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Review Paper

Capillary electrophoresis as a versatile tool for the bioanalysis of drugs—a review

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Abstract

This review article presents an overview of current research on the use of capillary electrophoretic techniques for the analysis of drugs in biological matrices. The principles of capillary electrophoresis and its various separation and detection modes are briefly discussed. Sample pretreatment methods which have been used for clean-up and concentration are discussed. Finally, an extensive overview of bioanalytical applications is presented. The bioanalyses of more than 200 drugs have been summarised, including the applied sample pretreatment methods and the achieved detection limits. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Capillary electrophoresis; Micellar electrokinetic chromatography; Bioanalysis; Drugs; Detection limit; Review

1. Introduction

The determination of drugs in body fluids is essential for therapeutic drug monitoring, forensic and clinical toxicology, as well as pharmacology related research. Analytical techniques suitable for these determinations in routine analysis should be simple in performance, suitable for automation, accurate, rapid, selective, and sensitive. About 90% of the drugs of clinical interest can be assayed by various types of immunoassays which have a high degree of automation. However, they lack specificity. Most of the drugs can also be analysed by chromatographic methods such as high performance liquid chromatography (HPLC) [1,2].

Capillary electrophoresis (CE) is a relatively new analytical technique based on the separation of charged analytes through a small capillary

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under the influence of an electric field. CE can be applied in different modes of separation and can be combined with several detection methods. The technique has gone through a stage of development in the 1980s and has now reached the stage of applications. It has several characteristics making it very attractive, such as high resolution, little sample consumption, and minimal solvent consumption. Additionally, CE is based on a separation mechanism which differs from all types of chromatography, which makes it either a potential alternative analytical technique capable of faster analysis and higher efficiency than HPLC, or a complementary technique to HPLC to augment the information obtained from the analysis.

Since its introduction, CE has gone through a period of rapid growth, which is evident in terms of the number of publications, scientific meetings and commercial instruments. CE is becoming popular for the analysis of pharmaceutical preparations. Methods have been developed to analyse drugs with CE in aqueous solutions, which, for instance, can be applied to pharmaceutical purity testing. The analysis of drugs in body fluids is, however, far more complicated, since these matrices present a variety of problems including a large number of potentially interfering compounds and low concentrations of the analyte of interest [2]. Although many laboratories apply CE for research purposes, few reports have appeared on the application of the technique in routine clinical or toxicological analysis [3]. This can be explained by the fact that an analytical technique can only make its way to routine analysis when analytes at the therapeutic level can be measured in biofluids.

A major disadvantage of CE is its low concentration sensitivity. The small diameter of the capillary allows for nanoliter injection volumes only. Sensitivity is expressed in terms of the limit of detection (LOD) or the limit of quantitation (LOQ), which are defined as the smallest concentration that can be detected or quantitated with reasonable certainty. Most commonly, LOD values are given as the concentration where the signal to noise ratio is for instance 3 or 5. LOQ values are arbitrarily assigned on the basis of the maximal allowable precision and the minimal accuracy in view of the application. For bioanalytical procedures precision should be better than 20% and accuracy between 80 and 120% at the LOQ. Using ultraviolet (UV) detection, which is commonly applied in CE, sensitivities are often in the μM range. For improvement of the detection limit a more sensitive detection method can be employed, such as laser induced fluorescence (LIF), electrochemical detection, or mass spectrometry (MS). Also, a sample pretreatment step prior to CE can be utilised for clean-up and preconcentration. Several sample pretreatment methods have been developed or adjusted from those which are used in combination with HPLC analysis.

The aim of this literature study is to investigate the strategies which improve the sensitivity in the bioanalysis of drugs using CE. The principles of CE, the various separation and detection modes, and sample pretreatment methods for clean-up and concentration will be briefly discussed. An overview of the applied methods in the bioanalysis of drugs, which is striven to be complete, will be presented.

2. Principles of capillary electrophoresis

2.1. Modes of operation

CE is a family of related electrodriven analytical separation techniques. It can be employed in different modes of operation with different separation mechanisms, which makes CE a flexible technique. In general, the different modes are accessed by simply altering the buffer composition (type, concentration, or pH). The basic mode of CE is capillary zone electrophoresis (CZE), which was developed around 1980 by Mikkers [4], Jorgenson [5], and Tsuda [6]. In CZE, components migrate under the influence of an electric field with different velocities, depending on their mobility, in a buffer with a certain pH and ionic strength. A schematic presentation of a CE system is given in Fig. 1. Analytes are separated in a small-diameter capillary (20–100 cm length; 25–100 μm i.d.) under the influence of a high electric field (10–30 kV). Samples are introduced by ei-

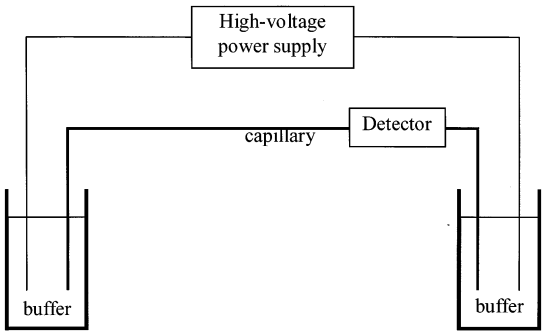

Fig. 1. Schematic representation of capillary electrophoresis.

ther electrokinetic or hydrodynamic techniques. A detection system is employed at the end of the capillary for quantitation or identification of the separated compounds.

Analytes migrate under the influence of the electric field as well as the electroosmotic flow (EOF). The EOF is the flow of the bulk liquid moving along the charged capillary wall. In the typical case of silica, the column will be negatively charged at a $pH > 3$ which causes the formation of an electrical double layer of positively charged ions. When an electric field is applied, the positive ions move towards the cathode, carrying with them the bulk liquid. In general, the EOF is higher than the electrophoretic velocity of any analyte, so all positive, neutral, and negative analytes migrate towards the cathode and can be detected [7,8].

A practically flat flow is obtained in CE by the uniformly distributed driving force along the capillary, this in contrast to pressure-driven separations such as HPLC. Consequently, sharp peaks are obtained in the electropherogram. CE has a good selectivity which can be altered through changes in running buffer composition such as pH changes, capillary surface characteristics, or by the addition of organic modifiers.

The versatility of CE is partially derived from its numerous modes of operation, which each have a different separation mechanism and can all be carried out using the same basic equipment. Besides CZE, other modes of CE are available, such as micellar electrokinetic chromatography (MEKC), isotachophoresis (ITP), capillary electrokinetic chromatography (CEC), capillary gel electrophoresis (CGE), and isoelectric focusing (IEF). The most applied modes for the quantitative analysis of pharmaceuticals in biofluids are CZE, MEKC, and, to a lesser extent, ITP. CEC is gaining interest from researchers and fast developments take place, but until now this CE mode does not play an important role in the bioanalysis of pharmaceuticals. CGE is used primarily for the separation of proteins and oligonucleotides. IEF is mostly used as a qualitative technique, giving an accurate estimation of the isoelectric point of the analytes. The separation of enantiomers can be achieved by the addition of a chiral selector to the buffer, which can be done in the CZE and MEKC modes. The CE modes other than CZE used for bioanalysis will now be briefly discussed.

MEKC, first introduced by Terabe [9,10] in 1984, involves simply the addition of surfactant ions above their critical micelle concentration (CMC) to the CE separation buffer. The most commonly used surfactant is sodium dodecyl sulfate, which is an anionic surfactant and is attracted by the anode. However, the EOF causes the micelles to migrate to the cathode, in a slower rate than the bulk of the liquid. The different velocities of the EOF and the micellar phase permit chromatographic separations, and provide a way to resolve charged as well as neutral molecules. Neutral solutes partition between the micelles and the background electrolyte, and are separated solely on chromatographic basis, whereas for ionic solutes separation is based on chromatography and electrophoresis. The micelles can be considered as a moving (pseudo) stationary phase. Selectivity in MEKC is dependent on the concentration of the micelle-forming agent, the buffer pH and the use of additives, including organic modifiers and salts. It should be noted that surfactants present in concentrations below the CMC also affect the mobility of analytes due to complexation of the analyte with one or more surfactant molecules.

ITP [11,12] is performed in a discontinuous buffer system. Samples migrate between a leading and a terminating electrolyte which have a higher, respectively, lower mobility than any ion in the

sample of interest. Separation occurs based on the individual ion mobilities. Sample ions migrate in consecutive zones having the same velocity (hence the prefix isotacho). The field strength varies in each band accordingly. An ion diffusing out of its zone will speed up or slow down, thereby rejoining its focused zone. Finally, the concentration in all the analyte zones is adapted to the concentration of the leading electrolyte. Since the concentration is constant in each zone, the length of the zones is proportional to the amount of analyte present. The boundary between the zones can be measured by a change in conductivity. In ITP, only one type of ion, either cation or anion, can be analysed in one run. Furthermore, the analysis time depends on the sample composition. ITP can be combined with CZE by first concentrating zones with ITP and then separating them by CZE, either using one capillary (transient ITP (tITP)) or two capillaries (ITP-CZE).

In CEC the separation column is packed with a silica-based chromatographic packing and separation of analytes is based on the differences in charge and in interaction with the sorbent [7,13]. The flow is provided by an existing EOF and the packing material acts as a real stationary phase. The main advantage of CEC are the low sample consumption and the high efficiency. The electrically driven flow approximates plug flow more closely than in a pressure-driven flow such as HPLC. Despite their potential, currently packed capillaries have not yet become a routinely used CE mode, mainly because of the practical difficulties involved in making and using micropacked columns [8].

Many pharmaceutical preparations are administered as a 1:1 racemic mixture of (R)- and (S)-enantiomers. In many cases there is a difference in pharmacological effect of each enantiomers. Furthermore, in the body, two enantiomers can behave completely different in stereoselective controlled processes such as drug absorption, distribution, metabolism, and elimination. The possibility of enantioselective separations was therefore a challenging demand in pharmacokinetic studies in biofluids. The addition of chiral selectors to the background electrolyte creates the possibility of enantiomeric separations of drugs by CE. Often chiral selectors can be used in CE that do not offer the required resolution in HPLC. Due to the substantial higher number of theoretical plates low selectivity values ($\alpha = 1.01$) suffice for CE applications. For HPLC $\alpha > 1.05$ are mandatory to obtain a resolution of > 1.5. Chiral selectors, which are being applied, are macromolecules such as proteins, carbohydrates, but mostly cyclodextrins (CDs).

Bressolle et al. have reviewed the principles and application of CDs in HPLC and CE [14]. CDs are cyclic oligosaccharides consisting of 1,4-linked glucose units, either 6 (α-CD), 7 (β-CD), or 8 (γ-CD). β-CD is the most often used CD. The hollow cone structure of CDs allows analytes to enter and form a transient inclusion complex. Since the CDs have no charge, the mobility of a charged analyte is decreased proportional to the partitioning into the CDs. The enantiomeric separation is based on small differences in CD partitioning coefficients of the two enantiomers. Methylated and 2-hydroxy-propylated β-CDs are the most frequently used modified CDs in CE. In MEKC enantiomeric separation of neutral racemic mixtures can be achieved by the addition of CDs which behave as an additional phase beside the micelles. In non-chiral separations the CDs, as well as other complexing agents, may improve the selectivity of the separation system due to differences in complexation with the analyte and matrix components [15].

2.2. Detection methods

Although CE nowadays is a versatile separation technique, next to GC and LC, and well suited for the determination of a wide variety of compounds in various matrices, the limited detection sensitivity and selectivity still is a problem. This is due to the fact that the small internal diameter of the capillary offers, on one hand, relatively low mass detection limits and a low sample volume, but on the other hand, the small detection volume results in relatively high concentration detection limits. In combination with CE mainly on-column detection techniques have been applied. So far the most frequently applied detection techniques are ultraviolet (UV) absorbance, diode-array (DA),

fluorescence (CIF), laser-induced fluorescence (LIF), electrochemical (AD), and mass spectrometric (MS) detection. A short description of these detection modes will be given, including a comparison with respect to sensitivity and selectivity of these detectors.

2.2.1. Ultraviolet (UV) absorbance and diode-array (DA) detection

UV absorbance is the most frequently applied detection principle in CE, because it is a relatively straightforward and universal technique. The major disadvantage of UV detection is the limited sensitivity because of the short optical path lengths that can be obtained. The cross-column pathlengths, which are in the order of 100 μm, are the reason that the concentration limits of detection obtained are normally in the μM range, which is in many cases not sufficient for the analysis of (biological) samples. A number of modifications of the capillary have been described to enlarge the optical pathlength at the detection point. Improving sensitivity by simply increasing the internal diameter of the capillary is limited because of the resulting increase in Joule heating—caused by the high current—which will disturb the electrophoretic process and because of additional band broadening which will result in a decreased efficiency of the system. Three alternative approaches have been described [16]:

1. a rectangular capillary being extended in the direction of the light path with a reduction in height, this to keep the volume to minimum;
2. a Z-shaped capillary having the light path aligned in the vertical section of the 'Z';
3. a capillary having a locally increased diameter in the detection region, which is called a 'bubble cell'.

These modifications all improve sensitivity by a factor of 3 to 5, however, there still are practical difficulties in the manufacturing process with respect to the reproducibility, in the availability, and the need to modify existing instrumentation. At the moment the Z-shaped capillaries provide the best compromise between gain in sensitivity and robustness of the system.

Multiwavelength UV absorbance or DA detection provides the possibility to obtain complete absorbance spectra of the migrating compounds. This means that in addition to quantitative data, information of the structure of the analyte can be obtained. In addition a DA detector is able to distinguish between (partially) overlapping peaks and to determine peak purity factors. Especially in CE where peak shapes strongly depend on mobility matching and mass overloading, a positive identification and purity confirmation by using a DA detector is an advantage. The sensitivity of modern DA detectors is in the same order as of single-wavelength UV absorbance detectors. Applications of DA detection can be found in the multiple wavelength detection of explosive and pesticide analysis in environmental samples, where functional group identification is applied by using selected wavelength detection. Spectra of compounds can be stored in a computer for automated library searches, for analyte identification and/or structure confirmation. Another possibility of DA detectors is the use of second derivative spectra to emphasise small differences in almost similar molecules. An example in the biosciences is the distinction between tryptophan and tyrosine in peptides.

2.2.2. Fluorescence (CIF) and laser-induced fluorescence (LIF)

LIF detection has proven to be almost perfectly suited as a detection technique in CE. The high energy excitation beam can be focused on the capillary, while collection of the resulting fluorescence is carried out on a 'dark background'. Although conventional fluorescence (CIF) detection already provides an increase of the sensitivity, LIF detection is even more powerful by allowing the detection of only a few molecules. The fact that only a limited number of molecules possesses native fluorescence is an advantage with respect to selectivity, but on the other hand it is a disadvantage with respect to the applicability. This means that in many cases a pre- or post-column derivatisation procedure should be incorporated [17].

Recent instrumental developments and applications of on- [18] and post-column [19] derivatisation procedures for, mainly, fluorescence and chemiluminescence detection show that there cer-

tainly is a future for this type of techniques, especially since the sensitivity of this type of procedures is comparable with pre-column derivatisations.

In order to be good fluorophore, a molecule should possess a high fluorescence quantum yield ($\varnothing_f$), a high absorptivity (ε) and a small width of the emission band [20]. Aromatic hydrocarbons and their corresponding heteroatomic analogues possessing a rigid planar structure normally fulfil these requirements. However, the presence or absence of certain functional groups, the composition and the pH of the run buffer, and the temperature strongly influence both the excitation and emission wavelengths as well as the quantum yields of fluorescence [21]. As a rule of thumb CIF is a factor 10–100 more sensitive compared with UV absorbance detection and the gain in sensitivity by using LIF detection can be a factor of 10^5 (Table 1) [22].

LIF is especially a favourable detection technique in combination with miniaturised systems because the fluorescence signal is proportional both to the detection pathlength and the irradiance. This explains the high gain in sensitivity just by focusing the beam on a spot small enough to fit inside of the CE capillary [23,24]. The most important limitations of using either fixed-wavelength or pulsed lasers are that only a limited number of wavelengths are available and that the traditionally used lasers are expensive and relatively unstable [25]. During the past 10 years the number of diode lasers is rapidly increasing. Nowadays diode lasers with emission wavelengths of 460 nm and higher are available and frequency-doubled diode lasers with emission wavelengths as low as 266 nm can be found. The main advantages of the use of diode lasers over the traditionally applied (gas) lasers are the increased stability, significantly lower costs, and longer lifetimes. Interesting applications have been described using infrared and near-infrared diode lasers for the determination of organic compounds in various samples either without or after labelling of the analytes with a suitable fluorophore [26,27]. The main limitation of this approach is, again, the applicability because excitation wavelengths over 600 nm must be used. The result is that a combi-

Table 1
Sensitivity of detection methods used in CE, based on 10 nl injections [22][a]

Method	Mass detection limits (moles)	Concentration detection limit (molar)	Advantages/disadvantages
UV/DA	10^{-13}–10^{-16}	10^{-5}–10^{-8}	Nearly universal Spectral information
CIF	10^{-15}–10^{-17}	10^{-7}–10^{-9}	Sensitive Usually requires derivatisation
LIF	10^{-18}–10^{-20}	10^{-14}–10^{-16}	Extremely sensitive Usually requires derivatisation (Expensive)
AD	10^{-18}–10^{-19}	10^{-10}–10^{-11}	Sensitive Limited applicability Requires system modification
C	10^{-15}–10^{-16}	10^{-7}–10^{-8}	Universal Requires system modification
MS	10^{-16}–10^{-17}	10^{-8}–10^{-9}	Sensitive Structural information Complicated interface between CE and MS
Indirect UV, CIF, AD	10–100 times less sensitive than direct method		Universal Limited sensitivity

[a] AD, amperometry; C, conductivity; CIF, fluorescence; DA, diode-array; LIF, laser-induced fluorescence; MS, mass spectrometry; UV, ultraviolet absorbance.

Table 2
Applied CE methods for the bioanalysis of drugs and metabolites[a]

Drug	Matrix (chiral selector)	Method	Pretreatment	LOD/sensivity remarks	Ref
Acarbose + metabolite	Urine	CZE-LIF	Derivatisation	0.03 μg/ml	[112]
Acebutolol	Urine	CZE-pulsed LIF	DSI	–	[113]
Acebutolol	Urine	MEKC-UV	DSI	10 μg/ml	[114]
Acebutolol	Serum	MEKC-UV	Enz. hydr. + precip.	LOD: 1–50 μg/ml	[115]
Acebutolol	Urine	MEKC-UV	DSI	LOD: 10–20 μg/ml	[116]
Acepromazine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Acepromazine	Urine	MEKC-UV	LLE	0.01 μg/ml	[103]
Acetazolamide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Acetazolamide	Urine	CZE-UV	DSI	<20 μg/ml	[117]
Acetohexamide	Urine	MEKC-DAD	LLE + SPE	0.05 μg/ml	[79]
Acetylmorphine	Urine	MEKC-DAD	SPE	0.1 μg/ml	[88]
Adamantanamine	Urine, plasma	MEKC-UV	DSI	–	[118]
Allobarbital	Urine	MEKC-DAD	SPE	–	[119]
Allobarbital	Serum	MEKC-DAD	SPE or LLE or DSI	–	[119]
Alprenolol	Urine	MEKC-UV	DSI	10 μg/ml	[114,116]
Alprenolol	Serum	MEKC-UV	Enz. hydr. + precip. or SPE	LOD: 1–50 μg/ml	[115,116]
Amiloride	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Amiloride	Urine	CZE-UV	DSI	<30 μg/ml	[117]
Amiloride	Urine	CZE-fluor.	DSI	0.7 μg/ml	[120]
Amiloride	Urine, serum	CITP-cond.	SPE	0.03–0.04 μg/ml	[100]
Amiodarone	Urine	MEKC-UV	LLE	0.01 μg/ml	[103]
Amiodarone	Serum	CZE-UV	DSI + stacking	0.05 μg/ml	[121]
Amitriptyline	Plasma	MEKC-UV	LLE	0.005–0.01 μg/ml	[122]
Amitriptyline	Urine	MEKC-UV	LLE	0.01 μg/ml	[103]
Amobarbital	Urine	MEKC-DAD	SPE	–	[119]
Amobarbital	Serum	MEKC-DAD	SPE or LLE	–	[119]
Amobarbital	Urine	MEKC-UV	LLE	–	[44]
Amphetamine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Amphetamine	Urine, serum	MEKC-UV	DSI or precip.	1.2 μg/ml	[87]
Amphetamine	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Amphetamine	Urine	CZE-DAD (HPCD)	SPE or LLE	<1 μg/ml	[123]
Amphetamine	Urine	CZE-DAD	SPE	0.4 μg/ml	[124]
Amphetamine	Urine, hair	CZE-UV (CD)	LLE	0.3 μg/ml (standard solution)	[125]
Amphetamine	Urine	CZE-DAD	LLE	0.2 μg/ml	[126]
Amphetamine	Hair	CZE-DAD	LLE + stacking	0.2 μg/ml	[126]
Amphetamine	Urine	CZE or MEKC-UV	LLE	0.9 μg/ml	[127]
Amphetamine	Urine	MEKC-LIF	SPE + derivatisation	0.001 μg/ml	[127]
Antipyrine	Plasma	MEKC-UV	DSI	1 μg/ml	[128]
Antipyrine	Serum	MEKC-UV	Precip.	Linear: 0.5–25 μg/ml	[129]
Antipyrine	Serum	MEKC-UV	Precip.	Linear: 0.5–25 μg/ml	[130]

Table 2 (*continued*)

Antipyrine	Saliva	MEKC-DAD	DSI	2 μg/ml	[131]
Aracytidine	Serum	MEKC-UV	DSI	0.8 μg/ml	[132]
Aspoxicillin	Plasma	MEKC-UV	DSI	1.3 μg/ml	[133]
Atenolol	Urine	MEKC-UV	DSI	10 μg/ml	[114,116]
Atenolol	Serum	MEKC-UV	Enz. hydr. + precip. or SPE	LOD: 1–50 μg/ml	[115,116]
Atenolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]
Atenolol	Urine	CZE-UV	DSI	0.5 μg/ml	[134]
Atenolol	Urine	CZE-UV	DSI	0.6 μg/ml	[117]
Atenolol	Urine	CZE-UV	SPE	LOQ: 0.1 μg/ml	[135]
Azaperone	Urine	MEKC-UV	LLE	–	[103]
Bambuterol	Plasma	CZE-UV	On-line SLM + stacking	18 μg/ml	[82]
Bambuterol	Urine	CZE-UV	On-line SLM	low μg/ml	[64]
Bambuterol	Plasma	CZE-UV	On-line SLM + stacking	0.001 μg/ml	[66]
Bambuterol	Plasma	CZE-UV (β-CD)	On-line SPE + stacking	0.001 μg/ml	[64]
Barbital	Urine	MEKC-DAD	SPE	–	[119]
Barbital	Serum	MEKC-DAD	SPE or LLE or DSI	–	[119]
Barbital	Urine	MEKC-UV	LLE	–	[44]
Bendroflumethiazide	Urine, serum	CZE-UV	precip. + SPE, spiking afterwards	–	[81]
Bendroflumethiazide	Urine	CZE-pulsed-LIF	DSI	–	[113]
Bendroflumethiazide	Urine	CZE-fluor.	DSI	0.2 μg/ml	[120]
Benzocaine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Benzoylecgonine	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Benzthiazide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Bromazepam	Urine	MEKC-DAD	Enz. hydr. + SPE	low μg/ml	[136]
Bromazepam	Serum	MEKC-UV	SPE or LLE	0.025 μg/ml	[137]
Bromhexine	Urine	CZE-DAD	Dilution or LLE	0.01 μg/ml	[138]
+ metabolite					
Bromhexine	Serum	CZE-DAD	Precip.	–	[138]
+ metabolite					
Brompheniramine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Bumetanide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Bumetanide	Urine	CZE-fluor.	DSI	1.3 μg/ml	[120]
Bupivacaine	Serum	MEKC-UV (DMCD)	LLE	0.19 μg/ml	[139]
Bupivacaine	Drain fluid	CZE-UV	LLE	5 μg/ml	[128]
Butacaine	Urine, plasma	CZE-UV	LLE	< 1 μg/ml	[86]
Butalbital	Urine	MEKC-DAD	SPE	–	[119]
Butalbital	Serum	MEKC-DAD	SPE or LLE or DSI	–	[119]
Butalbital	Urine, blood	MEKC-UV	SPE	linear: 3–60 μg/ml	[140]
Caffeine	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Caffeine	Serum	MEKC-UV	LLE	2 μg/ml	[90]
Caffeine + metabolites	Urine, serum, saliva	MEC-DAD	SPE or DSI or LLE	2 μg/ml	[92]
Caffeine metabolites	Urine	MEKC-UV	LLE or DSI	–	[141]
Caffeine metabolites	Urine	MEKC-DAD	Enz. hydr.	0.5 μg/ml	[142]
Caffeine	Urine, serum	MEKC-UV	Precip. or DSI	0.40 μg/ml	[87]
Caffeine + metabolites	Rat serum	MEKC-UV	DSI or LLE	1 μg/ml	[143]
Carbamazepine	Plasma	MEKC-DAD	LLE	linear: 5–40 μg/ml	[144]
Carbamazepine	Serum	MEKC-DAD	LLE	0.6 μg/ml	[145]
Carteolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]

Table 2 (*continued*)

Cefixime	Urine	CZE-UV	DSI	10–60 μg/ml	[146]
+ metabolites					
Cefodizim	Plasma	CZE-UV	DSI	4 μg/ml	[147]
Cefotaxim	Plasma	CZE-UV	DSI	2 μg/ml	[147]
Cefotaxime	Plasma	MEKC-UV	DSI	1 μg/ml	[148]
+ metabolite					
Cefotaxime	Plasma	CZE-UV	Precip.	2 μg/ml	[148]
+ metabolite					
Cefpiramide	Plasma	MEKC-UV	DSI	5 μg/ml	[149]
Cefpiramide	Plasma	MEKC-UV	DSI	10 μg/ml	[41]
Cefpirom	Plasma	CZE-UV	DSI	6 μg/ml	[147]
Cefuroxim	Plasma	CZE-UV	DSI	2 μg/ml	[147]
Cefuroxim	Serum	MEKC-UV	DSI	0.1 μg/ml	[150]
Celiprolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]
Chloroquine	Urine	CZE-UV	SPE + stacking	0.1 μg/ml	[151,152]
+ metabolites					
Chlorothiazide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Chlorpromazine	Urine	MEKC-UV	LLE	0.006 μg/ml	[103]
Chlorpropamide	Urine	MEKC-DAD	LLE + SPE	0.05 μg/ml	[79]
Chlortetracycline	Plasma, milk	CZE-LIF	Precip. + SPE	0.025 μg/ml	[105]
Chlorthalidone	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Chlorthalidone	Urine	CZE-UV	DSI	<20 μg/ml	[117]
Cianopramine	Urine	MEKC-UV	LLE	0.01 μg/ml	[103]
Cicletanine	Plasma	MEKC-UV (γ-CD)	LLE	0.02 μg/ml	[153,154]
Cimetidine	Serum	MEKC-UV	SPE	0.2 μg/ml	[155]
Ciprofloxacin	Plasma	CZE-LIF	Precip.	0.020 μg/ml	[156]
+ metabolite					
Clenbuterol	Urine	CZE-MS	IAC + SPE + ITP	0.002 μg/ml	[157]
Clomipramine	Urine	MEKC-UV	LLE	0.01 μg/ml	[103]
Clonazepam	Urine	MEKC-DAD	Enz. hydr. + SPE	low μg/ml	[136]
Clonazepam	Serum	MEKC-UV	LLE	0.01 μg/ml	[158]
Clopamide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Clozapine	Urine	MEKC-UV	LLE	0.009 μg/ml	[103]
Cocaine	Urine	MEKC–DAD	SPE	± 0.1 μg/ml	[88]
Cocaine	Hair	CZE-UV	LLE	1.5×10^{-4} μg/mg	[108,109]
Codeine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Codeine	Urine, serum	MEKC-UV	DSI or precip.	0.3 μg/ml	[87]
Codeine	Urine	MEKC-DAD	SPE	± 0.1 μg/ml	[88]
Codeine	Urine	CZE-DAD	SPE	0.007 μg/ml	[89]
Cortisol	Serum	MEKC-UV	Enz. hydr. + precip. + SPE	0.05 μg/ml	[159]
Cortisone	Serum	MEKC-UV	Enz. hydr. + precip. + SPE	0.05 μg/ml	[159]
Cytidine	Serum	MEKC-UV	DSI	0.8 μg/ml	[132]
Cytosine-β-D-arabinoside	Plasma	CZE-UV	SPE + stacking	0.1 μg/ml	[160]
Daunorubicin	plasma	CZE-LIF	LLE + stacking	5×10^{-5} μg/ml	[101]
Deacetylmetipranolol	Urine, serum	CITP-cond.	SPE	0.03–0.04 μg/ml	[100]
Debrisoquine	Urine	CZE-UV	LLE or SPE	1 μg/ml	[161]
+ metabolite					
Debrisoquine	Urine	CZE-UV (HTMCD)	SPE	0.150 μg/ml	[162]
+ metabolite					

Table 2 (*continued*)

Desethylamiodarone	Urine	MEKC-UV	LLE	0.005 μg/ml	[103]
Desipramine	Plasma	MEKC-UV	LLE	0.005-0.010 μg/ml	[122]
Desipramine	Urine	MEKC-UV	LLE	0.006 μg/ml	[103]
Dexamethasone + metabolite	Tears	CZE-DAD	DSI	0.5 μg/ml	[163]
Dexamethasone	Urine	CZE-UV	IASPE	0.0011 μg/ml	[83]
Dexamethasone	Serum	MEKC-UV	Enz. hydr. + precip. + SPE	0.05 μg/ml	[159]
Dextromethorphan + metabolites	Urine	MEKC-UV (β-CD)	Acid hydr. + LLE + SPE	0.02 μg/ml	[164]
Dextromethorphan	Urine	CZE-UV	Enz. hydr.	0.08 μg/ml	[165]
Dextromethorphan + metabolites	Urine	MEKC-DAD	Enz. hydr.	0.5 μg/ml	[142]
Diazepam	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Diazepam	Urine	MEKC-DAD	SPE	± 0.1 μg/ml	[88]
Diazepam	Urine	MEKC-DAD	Enz. hydr. + SPE	low μg/ml	[136]
Diazepam	Serum	MEKC-UV	SPE or LLE	0.025 μg/ml	[137]
Dichlorphenamide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Diclofensine	Urine	MEKC-UV	LLE	0.018 μg/ml	[103]
Digoxin	Serum	CZE-UV (ind.)	Incubation with antibody	5×10^{-4} μg/ml	[166]
Dihydrocodeine + metabolites	Urine	MEKC-DAD	DSI or SPE	2, 0.05 μg/ml resp.	[167]
Dihydrocodeine	Plasma	MEKC-UV	LLE + stacking	0.003 μg/ml	[168]
Dimethindene + metabolite	Urine	CZE-UV (HPCD)	LLE + stacking	0.001 μg/ml	[169]
Dimethindene + metabolites	Urine	CZE-UV	DSI	–	[170]
Dolastatin-10	Plasma	CZE-UV	SPE	0.25 μg/ml	[171]
Dothiepine	Urine	MEKC-UV	LLE	0.01 μg/ml	[103]
Doxapram	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Doxepine	Plasma	MEKC-UV	LLE	0.005-0.01 μg/ml	[122]
Doxepine	Urine	CZE-UV	SPE + on-line SPE	LOQ: 0.5 μg/ml	[52]
Doxepine	Urine	MEKC-UV	LLE	0.004 μg/ml	[103]
Doxorubicin	Plasma	CZE-LIF	LLE + stacking	3.5×10^{-5} μg/ml	[101]
[D-pen (2,5)]-enkephaline	Serum	CZE-UV	Precip. + SPE	0.25 μg/ml	[172]
[D-pen (2,5)]-enkephaline	Serum	CZE-LIF	Precip. + SPE + derivatisation	0.0006 μg/ml	[173]
Ephedrine	Urine	CZE-UV	DSI after dilution	2.6 μg/ml	[174]
Ephedrine	Urine	MEKC-UV (DV)	DSI	<100 μg/ml	[175]
Ephedrine	Urine	CZE-MS/UV (β-CD)	DSI	<30, 30 μg/ml resp.	[176]
Ephedrine	Urine	CZE-DAD	LLE	0.2 μg/ml	[126]
Ephedrine	Hair	CZE-DAD	LLE + stacking	0.2 μg/ml	[126]
Epirubicin	Plasma	CZE-LIF	LLE + stacking	7×10^{-5} μg/ml	[101]
Estazolam	Serum	MEKC-UV	SPE or LLE	0.025 μg/ml	[137]
Ethacrynic acid	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Ethosuximide	Plasma	MEKC-DAD	LLE	linear: 25-200 μg/ml	[144]
Ethosuximide	Serum	CZE or MEKC-DAD	Ultrafiltration	5 μg/ml	[99]

Table 2 (*continued*)

Ethosuximide	Serum	MEKC-UV	DSI	low μg/ml range	[177]
Ethosuximide	Serum	MEKC-DAD	DSI or LLE	linear: 20-100 μg/ml	[178]
Ethosuximide	Serum	MEKC-DAD	DSI	–	[1]
Ethosuximide	Serum	MEKC-DAD	LLE	linear: 20-100 μg/ml	[1]
Ethosuximide	Serum	MEKC-LIF	Incubation with antibody	–	[94]
Etodolac	Serum	MEKC-DAD	DSI		[1]
Famotidine	Urine	CZE-UV sieving polymer matrix	DSI	linear: 0.5–30 μg/ml	[179]
Felbamate	Serum	MEKC-UV	DSI	linear: 5–160 μg/ml	[180]
Fenoterol	Urine	CZE-MS	IAC + SPE + ITP	0.002 μg/ml	[156]
Fentanyl	Plasma	CZE-DAD	LLE	0.003 μg/ml	[181]
Flavoxate metabolite	Urine	CZE-UV	DSI	0.2 μg/ml	[182]
Fluconazole	Plasma	MEKC-UV	DSI or precip.	5 μg/ml	[77]
Fluconazole	Plasma	MEKC-UV	LLE	1 μg/ml	[77]
Fluconazole	Plasma	MEKC-UV	SPE	0.1 μg/ml	[77]
Flucytosine	Serum	MEKC-UV/DAD	DSI	linear: 20-120 μg/ml	[1,183]
Flumethasone	Urine	CZE-UV	IASPE	0.0027 μg/ml	[83]
Flunitrazepam	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Flunitrazepam	Urine	MEKC-DAD	Enz. hydr. + SPE	0.15 μg/ml	[136]
+ metabolite					
Flunitrazepam	Urine	MEKC-UV	SPME	–	[44]
Flunixin	Urine, serum	CZE-DAD	SPE	0.017, 0.003 μg/ml resp.	[184]
Flurazepam	Urine	CZE-UV/MS	LLE	–	[185]
+ metabolites					
Flurazepam	Serum	MEKC-UV	SPE or LLE	0.2 μg/ml	[137]
Flurazapam	Urine	CZE-UV	SPE + stacking	1 μg/ml	[186]
+ metabolites					
Fosfomycin	Serum,	CZE-UV (ind.)	Precip.	2.5 μg/ml	[187]
Furosemide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Furosemide	Urine	CZE-UV	DSI	0.15 μg/ml	[117]
Furosemide	Urine, serum	CITP-cond.	SPE	0.03 μg/ml	[100]
Furosemide	Urine	MEKC-DAD	LLE	LOQ: 0.025 μg/ml	[188]
Gabapentin	Serum	CZE-UV	Derivatisation + precip.	1 μg/ml	[189]
Glipizide	Urine	MEKC-DAD	LLE + SPE	0.05 μg/ml	[79]
Glipizide	Urine	MEKC-DAD	SPE	–	[190]
Glyburide	Urine	MEKC-DAD	LLE + SPE	0.05 μg/ml	[79]
Glyburide	Urine	MEKC-DAD	SPE	–	[190]
Glyphosate	Serum	CZE-UV	LLE + derivatisation	0.1 μg/ml	[191]
+ metabolite					
Haloperidol	Urine	CZE-MS	On-line SPE + stacking	–	[57,63]
+ metabolites					
Haloperidol	Urine	CZE-MS	Off-line SPE	$6 * 10^{-7}$ μg/ml	[36]
+ metabolite	Urine	CZE-MS/MS	Precip. + on-line SPE + stacking	–	[36]
Heroin	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Heroin	Urine, serum	MEKC-UV	DSI or precip.	0.52 μg/ml	[87]
Heroin + metabolites	Urine	CZE-DAD	SPE	0.004-0.009 μg/ml	[89]

Table 2 (*continued*)

Hydrochlorothiazide	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Hydroxycoumarin	Urine, serum	CZE-UV	LLE	1 μg/ml	[192]
Hydroxycoumarin	Urine	CZE-UV	MI-SPE	linear: 20-120 μg/ml	[84]
Ibuprofen	Serum	CZE-UV (MOS)	LLE	<8.5 μg/ml	[193]
Ibuprofen	Serum	CZE-UV	Precip.	10 μg/ml	[194]
Ibuprofen + metabolites	Urine	CZE-UV (dextrin 10 + HTMCD)	Enz. hydr. + SPE	–	[195]
Idarubicin + metabolite	Plasma	CZE-LIF	LLE	LOQ: 0.5 * 10^{-3} μg/ml	[196]
Imipramine	Plasma	MEKC-UV	LLE	0.005-0.01 μg/ml	[122]
Imipramine	Urine	MEKC-UV	LLE	0.007 μg/ml	[103]
Isocarboxazide	Urine	MEKC-UV	LLE	0.08 μg/ml	[103]
Isoproterenol	Plasma	CZE-EC	Microdialysis	0.63×10^{-3} μg/ml	[197]
Ketoprofen	Serum	CZE-UV	Precip. + stacking	1 μg/ml	[198]
Labetalol	Urine	MEKC-UV	DSI	10 μg/ml	[114]
Labetalol	Serum	MEKC-UV	Enz. hydr. + SPE	1–50 μg/ml	[116]
Labetalol	Urine	MEKC-UV	DSI	10–20 μg/ml	[116]
Labetalol	Urine, serum	CITP-cond.	SPE	0.03–0.04 μg/ml	[100]
Lamotrigine	Serum	CZE-UV	Precip.	0.3 μg/ml	[199]
Leukovorin + metabolites	Plasma	CZE-UV (γ-CD)	Precip. + ultrafiltration	low μg/ml	[200]
Levobunolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]
Lidocaine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Lorazepam	Urine	MEKC-DAD	Enz. hydr. + SPE	low μg/ml	[136]
LSD	Blood	CZE-LIF	LLE	$1–2 \times 10^{-4}$ μg/ml	[102]
MDA	Urine	CZE-DAD	LLE	0.2 μg/ml	[126]
MDA	Hair	CZE-DAD	LLE, stacking	0.2 μg/ml	[126]
MDA	Urine	CZE-DAD (HPCD)	SPE or LLE	<1 μg/ml	[123]
MDEA	Urine	CZE-DAD	LLE	0.2 μg/ml	[126]
MDEA	Hair	CZE-DAD	LLE + stacking	0.2 μg/ml	[126]
MDEA	Urine	CZE-DAD (HPCD)	SPE or LLE	<1 μg/ml	[123]
MDMA	Urine	CZE-DAD	LLE	0.2 μg/ml	[126]
MDMA	Urine	CZE-DAD (HPCD)	SPE or LLE	<1 μg/ml	[123]
MDMA + metabolites	Urine	CZE-UV (HPCD)	Enz. hydr. + SPE	0.02-0.05 μg/ml	[201]
MDMA	Hair	CZE-DAD	LLE + stacking	0.2 μg/ml	[126]
Meclizine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Medazepam	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Mephenytoin metabolites	Urine	MEKC-DAD	Enz. hydr.	0.5 μg/ml	[142]
Mephenytoin + metabolites	Urine	MEKC-UV/DAD (β-CD)	enz. hydr. + LLE	3, + 3 μg/ml resp.	[202]
Mepivacaine	Serum	CZE-UV (DMCD)	LLE	0.15 μg/ml	[203]
Methadone	Urine	MEKC-DAD	SPE	0.1 μg/ml	[88]
Methadone + metabolite	Urine	CZE-UV	SPE	0.02 μg/ml	[78]
Methadone + metabolite	Urine	CZE-UV	DSI	2 μg/ml	[78]
Methadone + metabolite	Urine	CZE-UV (DMCD)	LLE	0.01 μg /ml	[204]

Table 2 (*continued*)

Methadone + metabolite	Serum	CZE-UV (DMCD)	LLE	0.002 μg /ml	[204]
Methadone + metabolite	Hair	CZE-UV (DMCD)	LLE	–	[204]
Methadone + metabolite	Urine	CZE-LIF	Incubation with reactants for immunoassay	0.01 μg/ml	[205]
Methadone + metabolite	Urine	CZE-MS	DSI	–	[205]
Methamphetamine	Urine	CZE-UV + MS (off-line)	On-line SPE (antibody)	–	[48]
Methamphetamine	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Methamphetamine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Methamphetamine	Urine	CZE-DAD (HPCD)	SPE or LLE	<1 μg/ml	[123]
Methamphetamine	Urine	CZE-DAD	LLE	0.2 μg/ml	[126]
Methamphetamine	Hair	CZE-DAD	LLE, stacking	0.2 μg/ml	[126]
Methamphetamine	Urine	CZE-LIF	Derivatisation, competitive immunoassay	0.2 μg/ml	[206]
Methamphetamine	Urine	MEKC-LIF	SPE + derivatisation	0.017 μg/ml	[127]
Methamphetamine	Urine	CZE or MEKC-UV	LLE	1 μg/ml	[127]
Methapyrilene	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Methaqualone	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Methaqualone	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Methaqualone	Urine, blood, gastric content, hair	MEKC-UV	LLE	0.1 μg/ml	[111]
Methotrexate + metabolite	Serum	CZE-LIF	Precip. or SPE	0.0002 μg/ml	[207]
Methylphenidate	Urine	CZE-MS	LLE	0.0015 μg/ml	[104]
Metoprolol	Urine	MEKC-UV	DSI	10 μg/ml	[114,116]
Metoprolol	Serum	MEKC-UV	Enz. hydr. + precip. or SPE	LOD: 1-50 μg/ml	[115,116]
Metoprolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]
Metoprolol	Urine, serum	CITP-cond.	SPE	0.03–0.04 μg/ml	[100]
Metyrapone	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Midazolam	Urine	MEKC-DAD	Enz. hydr. + SPE	low μg/ml	[136]
Moclobemide	Urine	MEKC-UV	LLE	0.004 μg/ml	[103]
Morphine	Hair	CZE-UV	LLE	0.00015 μg/ml	[108]
Morphine	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Morphine	Urine	CZE-LIF	Competitive immunoassay	0.01 μg/ml	[208]
Morphine	Urine, serum	MEKC-UV	DSI or precip.	0.40 μg/ml	[87]
Morphine	Urine	CZE-DAD	SPE	0.008 μg/ml	[89]
Morphine + metabolites	Urine	MEKC-DAD	SPE	0.15 μg/ml	[209]
Morphine-3-glucuronide	Urine	CZE or MEKC-UV	DSI or SPE	20 μg/ml 1 μg/ml	[210]
Nadolol	Urine	MEKC-UV	DSI	10 μg/ml	[114]
Nadolol	Serum	MEKC-UV	Enz. hydr. + precip.	1–50 μg/ml	[115]
Nadolol	Urine	CZE-UV	DSI	0.7 μg/ml	[117]
Naproxen	Serum	MEKC-DAD	LLE or DSI	linear: 10–125 μg/ml	[1,178]
Naproxen	Serum	MEKC-UV/fluor.	DSI	0.2 μg/ml	[17]
Naproxen	Plasma	MEKC-LIF	Dilution	0.03 μg/ml	[211]
Naproxen	Liver, kidney	MEKC-LIF	Hydr. + LLE	0.07 μg/ml	[212]
Nitrazepam + metabolites	Urine	MEKC-UV	SPE	0.1–0.2 μg/ml	[213]

Table 2 (*continued*)

Nitrazepam	Serum	MEKC-UV	SPE or LLE	0.025 μg/ml	[137]
Nitrazepam	Urine	MEKC-UV	LLE	–	[44]
Nitrazepam	Urine	MEKC-UV	SPME	–	[44]
Nitrazepam	Serum	MEKC-UV	LLE	0.01 μg/ml	[158]
Norephedrine	Urine	CZE-UV	Dilution	2.3 μg/ml	[174]
Nortriptyline	Plasma	MEKC-UV	LLE	0.005-0.01 μg/ml	[122]
Norverapamil	Plasma	CZE-UV (TMCD)	LLE	LOQ: 0.002 μg/ml	[106]
Ordansetron	Serum	CZE-UV (HTMCD)	SPE	0.01 μg/ml	[214]
Oxazepam	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Oxazepam	Urine	MEKC-DAD	Enz. hydr.+SPE	low μg/ml	[136]
Oxprenolol	Urine	MEKC-UV	DSI	10 μg/ml	[114,116]
Oxprenolol	Serum	MEKC-UV	enz. hydr.+precip. or SPE	LOD: 1-50 μg/ml	[115,116]
Oxytetracycline	Plasma, milk	CZE-LIF	Precip.+SPE	0.025 μg/ml	[105]
Paclitaxel	Urine, plasma	MEKC-UV	LLE	0.020, 0.050 μg/ml resp.	[215]
Paracetamol	Urine, serum	MEKC or CZE or ITP-DAD	Dilution or ultrafiltration	1 μg/ml	[99]
Paracetamol	Serum	MEKC-DAD	DSI		[1]
Paracetamol	Serum	MEKC-LIF	Incubation with antibody	–	[94]
Paracetamol	Plasma	MEKC-UV	DSI	–	[216,217]
Paraxanthine	Serum	MEKC-UV	LLE	1 μg/ml	[90]
Paraxanthine	Serum, saliva	MEKC-DAD	DSI or LLE	low μg/ml	[92]
Paraxanthine	Rat serum	MEKC-UV	DSI or LLE	1 μg/ml	[143]
Penbutolol	Serum	MEKC-DAD	Ultrafiltration		[1]
Pentobarbital	Urine	MEKC-DAD	SPE	–	[119]
Pentobarbital	Serum	MEKC-DAD	SPE or LLE	linear:1–60 μg/ml	[119]
Pentobarbital	Serum	CZE-UV	Precip. or LLE	linear: 10–100 μg/ml	[218]
Pentobarbital	Serum	CZE-UV (HPCD)	SPE	1 μg/ml	[219]
Phencyclidine	Urine	CZE-LIF	Competitive immunoassay	0.001μg/ml	[208]
Phenmetrazine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Phenobarbital	Urine	MEKC-DAD	SPE	–	[119]
Phenobarbital	Serum	MEKC-DAD	SPE or LLE or DSI	–	[119]
Phenobarbital	Blood-, brain-dialysates	MEKC-UV	Microdialysis	2 μg/ml	[220]
Phenobarbital	Plasma	MEKC-DAD	LLE	linear: 5-60 μg/ml	[144]
Phenobarbital	Serum	CZE or MEKC-DAD	Ultrafiltration	5 μg/ml	[99]
Phenobarbital	Serum	MEKC-DAD	DSI or LLE	low μg/ml range	[1,177,178]
Phenobarbital	Serum	MEKC-DAD	LLE	2.5 μg/ml	[145]
Phenothiazine	Urine	MEKC-UV	LLE	0.009 μg/ml	[103]
Phenylbutazone	Serum	MEKC-DAD	DSI	–	[1]
Phenytoin	Plasma	MEKC-DAD	LLE	linear: 5–40 μg/ml	[144]
Phenytoin	Serum	MEKC-DAD	LLE	linear: 5–23 μg/ml	[178]
Phenytoin	Urine	MEKC-UV/DAD (β-CD)	Enz. hydr.+LLE	low μg/ml	[202]
Phenytoin	Serum	MEKC-DAD	LLE	0.8 μg/ml	[145]
Pherphenazine	Urine	MEKC-UV	LLE	0.005 μg/ml	[103]
Pimozide	Urine	MEKC-UV	LLE	0.006 μg/ml	[103]
Pindolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]
Pindolol	Urine	MEKC-UV	DSI	10 μg/ml	[114]
Pindolol	Serum	MEKC-UV	Enz. hydr.+precip.	1–50 μg/ml	[115]
Pindolol	Urine	MEKC-UV	DSI	10–20 μg/ml	[116]
Pindolol	Serum	MEKC-UV	Enz. hydr.+SPE	1–50 μg/ml	[116]

Table 2 (*continued*)

Piracetam	Plasma	CZE-UV (α-CD)	Precip.	1 μg/ml	[221]
Piretanide	Urine	MEKC-DAD	LLE	LOQ: 0.025 μg/ml	[188]
Prilocaine	Serum	CZE-UV (DMCD)	SPE	0.04 μg/ml	[222]
Primidone	Plasma	MEKC-DAD	LLE	linear: 5–40 μg/ml	[144]
Primidone	Serum	MEKC-UV/DAD	DSI or LLE	low μg/ml range	[1,177,178]
Primidone	Serum	MEKC-DAD	Ultrafiltration	4 μg/ml	[99]
Probenecid	Urine, serum	CZE-UV	Precip. + SPE, spiking afterwards	–	[81]
Procainamide	Urine	CZE-DAD	DSI	0.3–1.2 μg/ml	[223]
+ metabolite					
Procainamide	Urine	CZE-UV	Dilution	1.2 (metabolite: 0.4) μg/ml	[224]
+ metabolite					
Procaine	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Prochlorperazine	Urine	MEKC-UV	LLE	0.005 μg/ml	[103]
Prolintane	Urine	MEKC-UV (β-CD)	Dilution	1 μg/ml	[225]
+ metabolite					
Proguanil	Urine	CZE-UV	SPE + stacking	0.1 μg/ml	[151,152]
+ metabolites					
Promazine	Urine	MEKC-UV	LLE	0.007 μg/ml	[103]
Propanolol	Serum	MEKC-DAD	Ultrafiltration + precip.	–	[1]
Propanolol	Urine	MEKC-UV	DSI	10 μg/ml	[114,116]
Propanolol	Serum	MEKC-UV	Enz. hydr. + precip. or SPE	1 μg/ml	[115,116]
Quinidine	Urine, serum	MEKC-UV/fluor.	DSI	1.5 μg/ml	[17]
Quinidine	Serum	MEKC-LIF	Incubation with antibody	–	[94]
Salbutamol	Urine	CZE-MS	IAC + SPE + ITP	0.002 μg/ml	[157]
Salicylate	Urine, serum	MEKC or CZE or ITP-DAD	Dillution or ultrafiltration	1 μg/ml	[99]
Salicylate	Urine, serum	MEKC-UV-fluor.	DSI	–	[17]
Salicylate	Serum	MEKC-LIF	Incubation with antibody	–	[94]
Salicylate	Plasma	MEKC-UV	DSI	–	[216]
Salicylate	Urine	ITP-UV-fluor./DAD	DSI	–	[226]
+ metabolites					
Salicylic acid	Plasma	MEKC-UV	DSI	–	[217]
Salicyclic acid	Serum	CZE-DAD	LLE	1 μg/ml	[227]
Secobarbital	Serum	CZE-UV (HPCD)	SPE	1 μg/ml	[228]
Spironolactone	Urine	CZE-UV	DSI	0.5 μg/ml	[117]
Sufentanil	Plasma	CZE-DAD	LLE	0.003 μg/ml	[181]
Sulfamethoxazole	Serum	MEKC-DAD	DSI	–	[1]
Sulfamethoxazole	Plasma	MEKC-UV	DSI	–	[217]
Suramin	Serum	CZE-UV	Precip.	linear:50–500 μg/ml	[229]
Tacrine	Urine, serum	CZE-UV	Dilution + precip.	0.050 μg/ml	[230]
+ metabolite					
Tampxifen	Serum	CZE-UV	LLE	0.2 μg/ml	[231]
+ metabolites					
Temazepam	Urine	MEKC-DAD	Enz. hydr. + SPE	low μg/ml	[136]
Tenoxicam	Serum	MEKC-DAD	DSI		[1]
Terbutaline	Urine	CZE-MS/UV (β-CD)	DSI	<30, 30 μg/ml resp.	[176]
Terbutaline	Plasma	CZE-UV (β-CD)	Coupled CLC + stacking	0.001 μg/ml	[80]
Terbutaline	Urine	CZE-MS	IAC + SPE + ITP	0.002 μg/ml	[167]
Tetracycline	Plasma, milk	CZE-LIF	Precip. + SPE	0.025 μg/ml	[105]
Tetrahydrocannibol-carboxylic acid	Urine	MEKC-DAD	SPE	0.01 μg/ml	[232]

Table 2 (*continued*)

Tetrahydrozoline	Urine, plasma	CZE-UV	LLE	<1 μg/ml	[86]
Theobromine	Rat serum	MEKC-UV	DSI or LLE	1 μg/ml	[142]
Theophylline saliva	Urine, serum,	MEKC-DAD	DSI or SPE	1 μg/ml	[92]
Theophylline	Serum	CZE-UV	Precip.+stacking	linear: 3–40 μg/ml	[40]
Theophylline	Plasma	MEKC-UV	LLE	0.6 μg/ml	[90]
Theophylline	Plasma	CZE-UV	Precip.	2 μg/ml	[91]
Theophylline	Serum	MEKC-LIF	Incubation with antibody	1 μg/ml	[93,94]
Theophylline	Serum	MEKC-UV	DSI	linear: 2-30 μg/ml	[95]
Theophylline	Urine	CZE-UV	DSI+SPE	1 μg/ml	[96]
+metabolites					
Theophylline	Tissue-fluid	MEKC-UV	In vivo capillary ultrafiltration	low μg/ml	[97]
+metabolites					
Theophylline	Serum	CZE-UV	Precip.	2 μg/ml	[98]
Theophylline	Rat serum	MEKC-UV	DSI or LLE	1 μg/ml	[143]
Thiamphenicol	Plasma	MEKC-UV	LLE	LOQ: 0.1 μg/ml	[233]
Thiamphenicol	Plasma	MEKC-UV	LLE+stacking	LOQ: 0.1 μg/ml	[234]
Thiopental	Urine	MEKC-DAD	SPE	–	[119]
Thiopental	Serum	MEKC-DAD	SPE or LLE	linear: 1–60 μg/ml	[119]
Thiopental	Serum, plasma	MEKC-UV	LLE	2 μg/ml	[235]
Thiopental	Urine	MEKC-DAD	SPE	±0.1 μg/ml	[88]
Thioridazine	Urine	MEKC-UV	LLE	0.04 μg/ml	[103]
Thiothixene	Urine	MEKC-UV	LLE	0.016 μg/ml	[103]
Timolol	Urine	MEKC-UV	DSI	20 μg/ml	[114,116]
Timolol	Serum	MEKC-UV	Enz. hydr.+precip. or SPE	50 μg/ml	[115,116]
Timolol	Serum	MEKC-DAD	Ultrafiltration	–	[1]
Tolazamide	Urine	MEKC-DAD	LLE+SPE	0.05 μg/ml	[79]
Tolbutamide	Urine	MEKC-DAD	LLE+SPE	0.05 μg/ml	[79]
Tolbutamide	Plasma	MEKC-UV	DSI	–	[217]
Triamterene	Urine	CZE-pulsed-LIF	DSI	–	[113]
Triamterene	Urine, serum	CZE-UV	Precip.+SPE, spiking afterwards	–	[81]
Triamterene	Urine	CZE-fluor.	DSI	0.02 μg/ml	[81]
Triamterene	Urine	CZE-UV	DSI	0.06 μg/ml	[117]
Triazolam	Serum	MEKC-UV	SPE or LLE	0.025 μg/ml	[137]
Triazolam	Urine	MEKC-UV	SPME	–	[44]
Trichlormethiazide	Urine, serum	CZE-UV	Precip.+SPE, spiking afterwards	–	[81]
Trifluoperazine	Urine	MEKC-UV	LLE	0.043 μg/ml	[103]
Trimethoprim	Plasma	MEKC-UV	DSI	–	[217]
Trimipramine	Urine	MEKC-UV	LLE	0.006 μg/ml	[103]
Verapamil	Plasma	CZE-UV (TMCD)	LLE	LOQ: 0.002 μg/ml	[106]
Warfarin	Plasma	CZE-UV (MCD)	LLE	0.2 μg/ml	[236]
Zomepirac	Serum	MEKC-DAD	DSI	–	[1]
Zonisamide	Serum	MEKC-DAD	LLE	3.0 μg/ml	[145]
Zopiclone	Urine	CZE-LIF (β-CD)	LLE	<0.05 μg/ml	[237]

[a] Abbreviations used in the table: CD, cyclodextrin; cond., conductivity detection; DMCD, dimethyl-β-cyclodextrin; DV, (S)-*N*-dodecanoylvaline; EC, electrochemical detection; enz. hydr., enzymatic hydrolysis; fluor., fluorescence detection; HDMCD, heptakis-(2,6-dimethyl)-β-cyclodextrin; HPCD, (2-hydroxy)-propyl-β-cyclodextrin; HTMCD, heptakis-(2,3,6-trimethyl)-β-cyclodextrin; hydr., hydrolysis; IAC, immunoaffinity chromatography; IASPE, immunoaffinity solid phase extraction; ind., indirect detection fluor.; LSD, lysergic acid diethylamide; MCD, methyl-β-cyclodextrin; MDA, methylenedioxyamphetamine; MDEA,; methylenedioxyethylamphetamine; MDMA, methylenedioxymethamphetamine; MI, molecular imprinting; MOS, maltooligosaccharides; precip., precipitation; resp., respectively; TMCD, trimethyl-β-cyclodextrin

nation of diode LIF detection and a derivatisation procedure is a necessity. However, because of using wavelengths over 600 nm almost no matrix interferences are observed.

2.2.3. Electrochemical detection

Electrochemical detection in CE can be divided in potentiometric, and conductivity and amperometric detection [8]. The most popular mode of electrochemical detection, both in CE and LC, is amperometric detection (AD). One of the positive features of AD in CE over LC is the use of microelectrodes. This because the noise at a microelectrode decreases more rapidly than the signal when the size of the electrode is decreased, which means a better signal-to-noise ratio.

AD in CE is not as straightforward as in LC. The main challenge is to isolate the high electric field across the separation capillary from the low electric field used in the AD detector. In general two approaches can be followed to isolate the separation current from the detection current. The most simple one is producing a fracture in the CE capillary through which the separation current is grounded. The solvent in the fraction is grounded and the analytes are transported over the fracture to the detector by the flow generated by the separation capillary. Important is that the distance between the fracture and the detector is rather small to avoid additional band broadening. The second possibility is to isolate the detector from the separation current by keeping the electrode outside the capillary in such a way that the electric field is significantly decreased at the end of the capillary before it reaches the electrode. Using this approach the internal diameter should, preferably, be 25 μm or less.

Another critical parameter is the alignment of the electrode with the CE capillary. With a proper alignment and by using the wall-jet electrode configuration, the reproducibility of the detector can be in the order of 5%. Sensitivities are normally in the low μmol to high nmol range. For example, disulfides can be detected at the 0.2 μM level, using a mixed-valent ruthenium cyanide-modified electrode, and over a period of 8 h the decrease in response was not more than 85% [28].

In addition to AD, pulsed AD (PAD) has been applied [29]. Using PAD the detection potential is combined with pulsed potential steps to improve cleaning of the surface and surface activation. Analytes that rapidly inactivate the electrode surface (e.g. amines, carbohydrates) are determined by means of PAD techniques.

A few examples have been described using potentiometric detection. In this case the Nernst potential at the surface of an indicator electrode or across an ion-selective membrane is measured. This method requires the use of ion-selective electrodes, but can be rather sensitive provided that no interfering ions (e.g. other ions that permeate through the membrane) are present. In conductivity detection, the conductivity of the solution is measured by positioning a pair of electrodes in the capillary and measuring the resulting current between the electrodes as a function of the potential. This mode is almost universal, relatively cheap, but rather non-sensitive [30].

2.2.4. Mass spectrometric (MS) detection

The on-line coupling of CE with MS has been pioneered by Smith et al. [31] and Henion et al. [32]. When the high separation efficiency of CE is combined with the good sensitivity and high selectivity of MS, a powerful analytical technique is obtained. Analytes are identified by both their migration time and their molecular masses and/or fragmentation patterns. The main disadvantage of the MS detector is the high cost. A number of reviews have been published describing the on-line coupling of CE and MS [33,34].

In MS a number of ionisation techniques, capable of ionising compounds directly from the condensed phase without the need of using high temperatures, have been developed. These techniques allow the determination of a wide variety of compounds, including non-volatile and thermally labile biomolecules. In CE-MS the most critical parameter is the interface, allowing efficient on-line transfer of analytes from the CE capillary to the mass spectrometer. Two of the ionisation techniques that have been successfully coupled to CE are the continuous-flow fast atom

bombardment (CF-FAB) and electrospray ionisation (ESI).

The low flow-rates required to maintain the high efficiencies of CE are incompatible with, for example, the typical CF-FAB flow rates of 5 μl/min. To circumvent this problem, interfaces using a sheath-flow or liquid junction configuration have been introduced [34]. CF-FAB has been used for the determination of moderately high-molecular mass compounds.

ESI is the most suitable interface in the coupling of CE to MS, because it ionises as well as desolvates the analyte molecules [35]. In ESI charged droplets are formed which are rapidly reduced in size by evaporation, resulting in the formation of multiple-charged ions, which can be used for the determination of all kinds of high-molecular-mass compounds. An additional advantage of CE-ESI is that this method can be combined with various types of mass spectrometers (e.g. quadrupole, ion trap, magnetic sector, cyclotron resonance); the combinations which are also commercially available nowadays [8]. The use of surfactant additives as in MEKC, or other non-volatile reagents is not really compatible as they lead to suppression of analyte ionisation and fouling of the ion source [36].

The use of the sheath-flow principle can reduce the detectability, because of the introduction of additional ions to the system. The use of sheathless micro- or nanospray interfaces can circumvent this problem [37]. The outlet of the CE capillary is used as the micro- or nanospray, without the need of using a sheath flow. The tip of the capillary is then placed directly into the mass spectrometer. Comparison of this type of interface with a sheath-flow type of interface provides detection limits of low nmol/l, which is a gain in sensitivity of about one order of magnitude compared with the traditionally ESI devices.

Although mass spectrometers are generally much more expensive than other types of detectors, there certainly is a need for these systems because this combination of techniques offers a unique combination of high separation power and highly compound-selective and/or universal detection potential.

2.2.5. Concluding remarks

In general it can be stated that all detection principles used in LC can also be applied in CE and that in order to increase the sensitivity a derivatisation procedure can be incorporated. However, in combination with CE fluorescence (CIF or LIF) detection is preferred in combination with a labelling procedure. For the future it is expected that the various modes of fluorescence detection will overshadow the use of UV absorbance detection. The reason is the higher selectivity and sensitivity compared with absorbance detection. Furthermore, fluorescence detection can be used both in combination with aqueous and non-aqueous solvents, while the applicability of electrochemical detection is mainly limited to aqueous solutions. Another problem related to electrochemical detection is the inherent electrode contamination and fouling. Finally, fluorescence detection is more robust compared with chemiluminescence and electrochemical detection. However, all these detection modes have the same problem: limited identification and confirmation power. In this respect the use of CE-ESI-MS or CE-ESI-MS/MS systems will become more and more important in the future, especially because this combination provides good ionisation, and consequently, high sensitivity for polar, fragile and thermally labile compounds.

3. Sample pretreatment methods for clean-up and preconcentration

Drugs and their metabolites in the human body are commonly analysed in the body fluids urine, serum, or plasma. Less common matrices requiring analyses are saliva, tears, bile, cerebrospinal fluid, and tissue. Biological matrices consist of many components, which may include macromolecules such as proteins, carbohydrates, and lipids, as well as smaller molecules of widely different polarities. The complexity of the matrices and the low concentration of analytes often necessitates a clean-up and preconcentration step prior to the actual analysis. Components which are harmful to the separation system, for instance, undissolved particles that could block the capil-

lary or proteins which adsorb to the capillary wall, need to be removed, as well as substances which can interact with the analytes during the separation procedure and/or interfere with the detection. Equally important is the high detection limit in CE when combined with UV detection, caused by the short optical path length in the capillary. A trace amount of analytes present in the sample often necessitate some kind of preconcentration.

A variety of sample pretreatment methods is nowadays available and will be summarised below. For a more comprehensive discussion we refer to McDowell [38], who has reviewed sample treatment methods in general, and Lloyd [39], who has more recently reviewed pretreatment methods for the analysis of drugs in biofluids using CE.

3.1. Direct sample injection (DSI)

The direct sample injection (DSI) of biofluids involves no other pretreatment than a simple filtration or centrifugation step to remove matrix particles that would otherwise block the capillary. Thus, the time and effort of sample preparation are minimal. Important for DSI is that the analytes do not co-elute with the bulk of the matrix compounds, in other words, they must elute in an interference-free window.

The major problem of direct injection of plasma and serum is the high concentration of proteins [39]. Proteins are capable of adsorbing to the capillary wall causing a varying EOF and irreproducible migration times. Also, analyte binding to the surface-adsorbed proteins causes peak broadening. Another problem is the interference of the broad protein peaks with the analyte peaks [40].

However, the literature available does report direct injection of serum and plasma in CZE and MEKC. In CZE, proteinaceous samples can be diluted prior to analysis to reduce matrix effects, but analyte concentrations are often too low to allow dilution. Otherwise, vigorous reconditioning of the capillary is required to remove adsorbed proteins, which might take as long as an actual run, or a coated capillary can be used to reduce adsorption of proteins. MEKC has more potential for the direct injection of proteinaceous fluids. The proteins are solubilised by the micelles which minimises protein-wall interactions and causes the proteins to elute as a broad peak late in the electropherogram. Nagakawa and coworkers [41] were the first to perform DSI with MEKC, and since then quite a number of reports mentioning this approach have appeared. A slight drawback of DSI in MEKC is that the use of cationic surfactants should be avoided, since these are expected to precipitate the proteins [1].

Urine does not have a high protein concentration, but many endogenous compounds are present in high but highly variable concentrations. A good separation of the analytes from the interferences is therefore required. Specific detection modes, such as DAD or MS, can be useful in peak identification. Urine has a relatively high salt concentration, and the use of a high ionic strength buffer is required to reduce peak broadening.

3.2. Removal of endogenous compounds

Biological samples are often too complex to permit analysis by direct injection and require the removal of endogenous compounds. In general, pretreatment methods for the removal of endogenous compounds which are standard in HPLC are now being applied to CE. These methods include protein removal and analyte extraction.

3.2.1. Protein removal

Ralstone et al. [42] published a systematic study of deproteinisation methods for serum analysis with CE. The removal of proteins is often achieved by either ultrafiltration or precipitation.

Protein removal by ultrafiltration is performed with cone-shaped membranes that fit into centrifugation tubes. Samples are placed in the cones and centrifugated, thus allowing molecules smaller than the exclusion limit to pass through the membrane. Ultrafiltration allows for protein removal without diluting the samples but restricts the analysis to free drugs and metabolites only [38]. Drugs bound to proteins can be released by enzymatic hydrolysis, so that the total amount of drug present in the sample can be determined.

Protein precipitation agents may also liberate drugs from the proteins, and at the same time they can provide an excellent sample clean-up. Proteins are precipitated by the addition of a reagent and are then removed by centrifugation or filtration. Reagentia used for deproteinisation in CE are solvents such as trifluoroacetic acid, perchloric acid, alcohols, and, frequently used, acetonitrile. It must be noted that the addition of a relatively large amount of solvent leads to sample dilution and that analytes must therefore be present at a sufficiently high concentration. Otherwise the resulting supernatant should be evaporated and the residue reconstituted in a suitable solvent. The time required to precipitate proteins in a single sample can be reduced by pretreating several samples simultaneously.

3.2.2. Analyte extraction

Extraction methods are attractive for two reasons. They selectively collect the analyte of interest plus a part of the endogenous components, whereas the other endogenous components are removed. Secondly, the analyte can simultaneously be concentrated by one or two orders of magnitude. The main disadvantages of extraction techniques are the time and effort they require and the potential of losses of the analyte. Similar to protein precipitation, the procedure will be faster when more samples are pretreated at the same time.

In liquid–liquid extraction (LLE) hydrophobic sample components are extracted with a water–immiscible organic phase. Various organic solvents are being used, such as pentane, hexane, diethyl ether, ethyl acetate, chloroform, and methylene chloride. After extraction, the solvent can be evaporated and the residue reconstituted in a suitable buffer. The use of large amounts of organic solvent in LLE is a disadvantage as far as environmental and health aspects are concerned.

In solid phase extraction (SPE) the sample is passed over a disposable column or cartridge packed with a sorbent by which some of the components are retained [39]. After preconditioning of the sorbent bed, the sample is applied. The sorbent is then selectively washed to remove undesirable components without the loss of the analyte of interest. The analytes are then eluted using a minimum of solvent which can be evaporated and redissolved in a suitable solvent, or diluted with buffer. Using SPE, there is a possibility of the addition of impurities from the disposable column. An important advantage is that off-line SPE can (partly) be automated. As in LLE, the use of solvents is a disadvantage of SPE.

A relatively new extraction technique which is mainly used in combination with GC is solid phase micro extraction (SPME) [43]. A coated fibre is placed in the sample to allow analyte extraction, whereafter analytes are thermally desorbed simply by introducing the fibre in the injection port of the GC. For use in combination with LC or CE the analytes need to be desorbed by a solvent, which can be done using an SPME-LC interface or an off-line desorption chamber for CE [44]. The direct coupling of a fibre to a CE capillary has been successfully applied [45], however, the attachment of the fibre to the capillary was rather laborious and time-consuming, and the fibre had to be replaced after each analysis.

3.3. On-line extraction methods

The potential advantages of an on-line preconcentration and clean-up method are the minimised time and effort required in sample pretreatment, the possibility of automation, and the maximum transfer of analyte from the sample into the system. Various approaches have been made in the development of on-line sample pretreatment methods for CE, and most of them are discussed in two recent reviews [46,47]. Here we summarise those methods which were applied to the bioanalysis of drugs.

Guzman et al. [48] were the first to report the use of on-line extraction in CE. An antibody covalently bound to a solid support was used for specific on-line extraction of analytes from urine. A number of variations on on-line clean-up methods have been described since then. The coupling of (micro-)column liquid chromatography [49–51] using different interfaces has been described. Many other techniques utilise a small bed of packing material [52–56], (e.g. reversed-phase C_4 or C_{18}), or hydrophobic membranes [57] directly

at the inlet of the CE capillary. The bed of adsorptive phase is placed at the inlet of the capillary, held in place with for instance a plug of wool or glass frits. The adsorptive phase retains the analyte components, which enables the analysis of sample amounts that are in vast excess of the total capillary volume. Matrix components can be eluted from the phase and washed from the capillary prior to elution of the analytes of interest. This technique, which is sometimes referred to as on-line SPE, significantly enhances the LOD. However, it has been reported that it also can compromise CE performance. It may result in reduced analyte resolution, peak broadening and substantial component tailing [46,55,57–60]. These observations have been attributed to the increased analyte–analyte and analyte–wall interactions, due to the large amount of each component loaded on the capillary, and to the increased back-pressure induced in the capillary by the solid phase and frit material. Also, a relatively large amount of organic solvent is used to elute the analytes from the solid phase, and a reduction of the EOF has been reported which affects the CE performance [55].

Tomlinson and Naylor developed an impregnated membrane preconcentration (mPC) device to overcome the limitations of on-line extraction mentioned above [46,59–63]. The use of a suitably coated membrane minimised the bed volume of the sorbent. A reduced volume of organic solvent for elution is required, while the high adsorptive capacity of the impregnated membrane allows the analysis of large volumes (>100 ml). Compromised CE performance caused by the eluents was avoided by choosing stacking or tITP conditions (see Section 3.4) during electrophoresis. The method was successfully applied to the analysis of haloperidol in urine [57,63], and was combined with MS [57,59,60] for selective detection.

A supported liquid membrane (SLM) device is an on-line combination of LLE and dialysis. The analyte is extracted from an aqueous (donor) phase into an organic liquid immobilised in a porous membrane, followed by back-extraction into a second aqueous (acceptor) phase [64–66]. An efficient on-line LLE clean-up is thus achieved using a minimum of organic solvent. The technique was connected on-line to CE and successfully used for the analysis of the drug bambuterol in plasma by Pálmarsdóttir et al. [65,66].

3.4. Electrophoretic preconcentration

A unique advantage of the electrophoretic separation principle is the possibility to apply electrophoretic preconcentration, to increase the sample loading without sacrificing peak efficiency. Charged compounds can be concentrated (stacked) across an electrolyte discontinuity, either by isotachophoretic concentration (see Section 2.1), or by sample stacking due to the use of a sample buffer with a lower ionic strength than the electrophoretic buffer. ITP can be used prior to CE in a coupled column (dual column mode), or in the separation column (single column mode) as tITP. A careful choice of leading, terminating, and background electrolytes is required in performing tITP. In sample stacking, the electric field in the low-conductivity sample solution is higher than in the electrophoretic buffer. As a result, ions within the sample rapidly migrate to the interface between the sample and electrophoretic buffers. After passing the interface, the local field strength decreases, which causes the ions to slow down and stack in a zone much narrower than the original sample zone.

To remove the sample buffer from the capillary, high voltage with reversed polarity can be applied to the column after sample injection. The polarity of the voltage is then switched back to normal configuration and separation is performed.

Several variations of this concept are possible. In field amplified sample injection (FASI) a diluted sample buffer is used having the same composition as the background buffer, which induces an enhanced electric field strength at the injection point as the high voltage is applied. A short plug of diluted sample buffer is injected into the capillary prior to sample injection. After injection of the sample in the sample buffer, the inlet of the end of the column is transferred to the high-concentration running buffer to start the separation process. Using FASI, only positive ions can be analysed, since the high field strength at the injection point will push away the negative ions. By

polarity-switching FASI, both positive and negative ions can be separated in one run. A short plug of diluted buffer is injected before the sample. The positive ions are injected into the capillary first using a positive voltage with respect to the other end of the column. The negative ions are then injected using reversed polarity. After sample injection the polarity of the electrodes is switched back to the normal configuration and separation is performed as in normal FASI [12].

4. Applications of CE in the bioanalysis of drugs

The advantages of CE have stimulated many investigators to develop a variety of CE methods for the analysis of drugs and drug metabolites in biofluids. The pretreatment methods earlier discussed have all been successfully applied for clean-up and preconcentration. Many reviews have appeared dealing with the bioanalysis of drugs using various CE approaches [67–71], dealing specifically with analysis by MEKC [72–74], and dealing with specific classes of drugs such as diuretics [75] and cardiovascular drugs [76].

Here we present an overview of the applied CE methods on the analysis of drugs in body fluids described in the literature. The published methods are presented in Table 2, which is striven to be a complete overview. Sample pretreatment methods and remarks on concentration sensitivity or LOD values are included. For better accessibility of the table, drug substances are listed in alphabetical order.

Many methods have been described in the literature, which obviously cannot all be described here separately. Therefore, a selection of representative and/or innovative methods will be discussed below, which covers all pretreatment methods, separation modes, and detection methods which have been applied.

Although the direct injection of biofluids has been successfully applied, a clean-up or preconcentration method is often required to achieve sufficient sensitivity. Protein removal and extraction methods (LLE and SPE) are the most commonly applied pretreatment methods. The improvement of LOD values by extracting drugs from the matrix was clearly demonstrated by von Heeren et al. [77]. Different pretreatment methods were compared for the determination of the antifungal agent fluconazole in plasma by MEKC with UV detection (see Fig. 2). Direct injection of plasma was shown to permit the determination of fluconazole levels of 5 μg/ml. Injection of the supernatant after protein precipitation with acetonitrile resulted in an LOD of 5 μg/ml as well. With LLE employing dichloromethane, the LOD was about 1 μg/ml. When using SPE, drug levels as low as 100 ng/ml could be determined unambiguously.

Molteni et al. [78] compared the use of DSI and SPE in the analysis of methadone and its primary metabolite in the urine obtained from eight individuals undergoing methadone therapy. MEKC analysis could not determine the compounds, however, they separated rapidly under CZE conditions. After DSI methadone could be determined in only six urines, and the LOD value was 2 μg/ml. After an SPE clean-up, sensitivity was improved 100 times as drug concentrations down to ca 20 ng/ml could be monitored. Also, the drug and its metabolite could be determined in all urines, which shows that extraction is preferred for an unambiguous confirmation by CZE.

Nunez et al. [79] evaluated the use of MEKC for the separation of seven sulfonylurea drugs in urine. An LOD of 50 ng/ml was achieved, after a clean-up using a combination of LLE and SPE. The authors will attempt to decrease the effort required in the pretreatment procedure, by the development of an on-line extraction method.

Pálmarsdóttir et al. [80] described the use of analyte stacking based on field amplification enhancement as a means to increase the concentration sensitivity. Plasma samples were pretreated with SPE and analytes were separated by a coupled capillary LC-CZE device (see Section 3.3). Terbutaline enantiomers were separated using β-CD as a chiral selector. Microliter volumes could be injected into the capillary without significant loss of separation performance. A sensitivity gain of ca 400 could be obtained by concentrating a 3-ml sample as compared with a 7-nl sample.

Jumppanen et al. [81] developed a method to analyse 15 diuretics in urine and serum used in the

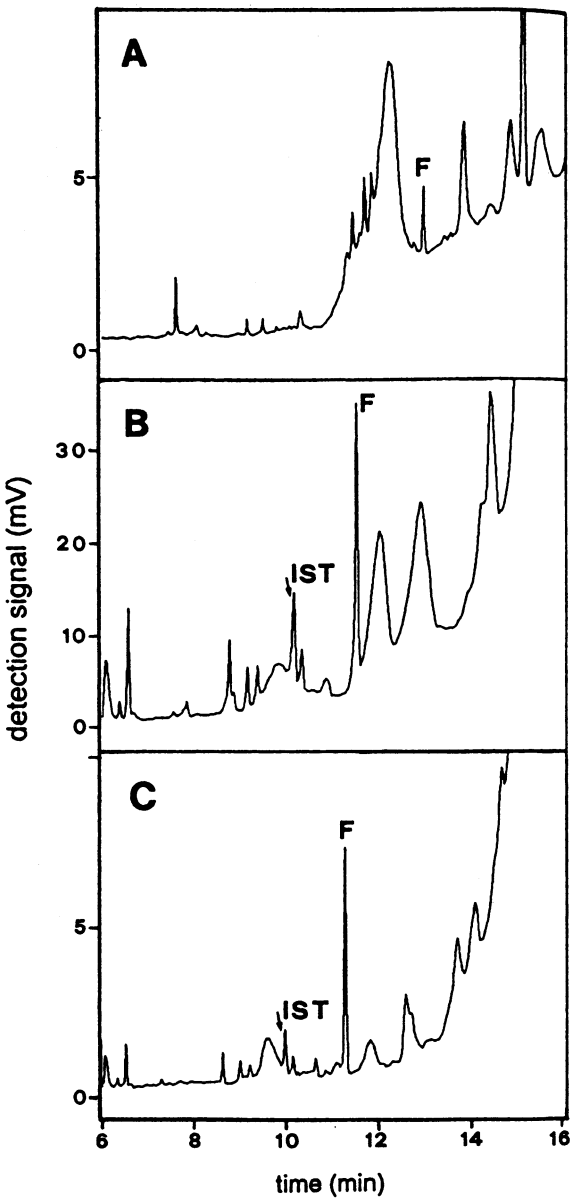

Fig. 2. Electropherograms obtained after different sample pretreatments having: (A) a patient plasma sample containing 22.1 μg/ml of fluconazole which was injected directly into the capillary; (B) bovine plasma containing 100 μg/ml of fluconazole and 40 μg/ml of internal standard after protein precipitation with acetonitrile; and (C) bovine plasma containing 25 μg/ml of fluconazole and 10 μg/ml of internal standard after extraction with dichloromethane. Conditions: for (A) capillary length 64 cm, 75 μm I.D., electric field 260 V/cm, detection at 200 nm; for (B) and (C) capillary length 70 cm, 75 μm I.D., electric field 250 V/cm, detection at 190 nm. Buffer: 75 mM SDS, 10 mM Na_2HPO_4, and 6 mM $Na_2B_4O_7$. [77]

treatment of cardiovascular diseases (see Fig. 3). Urine was treated using SPE for the removal of endogenous compounds. Serum samples were treated with methanol to precipitate proteins prior tot extraction. Samples were then analysed using CZE with UV detection. Owing to the heterogeneity of the family of diuretics two consecutive runs at different pH were required. Using this method urine and serum could be effectively screened for the presence of diuretics in < 30 min. No comments were made on the achieved sensitivity.

The group of Tomlinson and Naylor [36,57,63] has developed an on-line extraction method for the analysis of the neuroleptic drug haloperidol and its metabolites in urine (see Section 3.3). Analytes were further concentrated by sample stacking conditions. Compounds were detected with mass spectrometry. The use of an on-line chromatographic membrane preconcentration (mPC) device in conjunction with analyte stacking afforded optimal performance and no comprise in CE-MS performance.

In sample pretreatment steps we focus on removal of interferences from the matrix, compatibility of the solvent with the analytical system, and preconcentration. The success rate of the last element is crucial for widespread use of capillary electrophoretic separation techniques for the analysis of drugs and metabolites in biological matrices. Three approaches that may be particularly useful for preconcentration of particular drugs and removal of inorganic anions are supported liquid membrane techniques (SLM), immunoassay solid phase extraction (IASPE), and molecular imprint solid phase extraction (MISPE). Pálmarsdóttir et al. [65,66,82] showed that using SLM with 6-undecanone or a mixture of di-*n*-hexyl ether and tri-*n*-octyl phosphine as membrane liquid bambuterol could be readily extracted from plasma. Sample stacking substantially contributed to the sensitivity, due to the low ionic strength of the injected extract. Antibodies raised against a particular drug and immobilised on a carrier material can be used for a highly selective extraction of extremely low concentrations of that drug from large sample volumes. This IASPE approach has been successfully used off-line and on-line with liquid chromatographic

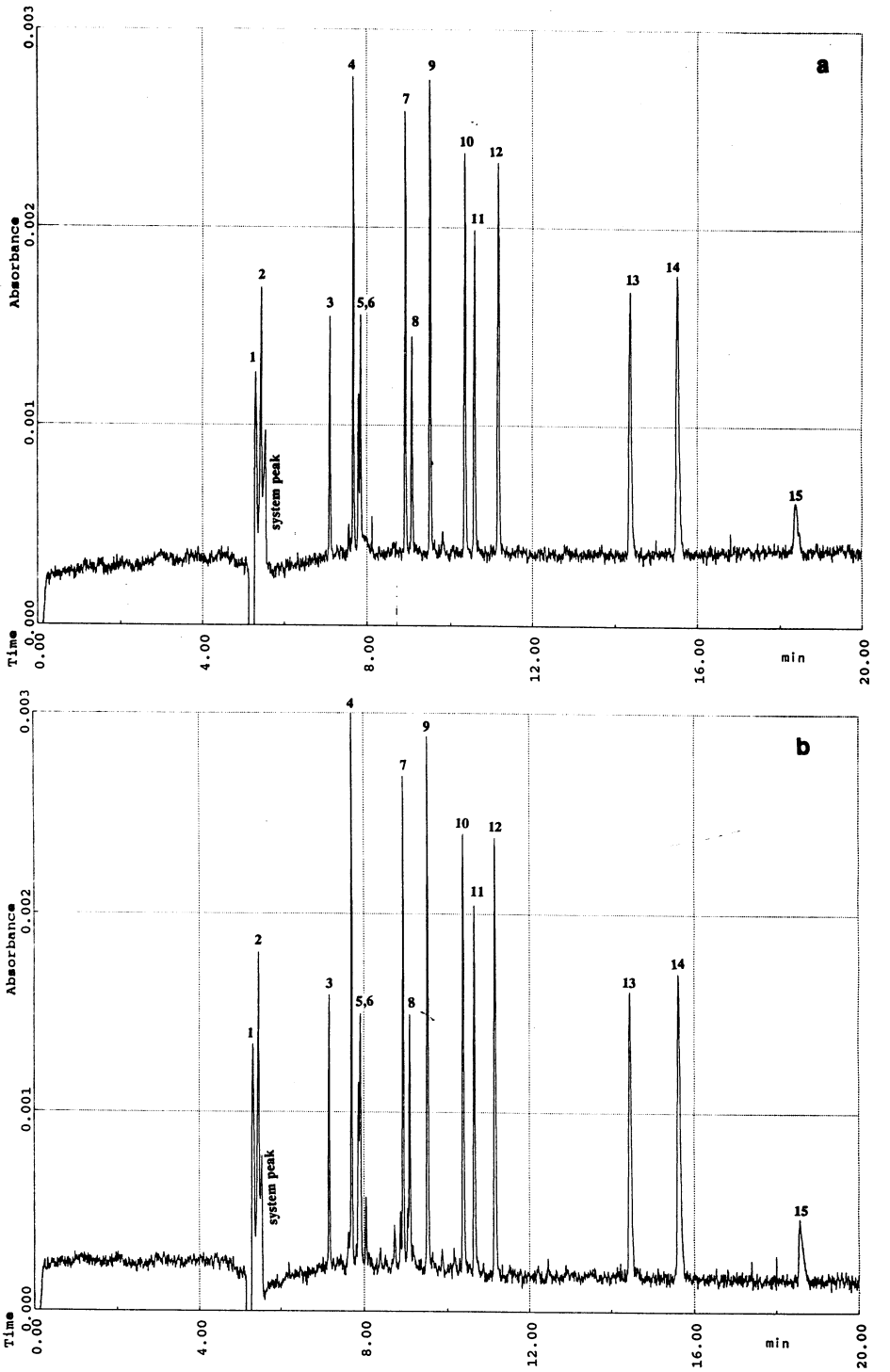

Fig. 3. Electropherograms of (a) serum and (b) urine spiked with 15 diuretics present at concentrations of 10 ppm. Conditions: capillary length 67 cm, 50 μm I.D., 25 kV, 20°C, CAPS 0.06 M, pH 10.6, 220 nm [81].

systems. It is no surprise that the IASPE approach also serves as an excellent preconcentration step for the CE analysis of dexamethasone and flumethasone in horse urine yielding limits of detection of 1.2 and 2.7 ng/ml, respectively [83]. If the antibodies are highly selective, the meaning of a separation step with CE in an analytical procedure will become questionable. This implies that particular antibodies or mixtures thereof that can collect a complete group of analytes are potentially interesting for preconcentration. Selective recognition and extraction can also be achieved by the use of molecular imprints of the analyte or of a structurally related analogue. Walshe et al. [84] showed that this concept works for the extraction of 7-hydroxycoumarin from urine and quantitation by CZE. The requirements of molecular imprints for application in different analytical techniques are described by Ensing and De Boer [85].

As can be seen from Table 2, the analysis of one specific drug can sometimes be achieved by choosing from a variety of pretreatment methods and CE modes. To illustrate this, two cases will be discussed in which a drug (codeine or theophylline) is analysed by different groups using different CE modes and pretreatment methods. Methods are being compared on the basis of concentration sensitivity.

The opiate codeine has been analysed by several investigators in urine, serum, and plasma by CZE and MEKC with UV detection using different pretreatment methods. Chee et al. [86] used CZE for the separation of 17 basic drugs of different classes, among which codeine. Drugs were extracted from urine and plasma by LLE. Measured concentrations were in the range of 1 μg/ml, but no comments were made on the LOD. Hyotylainen et al. [87] described a method for the screening and determination of opiates, including codeine in serum and urine. Adequate separation was achieved with MEKC. Serum proteins were precipitated with methanol, whereas urine samples were injected directly. The LOD for codeine was ca 1 μg/ml. Wernly et al. [88] analysed a mixture of many substances among which codeine. Analytes were extracted by SPE and separated by MEKC with DAD detection. Detection limits of 100 ng/ml were obtained. The lowest LOD for codeine was reached by Taylor et al. [89] who separated a mixture of opiates by CZE. Drugs were extracted from urine by SPE, and an LOD of 7 ng/ml was obtained for codeine.

Theophylline is one of the primary metabolites of caffeine and is widely used as a bronchodilating agent in the treatment of asthma. The analysis of theophylline using CE has been described by a number of groups. Lee et al. [90] separated theophylline and its analogues by MEKC in plasma. The analytes were extracted by LLE. A linear response was obtained for concentrations ranging from of 5–60 μg/ml. The effects of pH, surfactant concentration, applied voltage, and the temperature on the separation were studied. The authors indicate that this technique could be used as a reference or routine method for theophylline in therapeutic drug monitoring. Shihabi et al. [40] studied the effect of acetonitrile deproteinisation for the analysis of drugs by CZE, using theophylline in serum as an example. Acetonitrile was shown to be a good method for sample preparation, which also introduced a stacking effect permitting the injection of a larger volume into the capillary. The linearity of the method was 3–40 μg/ml. Johansson et al. [91] described the determination of theophylline in plasma by MEKC. Plasma proteins were precipitated with acetonitrile prior to injection. The method permitted the determination of theopylline at therapeutic concentrations of 4.5–20 μg/ml with high precision. The LOD obtained was 1.8 μg/ml, which was in the same order as the LOD obtained with a standard HPLC method (0.5 μg/ml). Reproducibility was in the same order as the HPLC method. Thormann et al. [92] reported that substituted purines, among which theophylline, were separated in one run with MEKC. Serum and saliva samples could be injected directly. Urine samples were pretreated with SPE. Concentration sensitivity was between 0.9–3.2 μg/ml. A combination of immunoassay with MEKC was described by the same group of Thormann [17,93,94]. Various drugs, among which theophylline, were monitored in serum. The assay was based on short time incubation of serum with a mixture of antiserum, containing the antibody raised against the drugs

and fluorescein labelled drugs (tracer). The mixture was analysed with MEKC using LIF detection, which separated free tracer and the antibody-tracer-complex. Zhang et al. [95] separated theopylline in serum with MEKC. Samples were injected directly and the method was linear from 2 to 28 μg/ml was obtained. Later the same group reported the analysis of theophylline and its metabolites in urine using CZE [96]. SPE was used for sample preparation, and a LOD of 1 μg/ml was reported. An unusual method for sample preparation was described by Linhares et al. [97], who performed a pharmacokinetic study on theophylline. Rats were impregnated with capillary ultrafiltration probes for in vivo sampling. Tissue fluid was collected every 15 min at a rate of 1–3 ml/min. Ultrafiltrates were free of protein and cell matter, and could be directly injected and analysed by MEKC. Tagliaro et al. [98] described the analysis of theophylline in serum. The sample pretreatment was rapid and simple, consisting only of deproteinisation by methanol. Linearity was good in the range of 2–120 μg/ml, and the LOD was 2 μg/ml. Sensitivity was lower than in HPLC, but comparable to enzyme immunoassays. Separation efficiency was at least 20 times higher than in HPLC, making this CE technique a promising alternative method for HPLC.

The above reported LODs of theophylline are all values which normally should not be too difficult to obtain. However, it was probably not the main goal of all researchers to reduce the LOD as much as possible.

Practically all bioanalytical methods are being performed in the CZE or MEKC mode, however, the use of ITP has been reported [99,100]. Caslavska et al. [99] compared the use of CZE, MEKC, and ITP for the rapid screening and confirmation of drugs in serum and urine of patients with medical drug overdoses. The drugs studied in these CE modes were salicylate and paracetamol. Serum and urine samples were obtained from two patients at the emergency unit. Urine samples were diluted whereas serum samples were ultrafiltered prior to injection. It was mentioned that ITP required very careful selection of buffer conditions and higher analyte concentrations than CZE and MEKC. In ITP, zone identification by migration time is not possible, since the detection time is dependent on the sample matrix. DAD was needed for unambiguous zone identification.

Sádecká et al. [100] presented a method for the analysis of diuretics and β-blockers in serum and urine using ITP with conductivity detection (see Fig. 4). SPE was used to almost totally remove endogenous compounds. LOD values ranging from 32–46 ng/ml were obtained. It was concluded that ITP could easily be used for the determination of the compounds in serum and urine.

LIF detection is known to be a sensitive method. Reinhoud et al. [101] developed a very sensitive method for the bioanalysis of some anthracycline antibiotics that are used for the treatment of cancers. Spiked plasma samples were pretreated and concentrated by LLE in two steps. Separation was performed with CZE under extensive sample stacking conditions. Using CZE-LIF, LOD-values as low as 125–250 pg/ml were obtained. Recently, Frost et al. [102] presented a method for the analysis of LSD in blood using CZE with LIF detection. LSD was extracted from whole blood samples in two steps by LLE (see Fig. 5), yielding an LOD of 100–200 pg/ml.

Aumatell and Wells [103] showed that high sensitivity can be achieved using UV detection. The development of an assay for the analysis of 26 tricyclic drugs in urine using MEKC with sodium taurodeoxycholate as a surfactant was described. Drugs were extracted from the matrix by LLE. Detection limits down to 4 ng/ml were reported.

In the detection step sensitivity can be improved as well. Tandem mass spectrometry can yield excellent concentration sensitivities as shown by Bach and Henion for methylphenidate [104]. However, the selection of buffers is a critical factor which implies that there will be a trade-off between the separation efficiency and the detector response. Another approach is the complexation of the analyte with a second compound in media that increase the detector response. Tjørnelund and Hansen [105] showed the complexation of tetracyclines with magnesium-ions in non-aqueous

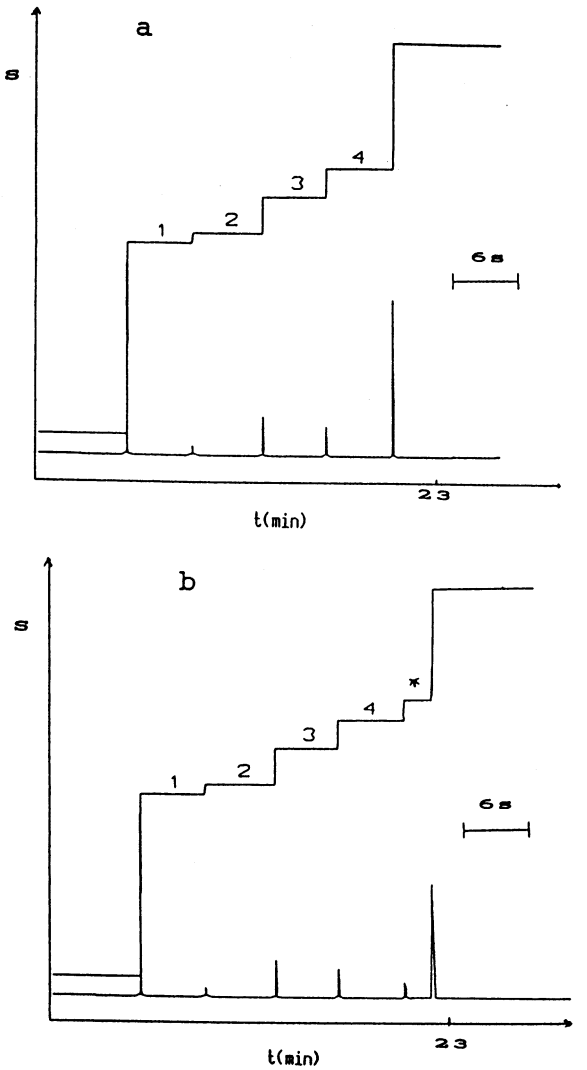

Fig. 4. Isotachopherograms obtained from extracts of (a) serum and (b) urine samples containing a mixture of five drugs (1, amiloride; 2, metoprolol; 3, deacetylmetipranolol; 4, labetalol; and 5, furosemide) at concentrations of 200 ng/ml each. Leading electrolyte, 10 mM sodium morpholinoethanesulfonic acid (pH 5.5) – 0.1% methylhydroxyethylcellulose; terminating electrolyte, 5 mM glutamic acid; driving current, preseparation column 150 μA, analytical column 20 μA (separation) and 10 μA (detection). [88]

media increased the fluorescence with a factor of 9 or even 34 in *N*-methylformamide and dimethylformamide, respectively.

Verapamil is a drug having antiarythmic, antianginal, and antihypertensive properties. Its metabolite norverapamil contributes to the therapeutic effect. As many synthetic drugs with a chiral centre, verapamil occurs in a racemic mixture. Dethy et al. [106] developed a method to determine simultaneously the verapamil and norverapamil enantiomers in plasma using CZE. Among the cyclodextrins tested as a chiral selector, only trimethyl-β-cyclodextrin was suitable to resolve the four enantiomers. The LOQ was as low as 2.5 ng/ml. Selectivity, linearity, precision, and accuracy were evaluated before the chiral method was successfully implemented for routine use to simultaneously determine the four enantiomers in several thousands of human plasma samples.

Most reports describe the analysis of drugs in matrices as urine and serum or plasma. Hair is a relatively new and upcoming matrix for the analysis of drugs of abuse [107–111]. Several drugs undergoing chronic use become embedded in the hair and remain fairly unaltered throughout the entire hair lifetime. The analysis of a few centimetres of hair can provide information on the subjects drug intake over several months. This is particularly important if one considers that usually drugs disappear from blood and urine within days. Tagliaro et al. [108–110] determined cocaine and its metabolites and morphine in hair. The drugs were extracted from 100 mg hair by LLE

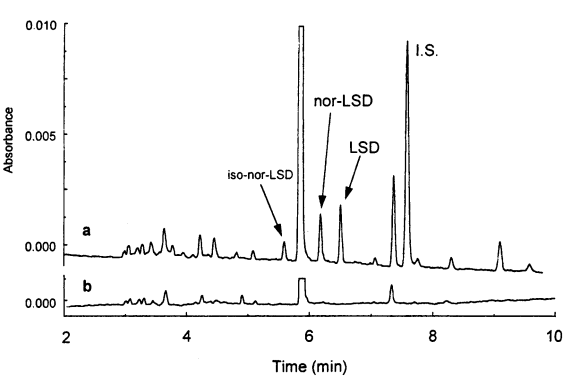

Fig. 5. Electropherogram of (a) human blood spiked with LSD and nor-LSD (1 ng/ml) and internal standard, (b) blank blood. Conditions: capillary length 37 cm, 50 μm I.D., 25 kV, 20°C, 250 mM citrc acid (adjusted to pH 4.0 with 250 mM sodium acetate)-methanol (10:70 v/v), laser induced fluorescence detection at 325 nm. [102]

and SPE. A CZE method with DAD was compared to analysis with HPLC. Levels of cocaine and morphine in hair as low as 0.15–0.30 ng/mg were detected by CZE, while HPLC allowed the determination of concentrations lower by one order of magnitude (0.015 ng/mg). However, the HPLC method did require an additional extraction. Sample throughput of CZE (7–10 injections/h) was higher than HPLC (2 injections/h).

The ultimate goal in toxicological and forensic analysis is to screen for as many different drugs as possible. Since even in one class of pharmaceuticals the heterogeneity can be enormous, this is a real challenge. Not all drugs can be resolved and analysed in one run. For the analysis of many classes of drugs in a single urine specimen, either extracts have to be analysed sequentially in different buffers, or multiple, specific extraction schemes have to be employed [88]. Several groups have presented methods for the separation of multiple classes of drugs in biofluids [1,4,86,88].

Chee et al. [86] described the efficient separation of 17 basic drugs of different classes in spiked urine and plasma samples. Compounds were extracted by LLE prior to injection, separated by CZE, and detected by UV absorbance. Separation was completed in 11 min. The use of CZE restricted the analysis to the separation of basic drugs only. Concentrations were measured in the range of 0.45–1.41 μg/ml.

Wernly et al. [88] presented a method for the confirmation testing of barbiturates, hypnotics, amphetamines, opioids, benzodiazepines, and metabolites of cocaine in a single aliquot of human urine. The samples were extracted using mixed-mode SPE and elution in two or three steps, thus separating acidic, neutral and basic compounds. The mixed-mode adsorbents exhibits hydrophobic and ionic interactions. The analytes are then sequentially analysed by MEKC with DAD detection. Excellent recoveries (80–90%) and detection limits (100 ng/ml) were obtained.

Schmutz et al. [1] showed the analysis of 25 compounds in serum, including antiepileptics, anti-inflammatory and β-blocking drugs. Using MEKC with DAD detection, samples were either injected directly (anti-inflammatory drugs), or after ultrafiltration (β-blockers) or LLE (anti-epileptics). The impact of physico-chemical drug properties on elution and peak shape was discussed. Analysis were made at drug concentrations of 50 mM. Seven chemically and pharmacologically different drugs were analysed in one aliquot of spiked serum using DSI.

Hudson et al. [3] reported the routine use of CZE in a forensic laboratory as a screening technique for the presence of 550 basic and 100 acidic drugs in blood. Prior to analysis, the analytes were extracted by either LLE (basic drugs) or SPE (acidic drugs). Using the electrophoretic mobility as an identification parameter, good reproducibilities were achieved (CV = 0.4%). This report shows that CE has a good chance to compete with chromatographic techniques for routine bioanalysis.

5. Conclusions

In this review we have shown the high versatility of CE techniques and their potential for the analysis of drugs in various biofluids. The well known advantages of capillary electrophoretic techniques are high speed analysis, high efficiency, and a separation mechanism other than in chromatographic methods, which can be easily altered by the use of various buffer additives. The emphasis of the review was laid on the strategies developed to overcome one of the main disadvantages of CE: the high LOD when used in combination with UV detection. Numerous groups have lowered the LOD either by the use of more advanced detection methods, or by the application of off-line or on-line preconcentration techniques.

Two aspects are most important to be considered when choosing an appropriate preconcentration method: the factor of concentration achieved and the extra time required prior to the actual analysis. The final choice usually should be a compromise between these aspects and depends on the actual application. For instance, a method resulting in an LOD of nM to pM using several extraction steps could take too much time to make it as a routine method.

We have summarised the concentration factor and the extra analysis time for the most generally

Table 3
Concentration factor, extra time required prior to the actual analysis, and number of samples treated in that time for some generally used preconcentration methods[a]

Method	Concentration factor	Extra time required (min)	Number of samples
DSI	1	0	1
Protein precipitation	<1	15 (5–20)	1–10
LLE	10 (5–20)	15 (5–20)	1
SPE	50 (10–100)	15–60	1–96
On-line extraction	50 (10–100)	5–10	1
Stacking, FASI	50 (10–100)	5–10	1

[a] DSI, direct sample injection; LLE, liquid–liquid extraction; SPE, solid phase extraction; FASI, field amplified sample injection.

used and previously described pretreatment methods in Table 3. The extra time required per sample depends on the number of samples that can be treated simultaneously. The best approach to achieve a sufficiently low LOD within acceptable time is, in our opinion, the combination of several preconcentration methods. This could be, for instance, an off-line extraction method followed by sample stacking within the capillary. The use of several preconcentration steps successively may result in a lower LOD. Also, the total analysis time will increase, but it does not necessarily have to lead to an unacceptably long run time when some automation or parallel pretreatment is applied.

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