monitoring alcohol abstinence, in serum after a simple protein-precipitation step. Consequently, enormous amounts of chloride and formate were present in the sample solution. However, unaffected detection was possible with an LOD of only 0.25 mg L^{-1} in only 15 min run time (see the section "Cost efficiency and green methods"). Caslavska et al. [160] confirmed these experiments via a CE-MS–MS coupling.

Huhn et al. [129] used MEKC coupled to UV and (UV)-LIF detection for the analysis of allylbenzenes in essential oils, which are used as precursors for the clandestine synthesis of amphetamine derivatives prevalent in ecstasy tablets. The method was capable of determining minor components, structurally similar to the main compound, present in a molar ratio of 1000:1, using only dilution with methanol and a surfactant as sample preparation (Fig. 8). High inter-capillary repeatability was obtained, despite this large excess, using a mobility axis (see also the section "Mobility axis").

Several prominent forensic applications are based on the determination of inorganic and small organic anions and cations present in trace amounts with one excess ion or in complex environmental matrices. An increasingly important application is the determination of typical inorganic cations (e.g. ammonium, potassium, sodium, calcium, magnesium, and strontium) and anions (especially nitrate, chlorate, perchlorate, and azide) related to improvised explosives in post-blast residues. Capillary electrophoresis is highly suitable for this application because of its potential for field-suitable analysis directly at the scene of crime using portable systems with capacitively-coupled contactless conductivity detection (C4D).

In pharmaceutical and illicit drug analysis, the determination of the salt form of the main active substance is of general interest because counterfeit pharmaceuticals sometimes contain the active substance in a different salt form compared with the original product. For illicit drugs, the salt-form composition can give information about the clandestine manufacturing process, e.g. the presence of methylsulfate in amphetamine sulfate. Figure 9 shows an example of inorganic and small organic ion analysis of a "Mitsubishi" ecstasy tablet by use of two indirect photometric CE procedures (study of Pütz et al. as a part of the EU project CHAMP: "Collaborative harmonisation of methods for profiling of amphetamine type stimulants", 6th framework programme of the EC, contract no. 502126). Anion analysis reveals the presence of significant amounts of chloride and sulfate. To ascertain the actual salt form of the main active substance, 3,4-methylenedioxymethamphetamine (MDMA), an additional cation profile was assessed, revealing an unexpectedly large excess of Mg^{2+}, small amounts of Ca^{2+}, Na^{+}, NH_4^{+}, and the

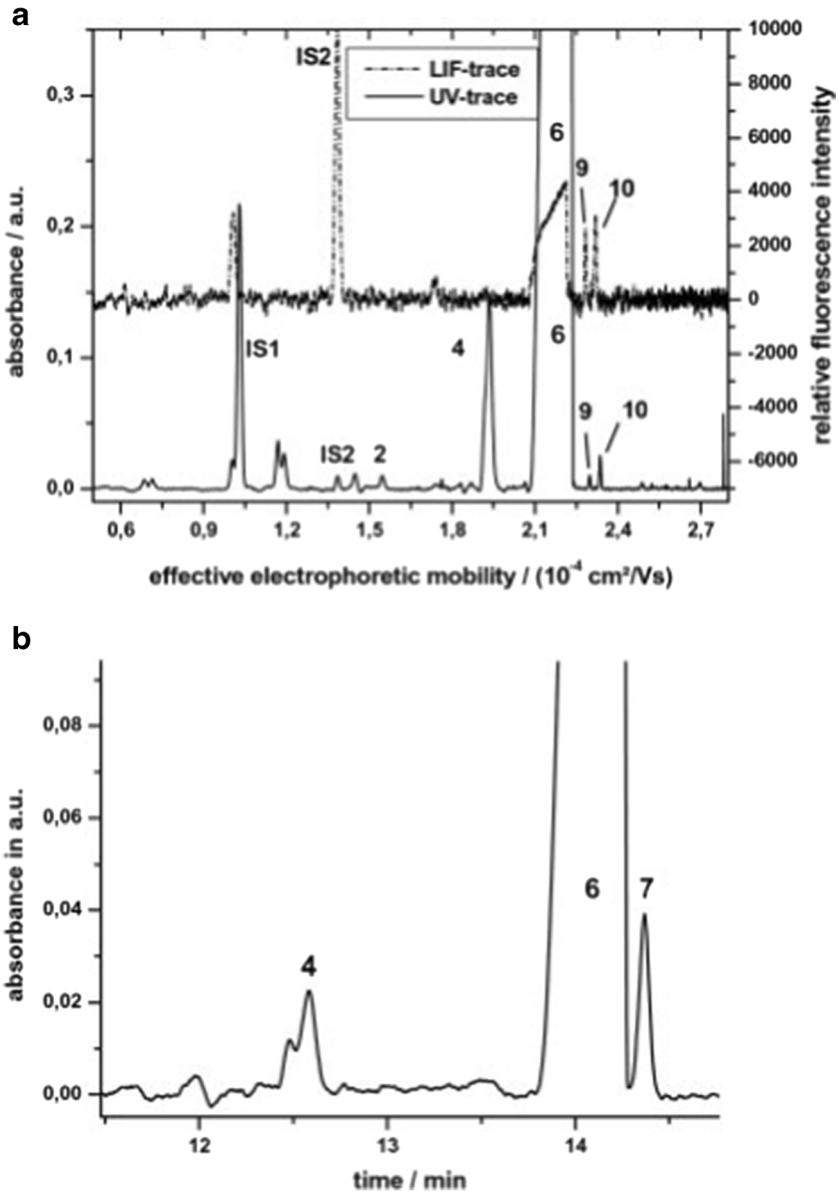

Fig. 8 Electropherograms of the analysis of *Sassafras albidum* oil, at different dilutions of the oil in a surfactant–methanol mixture. (**a**) 1:100 dilution, (**b**) 1:1000 dilution. The analysis was performed using a separation buffer composed of 60 mmol L^{-1} SDS, 7.5 mmol L^{-1} sodium tetraborate, 4 mol L^{-1} urea, and 0.5 mmol L^{-1} $CaCl_2$ at pH 9.2 with 20 % acetonitrile. Peak identification: (*IS1*) methyl-4-cyanobenzoate, (*IS2*) acridone, (*2*) eugenol, (*4*) methyleugenol, (*6*) safrole, (*7*) myristicin, (*9*) *cis* and *trans*-isosafrole, (*10*) anethol (with permission from [129])

presence of methylammonium as organic trace ion (indicating that a reductive amination synthesis procedure with methyl amine as reactant was performed). Quantification of anions and cations confirmed the salt form of MDMA to be hydrochloride, with magnesium sulfate as an unusual cutting agent in the examined ecstasy tablet.

The assessment of inorganic cation and anion profiles can also be used as a fingerprint to establish links between different seizures of drugs. Figure 10 illustrates such an application for the batch-to-batch comparison of four different types of ecstasy tablet from seizures in Finland, on the basis of CE-cation profiles. The relative peak areas for the four detected ions (K^+, Na^+, Ca^{2+}, Mg^{2+}) are similar for all four tablets, and the presence of significant amounts of potassium is unusual. As additionally proved by organic impurity profiling via GC–MS, all tablets originated from the same clandestine tableting lab and the quite uncommon cutting agent gelatin was the cause of the characteristic cation profile.

Another important forensic–toxicological application of cation and anion profiles is the evidence for or the exclusion of a manipulation of foodstuff, especially beverages that are suspected to have been poisoned. In this case the ion profiles of the product under question and a separately acquired original product (as reference) are directly compared in consecutive CE runs.

Fast method development and optimization strategies

The development and validation of analytical methods is often very time consuming and labor-intensive, and is chemical and/or solvent consuming and therefore expensive. Police seizures of items of evidence that are submitted to forensic analysis

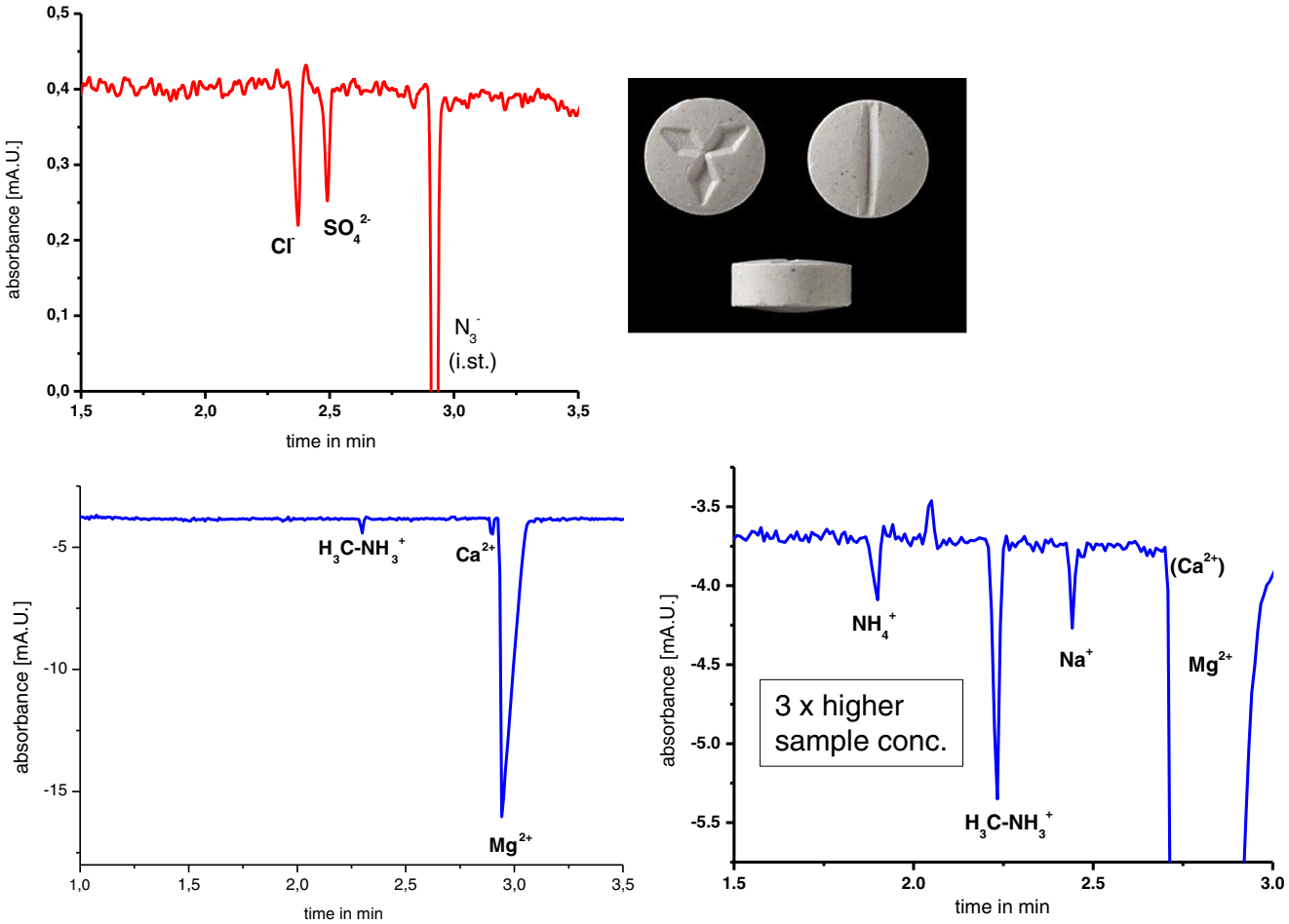

Fig. 9 Salt form determination and trace analysis of small anions and cations in an ecstasy tablet by capillary electrophoresis with indirect photometric detection. Anion analysis: BGE: 2.25 mmol L^{-1} sodium hydroxide, 6.5 mmol L^{-1} pyromellitic acid (probe ion), 1.6 mmol L^{-1} triethanolamine, 0.75 mmol L^{-1} hexamethonium hydroxide solution, adjusted to pH 7.7 with NaOH; bare-fused-silica capillary (i.d.: 50 μm, lengths: 47 or 40 cm); −28.0 kV, sample injection: 35 mbar/0.1 min; UV detection at 254 nm, i.s. sodium azide. Cation analysis: BGE: 10 mmol L^{-1} imidazole, 2 mmol L^{-1} crown ether 18-crown-6, adjusted to pH 4.0 with sulfuric acid; 50 μm i.d. bare-fused-silica capillary (57 or 50 cm); HV: +30.0 kV, sample injection: 35 mbar/0.1 min; UV detection at 214 nm

often consist of a large variety of different products and/or materials and come with a large amount of side-compounds, especially designer drugs, which may have totally different matrices, sometimes with the need for chiral separation. The high separation efficiency (high plate numbers) and high selectivity of electromigrative separation techniques, and the small volumes needed and the fact that nearly all types of separation-affecting compounds can simply be added to the running buffer, make electromigrative separation techniques very fast, easy, and cost effective to optimize [41, 42]. The number of possible optimization strategies in capillary electrophoresis is impressive, enabling the operator to quickly tune the different affecting factors of a separation (selectivity, resolution, efficiency), e.g. by differently functionalized capillary surfaces, coatings, complexing agents, surfactants, chiral selectors, pH value, induction of ion-pair formation, addition of organic modifiers, etc. However, mostly several, if not all, variables including resolution, speed, selectivity, and efficiency will be simultaneously altered as a result of changes of equilibria of the analyte (protonation, complexation, ion-pairing, chromatographic interaction) and of electroosmotic mobility.

An interesting example of for a method optimization using a one-factor-at-time approach is [161], which also includes a descriptive figure and discussions of the separation principles and effects induced by highly sulfated γ-cyclodextrins on the chiral analysis of nine chiral amphetamine-type stimulants. They used a short capillary (32 cm) and a high-ionic-strength buffer (50 mmol L^{-1} phosphate, 10 mmol L^{-1} highly sulfated-γ-cyclodextrins, pH 2.6), inducing a counter-electroosmotic separation by applying negative polarity. Their method development yielded electropherograms with well-resolved analyte peaks (several minutes between enantiomer pairs) and robust results with regard to migration times and peak areas. However, the achieved separation time of 32 min seems unnecessarily long, because they only used

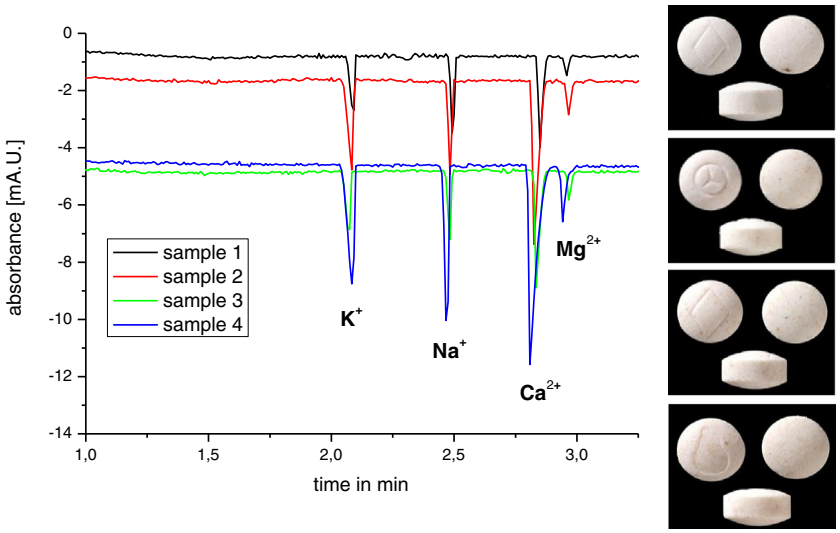

Fig. 10 Cation profiles of four ecstasy tablets with different logos via CE with indirect photometric detection (details of procedure, see Fig. 9)

−12 kV separation high voltage (there is deviation from Ohm's law at higher voltages and thus effects of excessive Joule heating are observed, with resulting peak broadening). Reducing the BGE concentration and/or the use of smaller-inner-diameter capillaries and capillary cooling would have enabled much faster separations.

Fast method development does not always lead to a globally optimized method; instead systematic experimental design strategies have to be applied and performed in a randomized setup to prevent any systematic errors. Design-of-experiment strategies are well suited for CE, because most conditions of the separation technique can easily be varied. Especially, changing buffers can be realized in CE in a short period of time (approx. 90 s for flushing the capillary), because no pump or column must be purged or conditioned as, e.g., in LC. Therefore, a high number of chemometrically optimized analytical methods can be found in literature.

A good and recent example of such a fast and efficient method development with multivariate data analysis, including a comparison to univariately optimized methods, is [14]: this presents the application of a statistical tool using both a Plackett–Burman and a central composite design for the optimization of six conditions for a CE–UV method with preconcentration (large-volume sample-stacking–sweeping) for the analysis of ten common illegal drug substances in urine. The results were compared with those of a method developed according to the standard one-factor-at-a-time approach. The method optimized by the statistical tools was revealed to be superior to the conventional approach in terms of resolution and speed of analysis, also providing a wider linear range for concentration (25–1500 ng mL^{-1} for six benzodiazepines, methamphetamine, and ketamine, and 50–3000 ng mL^{-1} for codeine and morphine). Additionally, the method development was found to be more efficient and less time consuming. The final conditions for the statistically optimized system were: 50 mmol L^{-1} NaH_2PO_4 buffer with 29 % methanol content for the separation (pH 2.3), 10 mmol L^{-1} NaH_2PO_4 added to the sample matrix, and 150 mmol L^{-1} SDS added to the sample. Another highly recommended publication in terms of method development is [162], which uses an experimental design and simplex optimization in an easy-to-follow and well-explained way. The separation of amphetamine, amphetamine derivatives, cocaine, and heroin was optimized (Fig. 11). They also described potential alternatives to their method, e.g. to speed it up when a lower resolution of the analytes is sufficient. The publication includes the validation of the method and the application to street samples. High linearity and low RSDs for peak areas and migration times were obtained.

Another well-presented method development, chemometric investigation, and optimization was performed in [163] for the analysis of amphetamines. By optimizing the analysis time (including shortening of the capillary) while maintaining baseline resolution, they were able to accelerate their separation from a run time of 8 min to a total run time of 1 min, conducting 31 experiments for a full-factorial and a face-centered central-composite design.

Cost-efficiency and green methods

When it comes to the implementation of techniques into routine analysis, it must be certain that said techniques are validated, sensitive, cover a wide concentration range of the analytes, and are cost-effective. The latter is the reason why many excellent methods using, e.g., LC–HRMS–MS or GC–GC–MS are not fit for routine analysis, although their intrinsic instrumental properties are excellently suited for the purpose. Additionally, developing countries might lack the financial

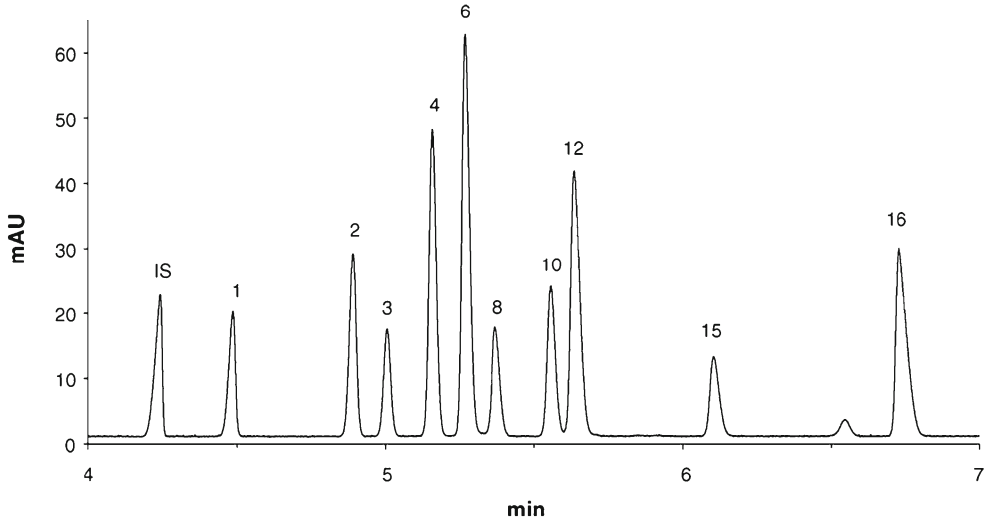

Fig. 11 Analysis of a standard mixture of compounds of forensic interest analyzed using a BGE composed of 30 mmol L^{-1} Tris–phosphate, pH 2.8 in a 45 cm capillary (eff. length: 37 cm, i.d: 50 μm). (i.s.) benzylamine. (*1*) Phenethylamine, (*2*) amphetamine, (*3*) methamphetamine, (*4*) 3,4-methylenedioxyamphetamine, (*6*) 3,4-methylenedioxymethamphetamine, (*8*) ephedrine, (*10*) *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butamine, (*12*) 3,4-methylenedioxyethylamphetamine, (*15*) cocaine, (*16*) heroin (adapted from [162] with permission from Elsevier)

resources to implement expensive high-end equipment, which is not always needed for the analytical task. Also, pure reagents, gases (for example pure hydrogen or helium for GC), or even pure water might not always be available. Usually simple GC–MS would be suitable for most applications, but even a stable electrical supply might not always be present, which is potentially harmful for vacuum pumps. Electromigrative separation techniques are absolutely low-cost techniques with regard to their maintenance and working costs [164], because of the low consumption of chemicals, the need only for typical household electricity, and low demands regarding chemical purity and water purity. All this makes CE the ideal separation technique for developing countries. In this subsection we will present some applications, where CE can be used as a low-cost alternative.

For the analysis and detection of non-UV-active ethyl glucuronide (EtG), a biomarker for recent alcohol consumption often used as diagnostic tool for the screening of blood samples for chronic alcohol abuse, GC–MS or LC–MS(–MS) have been successfully used [165]. Whereas LC–MS instruments can still be regarded as expensive, there are cheap benchtop GC–MS instruments available. However, GC methods usually need derivatization (silylation or perfluoropropionylation) for the analysis of EtG. In contrast with the numerous publications on analyzing EtG by LC–MS, Jung et al. [158] developed CE methods for the analysis of EtG using indirect UV detection (probe ion: nicotinic acid) [166, 167]. An alternative method based on hyphenated ITP–CE was published later [168]. The latest of the methods [158] used commercially available linear polyacrylamide-coated 75 μm capillaries, obtaining an LOD of 0.25 mg L^{-1} and an LOQ of 0.5 mg L^{-1} for EtG after a simple protein precipitation with 200 μL acetonitrile per 100 μL serum as sample preparation (Fig. 12). Although SPE was successfully tested, it was not implemented because it did not significantly improve the results and was more laborious and expensive. The BGE used was composed of 10 mmol L^{-1} nicotinic acid, adjusted to pH 4.65 with ε-amino caproic acid (EACA) (approx. 11 mmol L^{-1}) in a water–acetonitrile mixture (9:1). The method was cross-validated with an enzyme immunoassay, which turned out to be more sensitive, but was also more laborious and required ultrafiltration for sample pre-treatment. In summary, the developed CE method proved to be fast and fit for routine analysis, and was revealed to be robust in terms of precision and accuracy. Compared with the immunoassay, the consumption of chemicals and thus the costs were negligible. However, the authors recommended that questionable results should be confirmed by a more sensitive LC–MS method to avoid false positives.

Very small sample volumes

The use of capillary electrophoresis as separation technique results in the lowest absolute LOD of all routine separation techniques using the same detection strategy. Amounts of only a few femtograms on-column can still be detected and analyzed. The achievable limits of detection in terms of concentration are comparable to HPLC methods, if such detection techniques as MS or LIF are used instead of standard UV-detection, achieving only relatively high limits of detection because of the small optical path length of mostly 50 or 75 μm (Lambert–Beer's law). The work of Rittgen et al. [15] is a very good example of a highly repeatable analysis at extremely low analyte concentration in small sample volumes: they

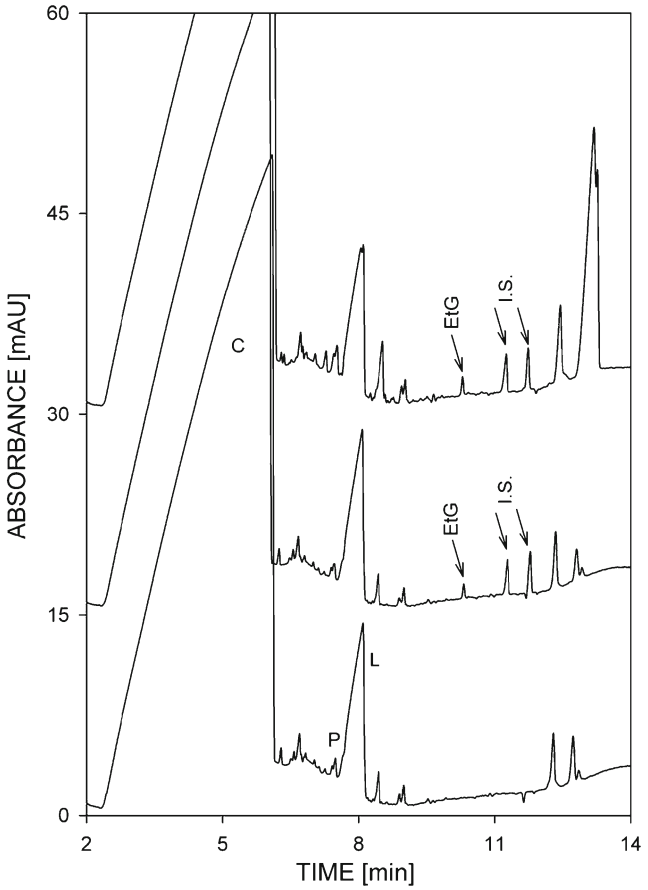

Fig. 12 Electropherogram of sera after protein precipitation. From *bottom* to *top*: blank serum, blank serum spiked with 4 mg L^{-1} ethylglucuronide and 20 mg L^{-1} internal standard and a real sample (determined ethylglucuronide concentration 4.72 mg L^{-1}, internal standard 20 mg L^{-1}). Analysis performed using a linear polyacrylamide-coated capillary (inner diameter: 75 μm, length: 60 cm) with 10 mmol L^{-1} nicotinic acid, EACA and 10 % acetonitrile as BGE at −30 kV (with permission from [158])

developed an NACE-MS method for the trace analysis of fentanyl derivates in seizures, in heroin (where it was used as substitute for or adulterant to heroin to enhance the potency), and from extracts of clothes contaminated with nebulized carfentanil. Although the size of the seizures were only 21 mg, 6.14 mg, and 3.21 mg of heroin, some of which contained only 0.3 ng fentanyl per mg (0.3 ppm), unambiguous identification and quantification was possible by NACE-MS, corroborated by isotope-dilution analysis. Also, *cis* and *trans*-methylfentanyl could be separated and identified. The buffer system used was a mixture of 200 mmol L^{-1} ammonium acetate in glacial acetic acid with acetonitrile (1:9) in a bare-fused-silica capillary. RSD values for migration times were below 0.07 % after correction with two internal standards and, linearity was in the range from 2.53 nmol L^{-1} to 6.33 μmol L^{-1} for carfentanil.

Another remarkable example of the analysis of small amounts of sample is the work of Tagliaro et al. in 1998 [169], who needed only 50–100 mg (approximately 4 cm) hair for a precise analysis of morphine, cocaine, and MDMA, achieving detection limits as low as 2–8 ng mL^{-1} (S/N =5): 0.01 ng mg^{-1} sample (10 ppb). The high sensitivity was achieved with a head-column field-amplified sample-stacking procedure [170, 171], in which analytes are preconcentrated via electrokinetic injection through a plug of 0.1 mmol L^{-1} solution of diluted acid (here, phosphoric acid). Separation was accomplished in a 100 mmol L^{-1} phosphate buffer (pH 2.5). The method was also characterized by a good linearity (0.025–0.5 ng mg^{-1}) and repeatability when internal standards and internal calibration were used. In 2007, the hair analysis using field-amplified sample stacking was transferred to a CE-MS hyphenation [172], thus adding a verification step by MS–MS while achieving similar LOD and LOQ. The high versatility of CE combined with head-column field-amplified sample stacking can also be seen in [27] in the analysis of opioids in bodyfluids (human plasma, serum, and urine) using only 20–100 μL sample volume in total. The article also presents a readily understandable introduction to head-column field-amplified sample stacking, and sums up the crucial factors and conditions for high repeatability and highly efficient online-preconcentration and sample preparation. Standards could be separated within 9 min in a 41 cm (effective length: 22 cm, i.d.: 50 μm) capillary with phosphate (75 mmol L^{-1} Na_2HPO_4 and 25 mmol L^{-1} NaH_2PO_4) in an ethylene glycol–water mixture (6:4 *v*/*v*) (pH 7.9). It was possible to detect opioids at a few ng mL^{-1}; however, the obtained results were strongly dependent on the pH during the extraction procedure, limiting the method to the analysis of closely related analytes and meaning only closely related opioids could be extracted simultaneously.

Immunoassays and immunoaffinity capillary electrophoresis

Immunoassays are usually very easy to use and fast, but also prone to cross-reactivity and high limits of detection. By transferring the assay to a capillary electrophoresis system, these weaknesses can be overcome via the introduction of a separation step to obtain additional selectivity and detect the enzyme and the product separately. CE provides the additional advantage of the absence of a stationary phase, removing possible interference with ligand binding. Furthermore, the implementation of different detection techniques enables low limits of detection and additional selectivity. Immunoaffinity capillary electrophoresis is regarded as an almost ideal enrichment tool for very-low-concentration analytes in complex biomatrices, for example blood, serum, or urine [173].

An early, thoroughly executed investigation of a CE-based immunoassay from 1998 can be found in [174]. They investigated and compared the feasibility of a micellar-electrokinetic-capillary-chromatography-based immunoassay (buffer: 6 mmol L^{-1} sodium tetraborate, 10 mmol L^{-1} disodium

hydrogenphosphate, and 75 mmol L^{-1} SDS, pH ~4.6) and a CE-based immunoassay (buffer: 50 mmol L^{-1} sodium tetraborate) with two previously developed CE–UV methods and a CE-MS method [60, 175] for the unambiguous identification of methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine in urine samples. Both novel electrokinetic-capillary-immunoassay approaches [60, 175] were capable of lowering the limit of detection to 10 ng mL^{-1} without exhaustive sample pretreatment or enrichment, and were judged as suitable for screening purposes. The capillary-zone-electrophoresis-immunoassay approach [60] increased selectivity because it separated the free tracer from the tracer–antibody complex, which comigrated in the micellar-electrokinetic-capillary-chromatography immunoassay. To confirm their findings the authors developed a CE-QqQ-MS method using a simple ammonium acetate buffer (20 mmol L^{-1} ammonium acetate, 20 mmol L^{-1} acetic acid, pH 4.6) and cross-validated it with an LC–MS approach. Even earlier than [174], in 1992 Bao and Regnier [176] transferred a standard immunoassay into a CE format. They combined electrophoretic mixing with the separation of the substrate, the enzyme, and the product. With the high resolution achieved, they obtained limits of detection as low as 46 amol for glucose-6-phosphate dehydrogenase, which was used as model analyte. Later, in 2000, Wey et al. [177] introduced an MEKC and a CE-based immunoassay for the analysis of opiates and metabolites thereof in urine; both were capable of recognizing opiates in urine (10 ng mL^{-1}) but not able to discriminate between different opiates. This was only achieved by a third CE method with MS detection. The importance of separation techniques for immunoassays was revealed during the MEKC-based analysis, in which the authors found out that the commercial fluorescence tracer was actually composed of two different tracers, which reacted competitively. Their final method was as sensitive as the immunoassay for most compounds, but an increase in selectivity by a factor of four was found. Nevertheless, the method could only prove the presence of opioids or codenoids; no quantitative data were obtained. A pure CE-MS method for the analysis of the analytes was developed later, superseding the need for the fluorescence reaction, at the cost of an LOD raised by a factor of four [178].

Orthogonality

"The identification of a substance has to be realized with several analytical techniques, independent from each other" (translation from [179]). This sentence is written in the guidelines for quality assurance in forensic-chemical analysis of medical drugs and controlled substances from the German Society of Toxicological and Forensic Chemistry, which was prepared by a working group of chemists from forensic science institutes of different police offices. It is logical and most reasonable to use a secondary analytical technique with an ideally orthogonal separation or detection principle. Electromigrative separation techniques are excellently suited as a complement to chromatographic techniques, because the sample matrix and solvent are usually compatible with both analytical methods, but the separation principle is entirely different. Indeed, several publications performing cross validation of an electromigrative separation technique with chromatographic methods are available, reporting at least equal performance of the electromigrative compared with the chromatographic techniques. This was shown in 1996 [180] for the analysis of cefotaxime and impurities. An MEKC analysis yielded comparable results to the reference HPLC analysis; consequently, the authors reported MEKC analysis to be a valuable orthogonal separation technique. Shortly after, [181] compared HPLC and CE for the analysis of amphetamines in drug seizures and evaluated the results using a student's paired *t*-test. They found no significant difference between both methods in terms of linearity (0.5–20 μg mL^{-1}), precision, accuracy, limits of detection and quantification (between 130–300 ng mL^{-1}), and speed of analysis (8 min for the compounds of interest). Another example is a CE method with UV-LIF detection for the analysis of tryptamines, which was compared with an HPLC method with DAD detection [90]. Both methods were found to be suitable for the analysis of psychotropic tryptamines, but HPLC proved to be more robust in terms of peak areas, whereas CE, probably somewhat surprisingly, was more stable in migration times. The CE method used a simple 20 mmol L^{-1} NaH_2PO_4, 15 mmol L^{-1} α-cyclodextrin buffer (pH 3) in a 66 cm (46.5 cm effective length) 75 μm bare-fused silica capillary.

Theobald et al. [3] used CE-MS in addition to GC–MS for studies on the metabolism of a new designer drug (4-iodo-2,5-dimethoxy-β-phenethylamine (2C-I)). They could identify most of the metabolites by GC–MS–MS, and proposed a scheme for the metabolism of 2C-I in rats. However, GC–MS failed to verify why the metabolite (2-hydroxy-4-iodo-5-methoxyphenyl)acetic acid was not derivatized in the sample preparation, in contrast with the other isomer. Additionally, it was not clear whether the detected lactone was an artifact of the harsh conditions in the GC-interface or present already in the sample. To solve these questions, CE-MS was used for its ability to separate and detect unstable free carboxylic-acid metabolites, and indeed an *O*-demethyl-deamino-carboxy metabolite was separated from the two isomeric *N*-acetyl-*O*-demethyl metabolites of 2C-I and identified via MS. A simple 20 mmol L^{-1} ammonium formate buffer (pH 10) containing 25 % isopropanol was used in a 75 μm bare-fused-silica capillary.

Electromigrative separation methods themselves have orthogonal separation principles, if e.g. MEKC and CE are used for the analysis of the same sample. Then the same analytical instrumentation can even be used: in 1996 Tagliaro et al. [182] studied the complementary use of two CE and one MEKC methods for the mutual confirmation of results in forensic drug analysis, using Spearman's test and principle component analysis to determine their degree of orthogonality. They found out that a CE method and an MEKC method were of high orthogonality and therefore well suited for comparative analysis, providing a more comprehensive analysis.

Mobility axis

Drift or unnstable migration times may occur in CE for several reasons:

1. changes in the BGE composition (evaporation of organic solvents, pH value changes caused by electrolysis); and
2. matrix effects with matrix or analyte compounds adhering to the capillary surface.

In case 1, both effective electrophoretic mobility of analytes and electroosmotic mobility change, so a more stable BGE (e.g. with better buffering capacity) has to be chosen or better sealing of the BGE vials against evaporation has to be achieved. Likewise, buffer-exchange or replenishment procedures may be used. In case 2, only the EOF velocity may be affected. Rinsing procedures may be implemented, or the use of high-ionic-strength buffers, addition of modifiers (organic solvents, detergents), or the use of capillary coatings may be helpful. Coatings may also aid in obtaining more stable migration times for BGEs of intermediate pH values (4–7), which are critical because they lie in the range of the pK_a values of the silanol groups of the capillary surface. This reveals the steepest dependence of the electroosmotic mobility on pH in this range, which can be circumvented by changing the capillary surface. When only the EOF is affected, repeatability problems can be minimized by avoiding compounds adsorbing onto the capillary surface, e.g. by increased ionic strength, addition of organic solvents or detergents, or shielding the silanol surface by capillary coatings. However, coatings cannot be used in all approaches, e.g. they are mostly not resistant to high contents of organic solvents in the BGE. Nevertheless, high repeatability can be achieved, because the analyte's effective electrophoretic mobility is a physicochemical property of the analyte in the given BGE and is therefore constant when the BGE composition does not change. A transformation of the time axis into an effective-electrophoretic-mobility axis effectively eliminates detected migration-time differences caused by EOF changes between runs. In some cases improved repeatability for quantitative results was also observed, as reported by Schmitt-Kopplin [183, 184]. The transformation can be described by Eq. 2 or Eq. 3, either using the EOF or an internal standard as reference point.

$$\mu_{eff} = \frac{L_d L_t (t_{EOF} - t_m)}{U t_m t_{EOF}} \tag{2}$$

$$\mu_{eff} = \mu_{\mathrm{int}} + \frac{L_d L_t (t_m - t_{\mathrm{int}})}{U t_m t_{\mathrm{int}}} \tag{3}$$

Equations 2 and 3 for the transformation of a time scale into a mobility scale, with μ_{eff} being the effective electrophoretic mobility, L_{d} being the capillary length to the detection system, L_{t} being the total capillary length, t_{EOF} being the migration time of the electroosmotic flow, t_{m} being the measured migration time, U being the applied voltage, and t_{int} being the migration time of the internal standard.

This transformation is also very useful for the alignment of electropherograms, when signals from different detection systems have to be evaluated, for example UV, LIF, and MS signals [129], that are necessarily acquired in different segments of the capillary (e.g. UV-detection on-column at ca. 8 cm from the capillary outlet, and MS-detection post-column resulting from the ESI-sprayer geometry). Unfortunately, this technique has attracted little attention in general and even less in the field of forensic science, even though the transformation is often incorporated into the data-handling software of the commercial instruments. As far as we are aware, only one article with a forensic background using a mobility axis is available [129]. The alignment of the signal of an LIF-detection system and a UV-detection system enabled the identification and quantification of minor and major sassafras-oil constituents within one run. For the sake of completeness, [185] is worth mentioning here because it features a freely available software package, which can be used to align an unlimited number of CE-MS electropherograms with each other even if they suffer from non-linear shifts in the migration times. This approach can also correct for differences in μ_{eff}; however, mass spectrometric detection is required.

Potential for on-site screening instruments

In today's globalized world, it is often financially inefficient to transport samples to a lab for routine analysis, for example from such a logistic high-throughput location as an airport or a port. If the sample is perishable it would also be beneficial to have on-site analytics. At a crime scene, on-site analytics could provide the investigator with "useful intelligence at an early stage in a criminal investigation" [58], and can also be very important for self-protection, e.g. during scene-of-crime

work in a clandestine laboratory for illicit drugs or even improvised explosives. At the moment, there are only a few handheld or easily transportable analytical instruments available, usually having a narrow range of applications or being optimized for a specific compound. However, within the general trend for lab-on-a-chip analytics and portable devices, electrophoresis has outstanding properties within all the techniques of separation science. This is because of the ease of its miniaturization, especially when using C4D, and is only outmatched by ion-mobility spectrometry or thin-layer chromatography. A more detailed discussion of portable CE instruments can be found in the very vivid review by Lewis et al. [186].

Wallenborg et al. [187] developed a method using 4-fluoro-7-nitrobenzofurazane for a very fast on-chip derivatization (3–5 min) to enable the chiral separation of amphetamine and related compounds by chip electrophoresis with LIF detection, with the potential to be used in a portable system. A 50 mmol L^{-1} phosphate buffer (pH 7.35) with 10 mmol L^{-1} highly sulfated γ-cyclodextrins and an SDS concentration of 1.5 mmol L^{-1} (probably inducing MEKC-based separation, because the CMC is very low at this high ionic strength) was used. Six of the seven investigated chiral compounds were baseline resolved within 7 min in a 160 mm separation channel.

In summary, one can conclude that the possibilities of electromigrative separation techniques in mobile devices are currently being more deeply investigated and the technical implementation is on-going for drug analysis. In different fields, e.g. in the analysis of explosives and small organic compounds, mobile [188] and even remotely controlled small analytical techniques are already available [189]. The presented methods reveal the high potential of portable devices in the field of forensics, and we are sure that as miniaturization proceeds, small, portable (chip)–CE instruments will become available.

Regarding future possibilities, several recent publications can be mentioned which feature methods that can easily be transferred to a mobile technique or handheld instrument. Blanco et al. [190], for example, developed a very fast (run time: 55 s) and reliable (RSD of the migration time for ten analytes in 240 injections: 0.61–2.07 %) method and instrumental setup for the analysis of 10 inorganic anions in, e.g., soil, which are relevant for pre and postblast analysis. A short capillary (35 cm long, 25 μm inner diameter), coated with hexadimethrine bromide was used with a buffer consisting of 50 mmol L^{-1} Tris, 50 mmol L^{-1} CHES, and 0.05 % poly(ethyleneimine) to ensure high repeatability. A C4D-system was used. Sarazin et al. [191, 192] developed methods for the analysis of ten inorganic anions well separated from seven interfering anions and carbohydrates, with the intention to transfer these methods to portable devices for postblast analysis.

A very well validated and optimized approach can be found in the work of Kobrin et al. [193], who used a purpose-built, portable CE instrument for the postblast analysis of residues from improvised explosives and commercial organic explosives from a variety of matrices within 4 min. By injecting the sample from both ends of the capillary, they were capable of analyzing anions and cations in one run, using a C4D as universal detection system. The instrument was equipped with a bare-fused-silica capillary (length: 50 cm, i.d.: 50 μm), and a mixture of 20 mmol L^{-1} MES, 20 mmol L^{-1} HIS, 30 μmol L^{-1} CTAB, and 2 mmol L^{-1} 18-crown-6 was used as BGE. Low limits of detection (lowest 3.4 μmol L^{-1} for K^+ up to 35.7 μmol L^{-1} for Cl^-) were found. A principle component analysis enabled the identification of the specific explosive.

Limitations

Some limitations of CE methods are summarized here. First, the loadability in CE is limited compared with liquid chromatography. Only a few nanoliters can be injected, which is only partially counteracted by the higher separation efficiency. Detection limits are thus usually higher than those of standard chromatography. Usually the loadability can easily be enhanced by a variety of preconcentration techniques: simple stacking, when the sample has a lower conductivity than the buffer, or transient ITP, where a transient leader (e.g. sodium or ammonium and chloride) is added to the sample. For well-defined sample matrices, e.g. in organic extracts, electrokinetic injection may be used, producing impressive detection limits [194]. Other preconcentration techniques may be used by more experienced users.

Intermediate precision can be affected by matrix components, which adsorb onto the capillary surface. Because this will affect the EOF, changes in migration times will occur, often with drift phenomena. Rinsing procedures, a mobility axis or coatings, or a more sophisticated sample cleanup may be used. A general disadvantage of commercially available CE instruments is the inherently limited precision of the hydrodynamic or electrokinetic sample-injection step compared with the highly reproducible sample injection via loops in HPLC instruments. However, this limitation is not overly problematic because the insufficient precision of the CE sample injection step can be easily corrected by normalization of analyte peak areas to a suitable internal standard. Modern CE equipment reaches similar quantitative precision as for HPLC.

When transferring CE–UV to CE-MS methods, one has to consider that the outlet vial is replaced by the sheath-liquid interface. The sheath-liquid composition is not the same as that of the BGE, and consequently ions from the sheath liquid may travel into the capillary and affect the separation [195]. Another aspect that has to be addressed is the unavoidable loss in separation efficiency because of a parabolic-flow

component caused by the impact of the sheath-liquid at the capillary end, which superimposes the plug-like flow of the CE separation and contributes to peak broadening.

With MS detection, the choice of buffer additives is limited, which is especially critical for MEKC. APCI as an alternative ionization method or partial-filling techniques may be used.

Quick starting guide for method development in capillary electrophoresis

Because the intention of this review is to introduce the reader to the wide applicability of electromigrative separation techniques, the authors want to provide some useful standard buffer compositions as starting points for further method development, suitable for a wide range of analytes (Table 2). For an easily comprehensible presentation, we have tried to structure the choice for a specific method and buffer composition in a table, including simple remarks to assist with decision making based on our own experience and publications in the field. A relatively complex flow chart derived from the principles of MEKC can be found in [122] for decision making in MEKC separation optimization.

Of course, our table does not include all possible combinations or approaches, nor does it guarantee that the chosen conditions will be applicable in all cases, however, we hope that this guideline will help inexperienced operators to quickly reach a sound working base.

For neutral compounds, we recommend the use of MEKC if MS detection is not required. Otherwise CEC may be used, although additional equipment is necessary. For relatively polar compounds, buffer System 1 is a good starting point. For SDS as the most common micellar phase and for all detergents, a change of the phase ratio between stationary and mobile phase for tuning of the retention factors is possible just by changing the concentration of the detergent in the BGE. This may also serve as a good tool for the optimization of the overall analysis time. If the analytes are detected too close to, or even together with, the micelle marker, the addition of acetonitrile or methanol (up to 30 %*v*/*v*) can reduce the polarity of the mobile phase. For compounds with an octanol–water-partition coefficient of approximately 4–5, the combination of acetonitrile and urea is a good compromise (see also [196]). Even more hydrophobic compounds can be well separated by use of MEEKC with the "traditional" buffer System 2. Selectivity tuning can easily be achieved using different detergents, the choice of which can be made on the basis of the analyte characteristics as described in the section "Universal separations modes: MEKC and MEEKC".

With MEKC, mixtures of neutral and charged compounds (both anions and cations) can also be separated; the chromatographic distribution of the charged compounds between the phases is then overlaid by their migration in the electric field. Care has to be taken with the choice of the detergent, which may have like or unlike charge and thus induce coulombic attraction or repulsion, respectively; likewise, very high or low retention factors may be obtained. In the authors' opinion, MEKC will achieve the separation of any pair of analytes because the number of possible optimization conditions is impressive. Mostly, however, changing one condition will affect many entities relevant for the separation, including resolution, migration time window, overall analysis time, and selectivity. But because of its impressive separation efficiency, normally a fast method development can be achieved. MEKC is ideal for the separation of structural isomers. Chiral separation may also be achieved using micelles with chiral head groups or via the inclusion of secondary complexation equilibria using cyclodextrins.

For the CE separations of charged compounds a large number of buffers have been used, with borate and phosphate being most often used and also among those with highest robustness (System 3). Other frequently encountered buffering compounds, with their corresponding pH ranges, are: phosphate (pH 1.2–3.2 and pH 6.2–8.2), ammonium acetate (pH 3.8–5.8), borate (pH 8.1–10.1), HCO_3^- and/or CO_3^{2-} (pH 5.4–7.4), Tris, glutamic acid (pH 7.3–9.3), MES (pH 5.2–7.2), HEPES (pH 6.6–8.6), and Tricin (pH 7.2–9.2); see [197] for further reading. In general, a good separation is achieved when the pH of the BGE is close to the pK_a or pI value of the analytes (to maintain a sufficient effective electrophoretic mobility, we recommend a pH not more than 0.8 pH units away from the pI or pK_a). Beside adjusting the pH, which is usually the first tuning condition, selectivity changes may be introduced using a large variety of additives, predominantly organic solvents (water-soluble ones including acetonitrile, simple alcohols, and formamide), complexing agents (trifluoro acetic acid, EDTA, etc.), chiral selectors (mostly α, β, or γ-cyclodextrins or modified versions of them, including charged (mostly highly sulfated) cyclodextrins). We recommend 10 mmol L^{-1} solutions of the BGE as a compromise regarding ionic strength for good separations and reduced adsorption to the capillary wall vs. problems with Joule heating. With these buffers, high voltages (+/−30 kV) can normally be used for fast analysis. The use of coatings is only recommended:

1. when required against adsorption;
2. for the analysis of fast anions;
3. for BGE systems with a pH of ~5–6 (the range of the pK_a of the silanol groups of the capillary surface); or
4. in rare cases with very complex separation problems, for resolution optimization via the electroosmotic flow velocity (see also: [198]).

Table 2 Common standard conditions as starting points for method development in electromigrative separation techniques

BGE systems and analytes	Separation technique	Buffer for the first experiments	Possible modifications	Detection modes
System 1 (neutral and charged analytes of low-to-medium hydrophobicity)	MEKC	80 mmol L^{-1} SDS, 8.5 mmol L^{-1} borate, 8.5 mmol L^{-1} phosphate buffer (pH 8.5) with 15 % acetonitrile	Type and concentration of detergent; organic solvents, urea, cyclodextrins; pH for charged analytes	UV, LIF
System 2 (neutral compounds of high hydrophobicity)	MEEKC	3.3 % SDS, 1.5 % *n*-hexane, 6.6 % *1*-butanol in 8.85 mmol L^{-1} sodium tetraborate solution (pH 9.5)	SDS concentration, concentration of *1*-butanol, pH	UV, LIF
System 3 (cations and anions)	CE	See buffering ions in text	pH, organic solvents, complexing agents, cyclodextrins	UV, LIF, MS
System 4 (cations, especially alkaloids)	NACE	35 mmol L^{-1} ammonium acetate in acetonitrile methanol (8:2) or 60 mmol L^{-1} ammonium formate and 1 mol L^{-1} acetic acid in acetonitrile	Variation of solvent ratios, of ammonium acetate concentration, and of pH	UV, LIF, MS
System 5 (cationic analytes)	ITP	LE: 5–10 mmol L^{-1} ammonium or potassium, pH 3 for analytes to be well charged (using e.g. acetic acid) TE: Tris or H^+ (diluted counterion, e.g. 20 mmol L^{-1} acetic acid) counterion: 10 mmol L^{-1} β-alanine	pH via counterion, addition of organic solvents, complexing agents	C4D, UV
System 6 (anionic analytes)	ITP	LE: 5–10 mmol L^{-1} chloride salt TE: borate or OH^- (diluted counterion solution (basic) counterion: e.g. Tris	pH via counterion, addition of organic solvents, complexing agents	C4D, UV
System 7 (absorbing and non-UV-absorbing anions)	CE	7 mmol L^{-1} sorbic acid–15 mmol L^{-1} arginine; dynamic coating with 0.001 % hexadimethrin hydroxide (Polybrene), pH 9.0 at +30 kV	Type and concentration of buffer; pH, organic solvents, complexing agents	C4D
System 8 (absorbing and non-UV-absorbing cations)	CE	25 mmol L^{-1} MES–25 mmol L^{-1} histidine with 1 mmol L^{-1} 18-crown-6-tetracarboxlic acid (for the separation of NH_4^+ and K^+), pH 6.1	Type and concentration of buffer; pH, organic solvents, complexing agents	C4D
System 9 (non-UV-absorbing anions)	CE	Anions (bromide, chloride, nitrate, sulfate, phosphate, internal standard azide): 2.25 mmol L^{-1} sodium hydroxide, 6.5 mmol L^{-1} benzene-1,2,4,5-tetracarboxylic acid (pyromellitic acid), 1.6 mmol L^{-1} triethanolamine, 0.75 mmol L^{-1} hexamethonium hydroxide solution (as a dynamic coating for EOF reversal), pH 7.7 (with NaOH); −28 kV, UV detection at 254 nm	Type and concentration of probe ion; pH, organic solvents, complexing agents	Indirect UV
System 10 (non-UV-absorbing cations)	CE	Cations (ammonia, potassium, sodium, calcium, magnesium, lithium, methyl amine): 10 mmol L^{-1} imidazole, 2 mmol L^{-1} crown ether 18-crown-6-tetracarboxylic acid (for the separation of NH_4^+ and K^+), adjusted to pH 4.0 with sulfuric acid; +30 kV; UV detection at 214 nm	Type and concentration of probe ion; pH, organic solvents, complexing agents	Indirect UV

In many cases, for basic analytes, NACE may give rise to reduced adsorption phenomena and very high separation selectivity for structural isomers and diastereomers. Two BGE systems are well known and often used (System 4). Optimization can be achieved by changing the solvent ratios and the concentration of the electrolyte [20, 30] and by choosing strong ion-pairing counterions.

Strong bases or permanent cations (quaternary ammonium compounds) normally give rise to very fast separations. The effective electrophoretic mobility of analytes, and thus the selectivity, does not change upon changes in pH of the BGE because full ionization is always present, especially with quaternary ammonium compounds. However, resolution also depends on the electroosmotic flow velocity [198, 199], which can be tuned by pH. The closer the effective electrophoretic mobility of analytes to the electroosmotic mobility, the higher the resolution. Because the coelectroosmotic migration is considered here, a reduction in EOF will be advantageous.

For the separation of weak bases the pH of the BGE must be adjusted to the pK_a or pI of the analytes, with good selectivity often obtained close to these values. However, care must be taken in an intermediate pH range, where coatings may help to increase migration-time repeatability (see the section "Mobility axis"). Good selectivity may often also be obtained for analytes which are (almost) fully charged (thus at very low pH values) with size-based separation. Here, selectivity tuning using organic solvents or complexing agents is possible.

Strong acids and inorganic anions must be classified on the basis of their effective electrophoretic mobility, because different EOF requirements prevail. Very fast anions overcome the EOF, and separation may be performed at reversed polarity (in CE-MS, coatings are required because the EOF must be towards the MS inlet). Problems occur when the analytes' effective electrophoretic mobility is similar to the electroosmotic mobility. Very high resolution may be obtained, but so may too-high analysis times. Coatings are recommended, e.g. via a sulfonation of the capillary surface to obtain a fast, pH-independent EOF.

Weak acids (normally with a low effective electrophoretic mobility) can be characterized in a similar manner to weak bases with all optimization strategies discussed above. Normal polarity can be used because these analytes are effectively transported to the detector by the EOF, which is high at this pH value, as is necessary for effective ionization. Because of counterelectroosmotic migration, resolution is usually relatively high.

For ITP, we recommend starting with a fast leading combined with a slow termination ion. Usually chloride–borate or hydroxide ions (dilute base) (LE–TE) and potassium or ammonium–Tris or H^+ ions (dilute acid) (LE–TE) are good choices to provide a very wide mobility window (see Systems 5 and 6). During optimization, this window may be reduced to speed up the analysis or to use leaders slower than common salts to cut off the sample matrix with high loads of chloride and sodium ions. For transient ITP as a preconcentration technique, it is simplest to add sodium or ammonium ions to the sample for cation analysis or chloride for anion analysis. These compounds then migrate as transient leaders. Often, some preconcentration is automatically obtained as a result of sample-induced transient ITP, because most samples contain substantial amounts of these ions anyhow. The optimization of selectivity proceeds as for CE for an increase of differences in the effective electrophoretic mobility. In literature there is a focus on complexing agents.

Special buffer system with a focus on detection methods

MS detection requires volatile BGEs. Suitable buffer components include acetic acid (pK_a 4.76), formic acid (pK_a 3.77), carbonic acid (pK_a 6.35, 10.33), citric acid (pK_a 3.09, 4.75, 5.41), and ammonia (pK_a 9.21), and sometimes ε-aminocaproic acid (EACA) (pK_a 4.43, 10.75) for anion analysis. Addition of organic solvents to the BGE normally facilitates the electrospray as a result of reduced surface tension. Also, additives should be volatile, although many publications also apply semi-volatile separation-enhancing substances, for example ion-pairing reagents (e.g. trifluoroacetic acid) or cyclodextrins (preferably hydroxylated ones). Partial-filling techniques may be used, although their use should be restricted to more experienced users. Many coating agents (predominantly physically adsorbed coatings or covalently attached coatings) are suitable for use to adjust the EOF. Details on the choice of coatings for CE-MS can be found elsewhere [198].

When laser-induced-fluorescence (LIF) detection is used, especially when using a UV laser (e.g. frequency-quadrupled Nd:YAG-Laser emitting at 266 nm) for the analysis of native fluorophors, special care has to be taken regarding the quality and the chemical purity of the buffer compounds, especially concerning such additives as surfactants, EOF modifiers, chiral selectors, and coating polymers which sometimes contain impurities that are natively fluorescent at UV excitation, resulting in disturbed base-lines and artifact peaks. MEKC buffers should be thoroughly and frequently filtered to avoid generation of excessive stray light.

C4D measures the differences between sample and BGE conductivity and thus relies on the differences in the effective electrophoretic mobility of the analyte ion and of its coion. In theory, the use of either high or low-conductivity buffers is possible, although to reduce Joule heating effects low-conductivity buffers are usually recommended. So-called Good buffers are usually suitable for use, because they have a high buffering capacity but a low specific conductivity: e.g. MES, HEPES, Tricin, histidine, and others. For small

inorganic and organic anions and cations, buffer Systems 7 and 8 may be suitable as a starting point.

Indirect UV detection of non-UV-absorbing analytes requires the addition of probe ions at a concentration of typically 5–10 mmol L^{-1} to the BGE, which should result in an overall low conductivity to achieve an effective displacement of the analyte. A compromise has to be found on low ionic strength vs. high buffering capacity and detection limits. The probe ions' effective electrophoretic mobility should match well with the analytes' effective electrophoretic mobility to avoid band broadening with typical saw-like peak shapes caused by electrodispersion. Probe ions often used are: a) anion separation: chromate (−0.80 cm^2 V s^{-1}, UV range 250–280 nm), sorbic acid (−0.24 cm^2 V s^{-1}, UV range <290 nm), dinitrobenzoic acid (−0.22 cm^2 V s^{-1}, UV range <260 nm), naphthylsulfonic acid (−0.16 cm^2 V s^{-1}, UV range 200–225 nm); b) cation separation: histamine (+0.53 cm^2 V s^{-1}, UV range <220 nm), imidazole (+0.46 cm^2 V s^{-1}, UV range <220 nm), 9-aminoacridin (0.42 cm^2 V s^{-1}, UV range 250–265 nm), 1-aminonaphthalin (+0.17 cm^2 V s^{-1}, UV range ~300 nm). Buffer Systems 9 and 10, as used in the German Federal Criminal Police Office for the analysis of small inorganic and organic anions and cations, may serve as a starting point (Fig. 10). Optimization may include changes of the pH, addition of organic solvents, and selecting the type and concentration of the probe ion. Indirect fluorescence detection is hardly used, and despite more expensive equipment is not advantageous with regard to the high noise at higher fluorescence signals.

Conclusion and outlook

From the preceding sections, it should be obvious that electromigrative separation techniques are capable of having a greater function in forensic science and forensic toxicology than they currently do, especially with regard to the potential use of CE for forensics in developing countries (discussed in the section "Cost efficiency and green methods") and for on-site screening. All points of criticism which are still associated with this technique can be regarded as outdated: many publications have revealed robustness and well-validated methods, with high accuracy, repeatability, and separation efficiency. The ease of method development, adjustment to new questions, and orthogonality are other aspects to be considered. We hope that this review will aid separation scientists who want an introduction to electromigrative separation techniques in forensic science, and enable an easy beginning for application of CE techniques, by providing appropriate literature examples and a simplified starting guide.

There is an ongoing discussion in Germany as to whether doping, especially the distribution of doping agents, will be regarded as a crime in the near future, thus further extending the large variety of analytes to deal with: from steroid solutions to complex glycoproteins including erythropoietin (EPO). Doping is already regarded as a crime in several European countries. Because electromigrative separation techniques have attracted a lot of attention in peptide and protein analysis lately and are among the top analytical techniques in biopharma, we are sure that they will be found in more and more forensic laboratories in the future.

Acknowledgments We thank the Helmholtz Initiative and Networking Fund for financial support. This article was funded within the program "Forschung für die zivile Sicherheit" of the Federal Ministry of Education and Research (BMBF; 13 N12012). C.H. also thanks the German Excellence Initiative commissioned by the German Research Foundation (DFG).

References

1. Favretto D, Pascali JP, Tagliaro F (2013) New challenges and innovation in forensic toxicology: focus on the 'New psychoactive substances'. J Chromatogr A 1287:84–95
2. Rosenbaum C, Carreiro S, Babu K (2012) Here today, gone tomorrow…and back again? A review of herbal marijuana alternatives (k2, spice), synthetic cathinones (bath salts), kratom, salvia divinorum, methoxetamine, and piperazines. J Med Toxicol 8(1): 15–32
3. Theobald DS, Pütz M, Schneider E, Maurer HH (2006) New designer drug 4-iodo-2,5-dimethoxy-beta-phenethylamine (2C-I): studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric and capillary electrophoretic/mass spectrometric techniques. J Mass Spectrom 41(7):872–886
4. Chee GL, Wan TSM (1993) Reproducible and high-speed separation of basic drugs by capillary zone electrophoresis. J Chromatogr B 612(1):172–177
5. Mohr S, Pilaj S, Schmid MG (2012) Chiral separation of cathinone derivatives used as recreational drugs by cyclodextrin-modified capillary electrophoresis. Electrophoresis 33(11):1624–1630
6. Weinberger R, Lurie IS (1991) Micellar electrokinetic capillary chromatography of illicit drug substances. Anal Chem 63(8):823–827
7. Iwamuro Y, Iio-Ishimaru R, Chinaka S, Takayama N, Kodama S, Hayakawa K (2010) Reproducible chiral capillary electrophoresis of methamphetamine and its related compounds using a chemically modified capillary having diol groups. Forensic Toxicol 28(1):19–24
8. Iio R, Chinaka S, Tanaka S, Takayamaa N, Hayakawab K (2003) Simultaneous chiral determination of methamphetamine and its metabolites in urine by capillary electrophoresis-mass spectrometry. Analyst 128:646–650
9. Iio R, Chinaka S, Takayama N, Hayakawa K (2005) Simultaneous chiral analysis of methamphetamine and related compounds by capillary electrophoresis/mass spectrometry using anionic cyclodextrin. Anal Sci 21(1):15–19
10. Iwata YT, Kanamori T, Ohmae Y, Tsujikawa K, Inoue H, Kishi T (2003) Chiral analysis of amphetamine-type stimulants using reversed-polarity capillary electrophoresis/positive ion electrospray ionization tandem mass spectrometry. Electrophoresis 24(11):1770–1776
11. Iio R, Chinaka S, Takayama N, Hayakawa K (2005) Simultaneous chiral analysis of methamphetamine and its metabolites by capillary

electrophoresis/mass spectrometry with direct injection of urine. J Health Sci 51(6):693–701
12. Liotta E, Gottardo R, Seri C, Rimondo C, Miksik I, Serpelloni G, Tagliaro F (2012) Rapid analysis of caffeine in "smart drugs" and "energy drinks" by microemulsion electrokinetic chromatography (MEEKC). Forensic Sci Int 220(1–3):279–283
13. Lurie IS, Meyers RP, Conver TS (1998) Capillary electrochromatography of cannabinoids. Anal Chem 70(15): 3255–3260
14. Ho Y-H, Wang C-C, Hsiao Y-T, Ko W-K, Wu S-M (2013) Analysis of ten abused drugs in urine by large volume sample stacking–sweeping capillary electrophoresis with an experimental design strategy. J Chromatogr A 1295:136–141
15. Rittgen J, Pütz M, Zimmermann R (2012) Identification of fentanyl derivatives at trace levels with nonaqueous capillary electrophoresis-electrospray-tandem mass spectrometry (MSn, n = 2, 3): Analytical method and forensic applications. Electrophoresis 33(11):1595–1605
16. Pütz M, Martin N Application of CE-ESI-MS in forensic toxicology: Identification of piperazine-derived designer drugs in Ecstasy tablets and of food colorants in illicit drugs. In: Pragst F, Aderjan R (eds) XV. GTFCh-Symposium 2007 Aktuelle Beiträge zur Forensischen und Klinischen Toxikologie, Bad Vibel, 2008. pp 487–501
17. Lurie IS, Hays PA, Parker K (2004) Capillary electrophoresis analysis of a wide variety of seized drugs using the same capillary with dynamic coatings. Electrophoresis 25(10–11):1580–1591
18. Lurie IS, Hays PA, Garcia AE, Panicker S (2004) Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis. J Chromatogr A 1034(1–2):227–235
19. Wen T, Zhao X, Luo G, Wang J, Wang Y, Yao B, Zhao J, Zhu J, Yu Z (2007) Comparison of microemulsion electrokinetic chromatography and solvent modified micellar electrokinetic chromatography on rapid separation of heroin, amphetamine and their basic impurities. Talanta 71(2):854–860
20. Posch TN, Müller A, Schulz W, Pütz M, Huhn C (2012) Implementation of a design of experiments to study the influence of the background electrolyte on separation and detection in nonaqueous capillary electrophoresis–mass spectrometry. Electrophoresis 33(4):583–598
21. Lurie IS, Panicker S, Hays PA, Garcia AD, Geer BL (2003) Use of dynamically coated capillaries with added cyclodextrins for the analysis of opium using capillary electrophoresis. J Chromatogr A 984(1):109–120
22. Meng L, Wang B, Luo F, Shen G, Wang Z, Guo M (2011) Application of dispersive liquid–liquid microextraction and CE with UV detection for the chiral separation and determination of the multiple illicit drugs on forensic samples. Forensic Sci Int 209(1–3):42–47
23. Lim J-T, Zare RN, Bailey CG, Rakestraw DJ, Yan C (2000) Separation of related opiate compounds using capillary electrochromatography. Electrophoresis 21(4):737–742
24. Aturki Z, D'Orazio G, Rocco A, Bortolotti F, Gottardo R, Tagliaro F, Fanali S (2010) CEC-ESI ion trap MS of multiple drugs of abuse. Electrophoresis 31(7):1256–1263
25. Aturki Z, D'Orazio G, Fanali S, Rocco A, Bortolotti F, Gottardo R, Tagliaro F (2009) Capillary electrochromatographic separation of illicit drugs employing a cyano stationary phase. J Chromatogr A 1216(17):3652–3659
26. Kohler I, Schappler J, Rudaz S (2013) Highly sensitive capillary electrophoresis-mass spectrometry for rapid screening and accurate quantitation of drugs of abuse in urine. Anal Chim Acta 780:101–109
27. Wey AB, Zhang C-X, Thormann W (1999) Head-column field-amplified sample stacking in binary system capillary electrophoresis: preparation of extracts for determination of opioids in microliter amounts of body fluids. J Chromatogr A 853(1–2):95–106
28. Lurie IS, Jason Bethea M, McKibben TD, Hays PA, Pellegrini P, Sahai R, Garcia AD, Weinberger R (2001) Use of dynamically coated capillaries for the routine analysis of methamphetamine, amphetamine, MDA, MDMA, MDEA, and cocaine using capillary electrophoresis. J Forensic Sci 46(5):1025–1032
29. Unger M, Stöckigt D, Belder D, Stöckigt J (1997) General approach for the analysis of various alkaloid classes using capillary electrophoresis and capillary electrophoresis-mass spectrometry. J Chromatogr A 767(1–2):263–276
30. Posch TN, Martin N, Pütz M, Huhn C (2012) Nonaqueous capillary electrophoresis–mass spectrometry: a versatile, straightforward tool for the analysis of alkaloids from psychoactive plant extracts. Electrophoresis 33(11):1557–1566
31. Lurie IS, Conver TS, Ford VL (1998) Simultaneous separation of acidic, basic, and neutral organic compounds, including strong and moderate acids and bases, by capillary electrochromatography. Anal Chem 70(21):4563–4569
32. Tagliaro F, Bortolotti F (2006) Recent advances in the applications of CE to forensic sciences (2001–2004). Electrophoresis 27(1):231–243
33. Tagliaro F, Bortolotti F (2008) Recent advances in the applications of CE to forensic sciences (2005–2007). Electrophoresis 29(1):260–268
34. Tagliaro F, Pascali J, Fanigliulo A, Bortolotti F (2010) Recent advances in the application of CE to forensic sciences: a update over years 2007–2009. Electrophoresis 31(1):251–259
35. Pascali JP, Bortolotti F, Tagliaro F (2012) Recent advances in the application of CE to forensic sciences, an update over years 2009–2011. Electrophoresis 33(1):117–126
36. Riekkola M-L, Jönsson JÅ, Smith RM (2004) Terminology for analytical capillary electromigration techniques. Pure Appl Chem 76(2):443–451
37. Tagliaro F, Manetto G, Crivellente F, Smith FP (1998) A brief introduction to capillary electrophoresis. Forensic Sci Int 92(2–3): 75–88
38. Lauer HH, Rozing G (eds) High Performance Capillary Electrophoresis - A Primer. Agilent Technologies
39. Engelhardt H, Beck W, Schmitt T, Gutnikov G (1996) Capillary electrophoresis: Methods and potentials. Vieweg, Wiesbaden
40. Tagliaro F, Smith FP (1996) Forensic capillary electrophoresis. TrAC Trends Anal Chem 15(10):513–525
41. Tagliaro F, Bortolotti F, Pascali J (2007) Current role of capillary electrophoretic/electrokinetic techniques in forensic toxicology. Anal Bioanal Chem 388(7):1359–1364
42. Cruces-Blanco C, García-Campaña AM (2012) Capillary electrophoresis for the analysis of drugs of abuse in biological specimens of forensic interest. TrAC Trends Anal Chem 31:85–95
43. Smyth WF, Brooks P (2004) A critical evaluation of high performance liquid chromatography-electrospray ionisation-mass spectrometry and capillary electrophoresis- electrospray-mass spectrometry for the detection and determination of small molecules of significance in clinical and forensic science. Electrophoresis 25(10–11):1413–1446
44. Fanigliulo A, Bortolotti F, Pascali J, Tagliaro F (2008) Chapter 15 Forensic toxicological screening with capillary electrophoresis and related techniques. In: Bogusz MJ (ed) Handbook of analytical separations, vol 6. Elsevier Science B.V, Amsterdam, pp 513–534
45. Petersen JR, Okorodudu AO, Mohammad A, Payne DA (2003) Capillary electrophoresis and its application in the clinical laboratory. Clin Chim Acta 330(1–2):1–30
46. Smyth WF, McClean S (1998) A critical evaluation of the application of capillary electrophoresis to the detection and determination

of 1,4-benzodiazepine tranquilizers in formulations and body materials. Electrophoresis 19(16–17):2870–2882
47. Smyth WF (2006) Recent applications of capillary electrophoresis-electrospray ionisation-mass spectrometry in drug analysis. Electrophoresis 27(11):2051–2062
48. Smyth WF (2005) Recent applications of capillary electrophoresis-electrospray ionisation-mass spectrometry in drug analysis. Electrophoresis 26(7–8):1334–1357
49. Northrop DM, McCord B, Butler JM (1994) Forensic applications of capillary electrophoresis. J Capillary Electrophor 001(2):158–168
50. Bjørnsdottir I, Tjørnelund J, Hansen SH (1998) Nonaqueous capillary electrophoresis - its applicability in the analysis of food, pharmaceuticals and biological fluids. Electrophoresis 19(12):2179–2186
51. Lurie IS (1998) Capillary electrophoresis of illicit drug seizures. Forensic Sci Int 92(2–3):125–136
52. von Heeren F, Thormann W (1997) Capillary electrophoresis in clinical and forensic analysis. Electrophoresis 18(12–13):2415–2426
53. Lemos NP, Bortolotti F, Manetto G, Anderson RA, Cittadini F, Tagliaro F (2001) Capillary electrophoresis: a new tool in forensic medicine and science. Sci Justice 41(3):203–210
54. Deyl Z, Mikik I, Tagliaro F (1998) Advances in capillary electrophoresis. Forensic Sci Int 92(2–3):89–124
55. Thormann W, Aebi Y, Lanz M, Caslavska J (1998) Capillary electrophoresis in clinical toxicology. Forensic Sci Int 92(2–3): 157–183
56. Manetto G, Crivellente F, Tagliaro F (2000) Capillary electrophoresis: a new analytical tool for forensic toxicologists. Ther Drug Monit 22(1):84–88
57. Boone CM, Franke J-P, de Zeeuw RA, Ensing K (1999) Evaluation of capillary electrophoretic techniques towards systematic toxicological analysis. J Chromatogr A 838(1–2):259–272
58. Anastos N, Barnett NW, Lewis SW (2005) Capillary electrophoresis for forensic drug analysis: a review. Talanta 67(2):269–279
59. Cruces-Blanco C, Gámiz–Gracia L, García-Campaña AM (2007) Applications of capillary electrophoresis in forensic analytical chemistry. TrAC Trends Anal Chem 26(3):215–226
60. Thormann W, Molteni S, Caslavska J, Schmutz A (1994) Clinical and forensic applications of capillary electrophoresis. Electrophoresis 15(1):3–12
61. Thormann W, Caslavska J (1998) Capillary electrophoresis in drug analysis. Electrophoresis 19(16–17):2691–2694
62. Plaut O, Staub C (2002) Capillary electrophoresis in forensic toxicology. CHIMIA Int J Chem 56(3):96–100
63. Porras SP, Riekkola M-L, Kenndler E (2003) The principles of migration and dispersion in capillary zone electrophoresis in non-aqueous solvents. Electrophoresis 24(10):1485–1498
64. Porras SP, Riekkola M-L, Kenndler E (2001) Electrophoretic mobilities of cationic analytes in non-aqueous methanol, acetonitrile and their mixtures - influence of ionic strength and ion-pair formation. J Chromatogr A 924:31–42
65. Porras SP, Kenndler E (2004) Capillary zone electrophoresis in non-aqueous solutions: pH of the background electrolyte. J Chromatogr A 1037(1–2):455–465
66. Porras SP, Kenndler E (2005) Are the asserted advantages of organic solvents in capillary electrophoresis real? A critical discussion. Electrophoresis 26(17):3203–3220
67. Geiser L, Veuthey J-L (2009) Non-aqueous capillary electrophoresis 2005–2008. Electrophoresis 30(1):36–49
68. Riekkola M-L, Jussila M, Porras SP, Valko IE (2000) Non-aqueous capillary electrophoresis. J Chromatogr A 892(1–2):155–170
69. Szumski M, Buszewski B (2013) Non-aqueous capillary electrophoresis. In: Buszewski B, Dziubakiewicz E, Szumski M (eds) Electromigration techniques, vol 105, Springer series in chemical physics. Springer, Berlin-Heidelberg, pp 203–213
70. Bjørnsdottir I, HonoréHansen S (1995) Comparison of separation selectivity in aqueous and non-aqueous capillary electrophoresis. J Chromatogr A 711(2):313–322
71. Amini A (2001) Recent developments in chiral capillary electrophoresis and applications of this technique to pharmaceutical and biomedical analysis. Electrophoresis 22(15):3107–3130
72. Chankvetadze B (1997) Separation selectivity in chiral capillary electrophoresis with charged selectors. J Chromatogr A 792(1–2): 269–295
73. Pyell U (2006) Electrokinetic chromatography: theory, instrumentation, and applications. John Wiley & Sons, Chichester
74. Somsen GW, Mol R, de Jong GJ (2003) On-line micellar electrokinetic chromatography–mass spectrometry: feasibility of direct introduction of non-volatile buffer and surfactant into the electrospray interface. J Chromatogr A 1000(1–2):953–961
75. Watarai H (1991) Microemulsion capillary electrophoresis. Chem Lett 20(3):391–394
76. Marsh A, Altria K, Clark B (2007) Microemulsion electrokinetic chromatography. In: Electrokinetic chromatography. John Wiley & Sons, Ltd, Hoboken, pp 115–135
77. Yang H, Ding Y, Cao J, Li P (2013) Twenty-one years of microemulsion electrokinetic chromatography (1991–2012): a powerful analytical tool. Electrophoresis 34(9–10):1273–1294
78. Ryan R, Donegan S, Power J, McEvoy E, Altria K (2009) Recent advances in the methodology, optimisation and application of MEEKC. Electrophoresis 30(1):65–82
79. Altria KD, Mahuzier P-E, Clark BJ (2003) Background and operating parameters in microemulsion electrokinetic chromatography. Electrophoresis 24(3):315–324
80. Hansen SH, Sheribah ZA (2005) Comparison of CZE, MEKC, MEEKC and non-aqueous capillary electrophoresis for the determination of impurities in bromazepam. J Pharm Biomed Anal 39(1–2):322–327
81. Mistry K, Krull I, Grinberg N (2002) Capillary electrochromatography: an alternative to HPLC and CE. J Sep Sci 25(15–17):935–958
82. Bartle KD, Myers P (eds) (2001) Capillary electrochromatography. Royal Society of Chemistry, Cambridge
83. Deyl Z, Svec F (eds) (2001) Capillary electrochromatography, Journal of chromatography library. Elsevier, Amsterdam
84. Boček P (1981) Analytical isotachophoresis. In: Analytical Problems, vol 95, Topics in Current Chemistry. Springer, Berlin Heidelberg, pp 131–177
85. Bocek P (1988) Analytical isotachophoresis. Electrophoresis library. VCH Verl. Ges., Weinheim
86. Everaerts FM, Becker JL, Verheggen TPEM (1976) Isotachophoresis: theory, instrumentation, and applications, vol 6, Journal of chromatography library. Elsevier Scientific Pub. Co, Amsterdam
87. Danková M, Kaniansky D, Fanali S, Iványi F (1999) Capillary zone electrophoresis separations of enantiomers present in complex ionic matrices with on-line isotachophoretic sample pretreatment. J Chromatogr A 838(1–2):31–43
88. Tomás-Barberán FA (1995) Capillary electrophoresis: a new technique in the analysis of plant secondary metabolites. Phytochem Anal 6(4):177–192
89. Frost M, Köhler H (1998) Analysis of lysergic acid diethylamide: comparison of capillary electrophoresis with laser-induced fluorescence (CE-LIF) with conventional techniques. Forensic Sci Int 92(2–3):213–218
90. Huhn C, Pütz M, Martin N, Dahlenburg R, Pyell U (2005) Determination of tryptamine derivatives in illicit synthetic drugs by capillary electrophoresis and ultraviolet laser-induced fluorescence detection. Electrophoresis 26(12):2391–2401
91. Bishop SC, Lerch M, McCord BR (2007) Detection of nitrated benzodiazepines by indirect laser-induced

fluorescence detection on a microfluidic device. J Chromatogr A 1154(1–2):481–484
92. Bailey CG, Wallenborg SR (2000) Indirect laser-induced fluorescence detection of explosive compounds using capillary electrochromatography and micellar electrokinetic chromatography. Electrophoresis 21(15):3081–3087
93. Melanson JE, Boulet CA, Lucy CA (2001) Indirect laser-induced fluorescence detection for capillary electrophoresis using a violet diode laser. Anal Chem 73(8):1809–1813
94. Wallenborg SR, Bailey CG (2000) Separation and detection of explosives on a microchip using micellar electrokinetic chromatography and indirect laser-induced fluorescence. Anal Chem 72(8): 1872–1878
95. Páez X, Hernández L (2001) Biomedical applications of capillary electrophoresis with laser-induced fluorescence detection. Biopharm Drug Dispos 22(7–8):273–289
96. Swinney K, Bornhop DJ (2000) Detection in capillary electrophoresis. Electrophoresis 21(7):1239–1250
97. Zemann AJ, Schnell E, Volgger D, Bonn GK (1998) Contactless conductivity detection for capillary electrophoresis. Anal Chem 70(3):563–567
98. Vio L, Crétier G, Chartier F, Geertsen V, Gourgiotis A, Isnard H, Rocca J-L (2012) Separation and analysis of lanthanides by isotachophoresis coupled with inductively coupled plasma mass spectrometry. Talanta 99:586–593
99. Schmitt-Kopplin P, Frommberger M (2003) Capillary electrophoresis - mass spectrometry: 15 years of developments and applications. Electrophoresis 24(22–23):3837–3867
100. Reinhoud NJ, Schroder E, Tjaden UR, Niessen WMA, de Brauw MCTN, Van der Greef J (1990) Static and scanning array detection in capillary electrophoresis-mass spectrometry. J Chromatogr A 516(1):147–155
101. Verheij ER, Tjaden UR, Niessen WMA, Van der Greef J (1991) Pseudo-electrochromatography-mass spectrometry: a new alternative. J Chromatogr A 554(1–2):339–349
102. Chang SY, Yeung ES (1997) Laser vaporization/ionization interface for capillary electrophoresis-time-of-flight mass spectrometry. Anal Chem 69(13):2251–2257
103. Hirabayashi Y, Hirabayashi A, Koizumi H (1999) A sonic spray interface for capillary electrophoresis/mass spectrometry. Rapid Commun Mass Spectrom 13(8):712–715
104. Gusev AI (2000) Interfacing matrix-assisted laser desorption/ ionization mass spectrometry with column and planar separations. Fresenius J Anal Chem 366(6–7):691–700
105. Musyimi Harrison K, Guy J, Narcisse Damien A, Soper Steven A, Murray Kermit K (2005) Direct coupling of polymer-based microchip electrophoresis to online MALDI-MS using a rotating ball inlet. Electrophoresis 26(24):4703–4710
106. Preisler J, Foret F, Karger BL (1998) On-Line MALDI-TOF MS using a continuous vacuum deposition interface. Anal Chem 70(24): 5278–5287
107. Maxwell EJ, Chen DDY (2008) Twenty years of interface development for capillary electrophoresis–electrospray ionization–mass spectrometry. Anal Chim Acta 627(1):25–33
108. Whitt JT, Moini M (2003) Capillary electrophoresis to mass spectrometry interface using a porous junction. Anal Chem 75(9):2188–2191
109. Maxwell EJ, Zhong X, Zhang H, van Zeijl N, Chen DDY (2010) Decoupling CE and ESI for a more robust interface with MS. Electrophoresis 31(7):1130–1137
110. Schmitt-Kopplin P, Englmann M (2005) Capillary electrophoresis - mass spectrometry: survey on developments and applications 2003–2004. Electrophoresis 26(7–8):1209–1220
111. Scriba GKE (2007) Nonaqueous capillary electrophoresis-mass spectrometry. J Chromatogr A 1159(1–2):28–41
112. Av B, Nicholson G, Bayer E (2001) Recent advances in capillary electrophoresis/electrospray-mass spectrometry. Electrophoresis 22(7):1251–1266
113. Unger M, Stöckigt J (1997) Improved detection of alkaloids in crude extracts applying capillary electrophoresis with field amplified sample injection. J Chromatogr A 791(1–2):323–331
114. Bjørnsdottir I, Hansen SH (1995) Determination of opium alkaloids in crude opium using non-aqueous capillary electrophoresis. J Pharm Biomed Anal 13(12):1473–1481
115. Bjørnsdottir I, Hansen SH (1997) Comparison of aqueous and non-aqueous capillary electrophoresis for quantitative determination of morphine in pharmaceuticals. J Pharm Biomed Anal 15(8):1083–1089
116. Bjørnsdottir I, Hansen SH (1999) Fast separation of 16 seizure drug substances using non-aqueous capillary electrophoresis. J Biochem Bioph Methods 38(2):155–161
117. Bjørnsdottir I, Hansen SH (1995) Determination of opium alkaloids in opium by capillary electrophoresis. J Pharm Biomed Anal 13(4–5):687–693
118. Nielsen MKK, Johansen SS, Dalsgaard PW, Linnet K (2010) Simultaneous screening and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC–TOF-MS. Forensic Sci Int 196(1–3):85–92
119. Badawi N, Simonsen KW, Steentoft A, Bernhoft IM, Linnet K (2009) Simultaneous screening and quantification of 29 drugs of abuse in oral fluid by solid-phase extraction and ultraperformance LC-MS/MS. Clin Chem 55(11):2004–2018
120. Dresen S, Ferreirós N, Gnann H, Zimmermann R, Weinmann W (2010) Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP LC-MS/MS system and library searching. Anal Bioanal Chem 396(7):2425–2434
121. Pyell U (2007) Theory of electrokinetic chromatography. In: Electrokinetic chromatography. John Wiley & Sons, Ltd, Hoboken, pp 1–31
122. Terabe S (1992) Selectivity manipulation in micellar electrokinetic chromatography. J Pharm Biomed Anal 10(10–12):705–715
123. Beckers JL, Boček P (2000) Sample stacking in capillary zone electrophoresis: principles, advantages and limitations. Electrophoresis 21(14):2747–2767
124. Malá Z, Křivánková L, Gebauer P, Boček P (2007) Contemporary sample stacking in CE: a sophisticated tool based on simple principles. Electrophoresis 28(1–2):243–253
125. Huhn C (2007) Optimierung von Probeninjektion und Trennung in der Kapillarelektrophorese, der micellaren elektrokinetischen Chromatographie und der Säulenkopplung Isotachophorese-Kapillarelektrophorese zur Analyse komplexer Proben in der forensischen Analytik. Philipps-Universität Marburg, Marburg an der Lahn
126. Himes S, Concheiro M, Scheidweiler K, Huestis M (2014) Validation of a novel method to identify in utero ethanol exposure: simultaneous meconium extraction of fatty acid ethyl esters, ethyl glucuronide, and ethyl sulfate followed by LC-MS/MS quantification. Anal Bioanal Chem 406(7):1945–1955
127. Albermann ME, Musshoff F, Madea B (2012) A high-performance liquid chromatographic–tandem mass spectrometric method for the determination of ethyl glucuronide and ethyl sulfate in urine validated according to forensic guidelines. J Chromatogr Sci 50(1):51–56
128. Cherkaoui S, Bekkouche K, Christen P, Veuthey J-L (2001) Non-aqueous capillary electrophoresis with diode array and electrospray mass spectrometric detection for the analysis of selected steroidal alkaloids in plant extracts. J Chromatogr A 922(1–2):321–328
129. Huhn C, Pütz M, Holthausen I, Pyell U (2008) Separation of very hydrophobic analytes by micellar electrokinetic chromatography. Electrophoresis 29(2):526–537

130. Poole CF, Poole SK (1997) Interphase model for retention and selectivity in micellar electrokinetic chromatography. J Chromatogr A 792(1–2):89–104
131. Poole SK, Poole CF (1997) Characterization of surfactant selectivity in micellar electrokinetic chromatography. Analyst 122:267–274
132. Poole CF, Poole SK, Abraham MH (1998) Recommendations for the determination of selectivity in micellar electrokinetic chromatography. J Chromatogr A 798(1–2):207–222
133. Cherkaoui S, Mateus L, Christen P, Veuthey J (1999) Nonaqueous capillary electrophoresis for the analysis of selected tropane alkaloids in a plant extract. Chromatographia 49(1):54–60
134. Cherkaoui S, Varesio E, Christen P, Veuthey J-L (1998) Selectivity manipulation using nonaqueous capillary electrophoresis. Application to tropane alkaloids and amphetamine derivatives. Electrophoresis 19(16–17):2900–2906
135. Vindevogel J, Sandra P (1992) Introduction to micellar electrokinetic chromatography. Hüthig, Heidelberg
136. Lucangioli SE, Hermida LG, Tripodi VP, Rodríguez VG, López EE, Rouge PD, Carducci CN (2000) Analysis of cis–trans isomers and enantiomers of sertraline by cyclodextrin-modified micellar electrokinetic chromatography. J Chromatogr A 871(1–2):207–215
137. Huhn C, Pütz M, Pyell U (2008) Separation of very hydrophobic analytes by MEKC. II. Carbon number equivalents as analyte descriptors – influence of the composition of the separation electrolyte. Electrophoresis 29:567–575
138. Bjørnsdottir I, Hansen SH, Terabe S (1996) Chiral separation in non-aqueous media by capillary electrophoresis using the ion-pair principle. J Chromatogr A 745(1–2):37–44
139. Hansen SH, Bjørnsdottir I, Tjørnelund J (1997) Separation of cationic cis-trans (Z-E) isomers and diastereoisomers using nonaqueous capillary electrophoresis. J Chromatogr A 792(1–2):49–55
140. Iwata YT, Inoue H, Kuwayama K, Kanamori T, Tsujikawa K, Miyaguchi H, Kishi T (2006) Forensic application of chiral separation of amphetamine-type stimulants to impurity analysis of seized methamphetamine by capillary electrophoresis. Forensic Sci Int 161(2–3):92–96
141. Lee JS, Yang WK, Han EY, Lee SY, Park YH, Lim MA, Chung HS, Park JH (2007) Monitoring precursor chemicals of methamphetamine through enantiomer profiling. Forensic Sci Int 173(1):68–72
142. Inoue H, Iwata YT, Kuwayama K (2008) Characterization and profiling of methamphetamine seizures. J Health Sci 54(6):615–622
143. Fanali S (2000) Enantioselective determination by capillary electrophoresis with cyclodextrins as chiral selectors. J Chromatogr A 875(1–2):89–122
144. Zaugg S, Thormann W (2000) Enantioselective determination of drugs in body fluids by capillary electrophoresis. J Chromatogr A 875(1–2):27–41
145. Caslavska J, Thormann W (2011) Stereoselective determination of drugs and metabolites in body fluids, tissues and microsomal preparations by capillary electrophoresis (2000–2010). J Chromatogr A 1218(4):588–601
146. Fillet M, Servais A-C, Crommen J (2003) Effects of background electrolyte composition and addition of selectors on separation selectivity in nonaqueous capillary electrophoresis. Electrophoresis 24(10):1499–1507
147. Blanco M, Valverde I (2003) Choice of chiral selector for enantioseparation by capillary electrophoresis. TrAC Trends Anal Chem 22(7):428–439
148. Sabbah S, Süß F, Scriba GKE (2001) pH-dependence of complexation constants and complex mobility in capillary electrophoresis separations of dipeptide enantiomers. Electrophoresis 22(15):3163–3170
149. Sabbah S, Scriba GKE (2000) Influence of the structure of cyclodextrins and amino acid sequence of dipeptides and tripeptides on the pH-dependent reversal of the migration order in capillary electrophoresis. J Chromatogr A 894(1–2):267–272
150. Süß F, Poppitz W, de Griend CES-v, Scriba GKE (2001) Influence of the amino acid sequence and nature of the cyclodextrin on the separation of small peptide enantiomers by capillary electrophoresis using randomly substituted and single isomer sulfated and sulfonated cyclodextrins. Electrophoresis 22(12):2416–2423
151. Sidamonidze N, Süß F, Poppitz W, Scriba GKE (2001) Influence of the amino acid sequence and nature of the cyclodextrin on the separation of small peptide enantiomers by capillary electrophoresis using α-, β-, and γ-cyclodextrin and the corresponding hydroxypropyl derivatives. J Sep Sci 24(9):777–783
152. Loukas YL, Sabbah S, Scriba GKE (2001) Method development and validation for the chiral separation of peptides in the presence of cyclodextrins using capillary electrophoresis and experimental design. J Chromatogr A 931(1–2):141–152
153. Sabbah S, Scriba GKE (2001) Separation of dipeptide and tripeptide enantiomers in capillary electrophoresis using carboxymethyl-β-cyclodextrin and succinyl-β-cyclodextrin: influence of the amino acid sequence, nature of the cyclodextrin and pH. Electrophoresis 22(7):1385–1393
154. Süß F, Kahle C, Holzgrabe U, Scriba GKE (2002) Studies on the chiral recognition of peptide enantiomers by neutral and sulfated β-cyclodextrin and heptakis-(2,3-di-O-acetyl)-β-cyclodextrin using capillary electrophoresis and nuclear magnetic resonance. Electrophoresis 23(9):1301–1307
155. Rudaz S, Calleri E, Geiser L, Cherkaoui S, Prat J, Veuthey J-L (2003) Infinite enantiomeric resolution of basic compounds using highly sulfated cyclodextrin as chiral selector in capillary electrophoresis. Electrophoresis 24(15):2633–2641
156. Dieckmann S, Pütz M, Pyell U (2008) Enantiomeric identification of chiral drugs, adulterants and impurites by capillary electrophoresis-ESI-mass spectrometry (CE-ESI-MS). Paper presented at the XVI. Symposium der GTFCh, Mosbach
157. Cherkaoui S, Rudaz S, Varesio E, Veuthey J-L (2001) On-line capillary electrophoresis-electrospray mass spectrometry for the stereoselective analysis of drugs and metabolites. Electrophoresis 22(15):3308–3315
158. Jung B, Caslavska J, Thormann W (2009) Determination of ethyl glucuronide in human serum by capillary zone electrophoresis and an immunoassay. J Sep Sci 32(20):3497–3506
159. Schmitt T, Engelhardt H (1995) Optimization of enantiomeric separations in capillary electrophoresis by reversal of the migration order and using different derivatized cyclodextrins. J Chromatogr A 697(1–2):561–570
160. Caslavska J, Jung B, Thormann W (2011) Confirmation analysis of ethyl glucuronide and ethyl sulfate in human serum and urine by CZE-ESI-MSn after intake of alcoholic beverages. Electrophoresis 32(13):1760–1764
161. Iwata YT, Garcia A, Kanamori T, Inoue H, Kishi T, Lurie IS (2002) The use of a highly sulfated cyclodextrin for the simultaneous chiral separation of amphetamine-type stimulants by capillary electrophoresis. Electrophoresis 23(9):1328–1334
162. Dahlén J, von Eckardstein S (2006) Development of a capillary zone electrophoresis method including a factorial design and simplex optimisation for analysis of amphetamine, amphetamine analogues, cocaine, and heroin. Forensic Sci Int 157(2–3):93–105
163. Varesio E, Gauvrit JY, Longeray R, Lantéri P, Veuthey JL (1999) Optimization of fast CE analyses of ecstasy derivatives by use of experimental designs. Chromatographia 50(3–4):195–201
164. Tagliaro F, Antonioli C, Moretto S, Archetti S, Ghielmi S, Marigo M (1993) High-sensitivity low-cost methods for determination of cocaine in hair: high-performance liquid chromatography and capillary electrophoresis. Forensic Sci Int 63(1–3):227–238
165. Weinmann W, Schaefer P, Thierauf A, Schreiber A, Wurst FM (2004) Confirmatory analysis of ethylglucuronide in urine by

liquid-chromatography/electrospray ionization/tandem mass spectrometry according to forensic guidelines. J Am Soc Mass Spectrom 15(2):188–193
166. Jung B, Caslavska J, Thormann W (2008) Determination of ethyl sulfate in human serum and urine by capillary zone electrophoresis. J Chromatogr A 1206(1):26–32
167. Krivankova L, Caslavska J, Malaskova H, Gebauer P, Thormann W (2005) Analysis of ethyl glucuronide in human serum by capillary electrophoresis with sample self-stacking and indirect detection. J Chromatogr A 1081(1):2–8
168. Nováková M, Křivánková L (2008) Determination of ethyl glucuronide in human serum by hyphenation of capillary isotachophoresis and zone electrophoresis. Electrophoresis 29(8): 1694–1700
169. Tagliaro F, Manetto G, Crivellente F, Scarcella D, Marigo M (1998) Hair analysis for abused drugs by capillary zone electrophoresis with field-amplified sample stacking. Forensic Sci Int 92(2–3):201–211
170. Zhang C-X, Thormann W (1996) Head-column field-amplified sample stacking in binary system capillary electrophoresis: a robust approach providing over 1000-fold sensitivity enhancement. Anal Chem 68(15):2523–2532
171. Zhang C-X, Thormann W (1998) Head-column field-amplified sample stacking in binary system capillary electrophoresis. 2. Optimization with a preinjection plug and application to micellar electrokinetic chromatography. Anal Chem 70(3):540–548
172. Gottardo R, Bortolotti F, De Paoli G, Pascali JP, Miksik I, Tagliaro F (2007) Hair analysis for illicit drugs by using capillary zone electrophoresis-electrospray ionization-ion trap mass spectrometry. J Chromatogr A 1159(1–2):185–189
173. Guzman NA, Blanc T, Phillips TM (2008) Immunoaffinity capillary electrophoresis as a powerful strategy for the quantification of low-abundance biomarkers, drugs, and metabolites in biological matrices. Electrophoresis 29(16):3259–3278
174. Thormann W, Lanz M, Caslavska J, Siegenthaler P, Portmann R (1998) Screening for urinary methadone by capillary electrophoretic immunoassays and confirmation by capillary electrophoresis-mass spectrometry. Electrophoresis 19(1):57–65
175. Lanz M, Thormann W (1996) Characterization of the stereoselective metabolism of methadone and its primary metabolite via cyclodextrin capillary electrophoretic determination of their urinary enantiomers. Electrophoresis 17(12):1945–1949
176. Bao J, Regnier FE (1992) Ultramicro enzyme assays in a capillary electrophoretic system. J Chromatogr A 608(1–2): 217–224
177. Wey AB, Caslavska J, Thormann W (2000) Analysis of codeine, dihydrocodeine and their glucuronides in human urine by electrokinetic capillary immunoassays and capillary electrophoresis-ion trap mass spectrometry. J Chromatogr A 895(1–2):133–146
178. Wey AB, Thormann W (2001) Capillary electrophoresis-electrospray ionization ion trap mass spectrometry for analysis and confirmation testing of morphine and related compounds in urine. J Chromatogr A 916(1–2):225–238
179. W-R- B, Perez SB, El-Khadra-Kluth N, Fritsch R, Hindorf G, Jacobsen-Bauer A, Klein B, Naujoks E, Standke U, Stein K, Westphal F, Zerell U (2012) Richtlinie zur qualitätssicherung bei forensisch-chemischen untersuchungen von betäubungs- und arzneimitteln. Toxichem Krimtech 79(3):42
180. Castaneda Penalvo G, Julien E, Fabre H (1996) Cross validation of capillary electrophoresis and high-performance liquid chromatography for cefotaxime and related impurities. Chromatographia 42(3–4):159–164
181. Sadeghipour F, Varesio E, Giroud C, Rivier L, Veuthey JL (1997) Analysis of amphetamines by capillary electrophoresis and liquid chromatography: application to drug seizures and cross-validation. Forensic Sci Int 86(1–2):1–13
182. Tagliaro F, Smith FP, Turrina S, Equisetto V, Marigo M (1996) Complementary use of capillary zone electrophoresis and micellar electrokinetic capillary chromatography for mutual confirmation of results in forensic drug analysis. J Chromatogr A 735(1–2):227–235
183. Schmitt-Kopplin P, Garmash AV, Kudryavtsev AV, Menzinger F, Perminova IV, Hertkorn N, Freitag D, Petrosyan VS, Kettrup A (2001) Quantitative and qualitative precision improvements by effective mobility-scale data transformation in capillary electrophoresis analysis. Electrophoresis 22(1):77–87
184. Schmitt-Kopplin P, Fekete A (2008) The CE way of thinking. In: Schmitt-Kopplin P (ed) Capillary electrophoresis, vol 384, Methods in molecular biology. Humana Press, Totowa, pp 611–629
185. Nevedomskaya E, Derks R, Deelder A, Mayboroda O, Palmblad M (2009) Alignment of capillary electrophoresis–mass spectrometry datasets using accurate mass information. Anal Bioanal Chem 395(8):2527–2533
186. Lewis AP, Cranny A, Harris NR, Green NG, Wharton JA, Wood RJK, Stokes KR (2013) Review on the development of truly portable and in-situ capillary electrophoresis systems. Meas Sci Technol 24(4):042001
187. Wallenborg SR, Lurie IS, Arnold DW, Bailey CG (2000) On-chip chiral and achiral separation of amphetamine and related compounds labeled with 4-fluoro-7-nitrobenzofurazane. Electrophoresis 21(15):3257–3263
188. Fernández-la-Villa A, Sánchez-Barragán D, Pozo-Ayuso DF, Castaño-Álvarez M (2012) Smart portable electrophoresis instrument based on multipurpose microfluidic chips with electrochemical detection. Electrophoresis 33(17):2733–2742
189. da Costa ET, Neves CA, Hotta GM, Vidal DTR, Barros MF, Ayon AA, Garcia CD, do Lago CL (2012) Unmanned platform for long-range remote analysis of volatile compounds in air samples. Electrophoresis 33(17):2650–2659
190. Blanco GA, Nai YH, Hilder EF, Shellie RA, Dicinoski GW, Haddad PR, Breadmore MC (2011) Identification of inorganic improvised explosive devices using sequential injection capillary electrophoresis and contactless conductivity detection. Anal Chem 83(23):9068–9075
191. Sarazin C, Delaunay N, Varenne A, Vial J, Costanza C, Eudes V, Minet J-J, Gareil P (2010) Identification and determination of inorganic anions in real extracts from pre- and post-blast residues by capillary electrophoresis. J Chromatogr A 1217(44):6971–6978
192. Sarazin C, Delaunay N, Costanza C, Eudes V, Gareil P (2013) On the use of capillary electrophoresis for the determination of inorganic anions and cations, and carbohydrates in residues collected after a simulated suicide bombing attack. Talanta 103:301–305
193. Kobrin E-G, Lees H, Fomitšenko M, Kubáň P, Kaljurand M (2014) Fingerprinting postblast explosive residues by portable capillary electrophoresis with contactless conductivity detection. Electrophoresis 35(8):1165–1172
194. Manetto G, Tagliaro F, Crivellente F, Pascali VL, Marigo M (2000) Field-amplified sample stacking - capillary zone electrophoresis applied to the analysis of opiate drugs in hair. Electrophoresis 21(14):2891–2898
195. Foret F, Thompson TJ, Vouros P, Karger BL, Gebauer P, Bocek P (1994) Liquid sheath effects on the separation of proteins in capillary electrophoresis/electrospray mass spectrometry. Anal Chem 66(24):4450–4458
196. Huhn C, Pyell U (2008) Separation of very hydrophobic analytes by micellar electrokinetic chromatography: IV. Modeling of the effective electrophoretic mobility from carbon number equivalents and octanol-water partition coefficients. J Chromatogr A 1198–1199: 208–214
197. Reijenga JC, Verheggen TPEM, Martens JHPA, Everaerts FM (1996) Buffer capacity, ionic strength and heat dissipation in capillary electrophoresis. J Chromatogr A 744(1–2):147–153

198. Huhn C, Ramautar R, Wuhrer M, Somsen GW (2010) Relevance and use of capillary coatings in capillary electrophoresis–mass spectrometry. Anal Bioanal Chem 396:297–314

199. Kenndler E (1998) Dependence of analyte separation on electroosmotic flow in capillary zone electrophoresis: quantitative description by the reduced mobility. J Microcolumn Sep 10(3):273–279