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Capillary electrophoresis and related techniques in the determination of drugs of abuse and their metabolites

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ABSTRACT

Nowadays, so-called drugs of abuse (DOAs) are widely used and pose an undeniable problem at a social level. These compounds have incalculable consequences for society, such as the cost of medical treatment, higher incidence of criminality and economic repercussions. Innovative, modern separation and determination techniques are therefore required for analysis of DOAs. In this work, we review methods based on capillary electrophoresis applied to the determination of illicit drugs and new psychoactive substances in different kinds of matrix, published between 2007 and 2015. We critically discuss the main strengths and weaknesses of all of these approaches by means of relevant applications in clinical practice, forensics, pharmacokinetics, metabolic monitoring and environmental analyses.

Contents

1. Introduction

Drugs of abuse (DOAs) is a term given to drugs used excessively on a persistent or sporadic basis inconsistent with or unrelated to acceptable medical practice, the abuse of which can generally lead to physical and mental damage, and, in some cases, to dependence and addiction [1]. Consequently, there is a need for the continuous development of methods for the efficient determination of DOAs and their metabolites in biological samples. The matrices most commonly used for this purpose are urine, saliva, hair, blood and sweat, while breath is most commonly used for alcohol testing. Each matrix has particular strengths and weaknesses, which have already been discussed elsewhere [2,3]. The choice of sample is influenced by sample-collection procedures, costs, windows of drug detection and the degree of incorporation of drugs and their

Abbreviations: ATS, Amphetamine-type stimulant; BGE, Background electrolyte; BZD, Benzodiazepine; C^4D, Capacitively-coupled contactless conductivity detection; CD, Cyclodextrin; CE, Capillary electrophoresis; CEC, Capillary electrochromatography; CSEI, Cation-selective exhaustive injection; CZE, Capillary-zone electrophoresis; DLLME, Dispersive liquid-liquid microextraction; EC, Electrochemical; ECL, Electrochemiluminescence; EKS, Electrokinetic supercharging; ESI, Electrospray ionization; FASI, Field-amplified sample injection; i.d, Internal diameter; IT, Ion trap; LIF, Laser-induced fluorescence; LLE, Liquid-liquid extraction; LOD, Limit of detection; LOQ, Limit of quantitation; LVSS, Large-volume sample stacking; MEKC, Micellar electrokinetic chromatography; MEEKC, Microemulsion electrokinetic chromatography; MS, Mass spectrometry; MS/MS, Tandem mass spectrometry; MSPE, Magnetic solid-phase extraction; QqQ, Triple quadrupole; SDME, Single-drop microextraction; SDS, Sodium dodecyl sulfate; SPE, Solid-phase extraction; TOF, Time-of-flight; RSD, Relative standard deviation; USAEME, Ultrasound-assisted emulsification microextraction; UV, Ultraviolet.

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metabolites into the biological matrix, among other factors. However, as a consequence of their use, these compounds can reach wastewater-treatment plants and can eventually be released to environmental waters [4]. As a result, DOAs have been found in different surface waters at ng/L-concentration levels [4,5].

Identification and determination of the broad range of DOAs and their metabolites in different kinds of matrix has become a challenging task for analytical chemists. In this context, organizations such as European Workplace Drug Testing Society, Society of Hair Testing and Substance Abuse, and Mental Health Services Administration, have established cut-off concentrations, above which any analytical finding indicates positive for drug use in order to avoid false-positive results. Analytical methods must therefore provide accurate, reliable results from the test sample. In this regard, several analytical procedures have been reported in the scientific literature, most of which are based on gas chromatography (GC) or liquid chromatography (LC) [6–8]. Besides these techniques, capillary electrophoresis (CE) and related techniques, such as micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC) and capillary electrochromatography (CEC), have also been successfully applied for this purpose, shown by the large number of applications developed [8–11]. This increasing interest in CE-based techniques as analytical tools is certainly based on their high efficiency, high resolution power, low reagent and sample consumption, automation and the fact that they represent low-cost candidates, compared to other chromatographic techniques.

However, the widespread use of chromatography-based methodologies is due to limitations that CE-based methodologies display in relation to their relative lack of sensitivity. The development of strategies that increase this sensitivity has therefore become an important issue and numerous approaches have been published [12–14].

The goal of this review is to provide an overview of the state of the art and the latest trends of the CE-based methods proposed since 2007 to the present for the determination of DOAs and their metabolites. Recently, Cruces-Blanco et al. [15] reviewed the use of CE and related techniques for the analysis of DOAs and their metabolites in biological specimens of interest in forensic toxicology, particularly blood, urine and hair samples, focusing on some sample-preconcentration methods to enhance sensitivity and the use of different detection modes.

Our review provides the most relevant improvements and innovations in the CE-based methodologies for the analysis of a range of commonly-encountered illicit substances in different types of matrix, such as seized materials, and biological and environmental samples. We compare the different electrophoretic methods published during the period covered by this review in terms of separation efficiency, sensitivity and time of analysis and/or potential for automation. We also address the most recently introduced sample preparation and preconcentration techniques. We critically discuss the main strengths and weaknesses of all of these approaches by means of relevant applications.

We divide the article into drug classes with comprehensive, up-to-date tables, providing the reader with more detailed information related to the new trends in the analysis of a specific group of compounds in which she/he can be interested by means of CE-based methodologies. We selected stimulants, such as amphetamine-type stimulants (ATSs) and cocaine, depressants, such as barbiturates and benzodiazepines (BZDs), opium-related compounds, cannabinoids and new psychoactive substances as the target drugs because they are the most frequently-abused, illicit drugs.

2. Stimulants

Stimulants are a class of psychoactive substances that increase activity in the brain, causing temporary changes in alertness, mood and awareness. These drugs were historically used for treatment (e.g., respiratory problems, obesity, and neurological disorders), but, as their potential for abuse and addiction became apparent, medical use of stimulants began to decrease. Nowadays, stimulants are prescribed to treat only a few health conditions, such as attention deficit hyperactivity disorders (ADHDs), narcolepsy and, occasionally, depression. The most commonly-used street drugs that fall into this category are ATSs and cocaine [16]. With this in mind, this section consists of two parts, the first focusing on ATSs and the second on cocaine, with the CE-based analytical methods currently used being reviewed and summarized in Tables 1 [17–33] and 2 [34–44], respectively.

In view of the tendency for polydrug consumption, multi-analyte methods have also been reported, including those with different chemical and physical properties. In this respect, Table 1 shows analytical methods that determine ATSs together with other drugs, while Table 2 shows methods designed to detect cocaine together with other drugs.

2.1. Amphetamine-type stimulants

ATSs are a group of drugs mainly including amphetamine and methamphetamine. Besides these drugs, other substances also fall into this group, such as fenethylline, ephedrine, pseudoephedrine, methylphenidate and 3,4-methylenedioxymethamphetamine. The abuse of ATSs is global and a growing phenomenon, and, recently, there was an increase in the production and abuse of ATSs worldwide [16], so analytical methods for their efficient, accurate determination are needed.

These illicit substances have been widely studied by CE, with capillary-zone electrophoresis (CZE) [17–24,27–31] being the most commonly used separation mode, although MEKC [25,26,32] and MEEKC [33] have also been reported as approaches for separating these compounds. The most extensively-used detector is ultraviolet (UV) but other detectors, such as electrochemical (EC) [17], laser-induced fluorescence (LIF) [18], mass spectrometry (MS) [19] and capacitively-coupled contactless conductivity (C^4D) [20,21], have also been employed for this purpose.

With respect to the samples analyzed, the existing literature mainly focuses on biological samples, particularly urine [17–19,21,22,27–30,32], as well as blood [18], saliva [24] and hair [26]. However, there are other types of sample, such as street-grade tablets, banknotes, Kraft paper, plastic bags, silver paper and drug seizures, which have also been studied [20,23,31]. In the tables in this article, unless otherwise indicated, the term urine refers to human urine.

The use of CE for chiral analysis is also gaining momentum. In this respect, a review of the chiral analysis of ATSs in biological samples was published in 2011, including a discussion of CE applications [45]. To summarize, the most commonly-employed method for this purpose is to include native cyclodextrins (CDs) in the background electrolyte (BGE), with β-cyclodextrin (β-CD) [45] being the most widely used, although crown ethers, such as 18-crown-6-tetracarboxylic acid [21,30], have also been used. Chemically-modified CDs, especially the sulfated derivatives of β-CD [i.e., sulfated-β-CD, hydroxypropyl-β-CD, heptakis-(2,6-*O*-dimethyl)-β-CD and heptakis-(2,3,6-*O*-trimethyl)-β-CD], have also been shown to provide good enantioselectivity [20–22,24].

Recently, a research paper was published dealing with the optimization of a fast separation technique (8 min) for six enantiomeric pairs of ATSs using highly-sulfated-γ-CD as the chiral selector [23]. To improve control of the electroosmotic flow and to avoid the consequent shift in analyte peaks, and to improve the reproducibility of migration times for analytes by CE, four types of commercial chemically-modified capillaries were evaluated, namely, FunCap-CE/Type D (possessing diol groups), Type A (amino groups), Type C (carboxyl groups) and Type S (sulfate groups). The Type S capillary

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Table 1
Overview list of capillary electrophoresis (CE) methodologies for determination of amphetamine-type stimulants (ATSs)

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Methamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxymethamphetamine	**CZE** Fused-silica capillary: 50 cm × 25 μm i.d.; BGE: 50 mM phosphate buffer; detection buffer: 100 mM phosphate buffer (pH 9.0) + 5 mM Tris(bipyridine)ruthenium (II) chloride; separation voltage: 25 kV; temperature: 25°C	EC/ECL	1.6 10^{-7}–3.3 10^{-8} mol/L [a]	Urine	LLE with ethyl acetate	[17]
Pseudoephedrine; ephedrine; amphetamine; methamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxymethylamphetamine	**CZE** Fused-silica capillary: 50 cm × 75 μm i.d.; BGE: 20 mM borate buffer + sodium hydroxide (pH 12.0); separation voltage: 25 kV; temperature: 25°C	LIF	0.2 ng/mL [b]	Blood and urine	SPE with Oasis hydrophilic-lipophilic-balance cartridge	[18]
2,5-Dimethoxy-4-methylamphetamine; 2,5-dimethoxy-4-ethylamphetamine; 2,5-dimethoxy-4-propylamphetamine	**CZE** Fused-silica capillary: 120 cm × 50 μm i.d.; BGE: 10 mM phosphate buffer (pH 4.5); separation voltage: 10 kV; temperature: 25°C	MS	3.9–4.6 ng/mL [b]	Urine	SPE with Bond Elut C_{18} cartridge	[19]
2-(4-Methoxyphenyl) ethylamine; 2-bromo-N-methylbenzylamine; 2-methoxy-N-methylaniline; 2-phenethylamine; 5-aminomethyl-7-chloro-1,3-benzodioxole; 2-methoxyphenethylamine; 3-methyl-N-methylbenzylamine; amphetamine; dextroamphetamine; methamphetamine; 3,4-methylenedioxymethamphetamine	**CZE** Fused-silica capillary: 90 cm × 50 μm i.d.; BGE: 25 mM sodium acetate + 75 mM acetic acid (pH 4.55) + 30 mM hydroxypropyl-β-CD; separation voltage: 30 kV; temperature: 25°C; UV detection at 200 nm	C^4D-UV	1 300 ng/mL [a]	Street-grade tablets	–	[20]
Amphetamine; methamphetamine; ephedrine; pseudoephedrine; norephedrine; norpseudoephedrine	**CZE** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 500 mM acetic acid (pH 2.6) + 5 mM carboxymethyl-β-CD + 5 mM 18-crown-6-tetracarboxylic acid; separation voltage: 15 kV; temperature: 25°C	C^4D	410–940 ng/mL [a]	Urine	LLE with ethyl acetate	[21]
Methamphetamine; amphetamine; dimethylamphetamine; β-hydroxymethamphetamine; ephedrine; norephedrine; methylephedrine; 3,4-methylenedioxymethamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxy-N-ethylamphetamine	**CZE** Poly(vinyl alcohol)-coated capillary (homemade) and chemically modified capillary with diol groups (commercial): 64.5 cm × 50 μm i.d.; BGE: 125 mM Tris (pH 6.15) + 6 mM heptakis(2,6-di-*O*-methyl)-β-CD + 12 mM β-CD; separation voltage: 30 kV; temperature: 25°C; UV detection at 195 nm	UV	–	Urine	LLE	[22]
Methamphetamine; amphetamine; ephedrine; pseudoephedrine; norephedrine; norpseudoephedrine	**CZE** Chemically modified capillary with sulfate groups (commercial): 32.5 cm × 50 μm i.d.; BGE: 50 mM phosphate buffer (pH 4.5) + 10 mM highly sulfated-γ-CD; separation voltage: 10 kV; temperature: 15°C; UV detection at 195 nm	UV	–	Drug seizures	–	[23]
Threo-methylphenidate	**FASI-CZE** Fused-silica capillary: 40 cm × 50 μm i.d.; BGE: 50 mM phosphate buffer (pH 3.0) + 30 mM triethanolamine + 20 mM 2-hydroxypropyl-β-CD; separation voltage: 20 kV; temperature: 25°C; UV detection at 200 nm	UV	LOQ: 0.5 ng/mL [b]	Saliva	LLE with hexane	[24]
Amphetamine; methamphetamine; 3,4-methylenedioxymethamphetamine	**CSEI-Sweeping-MEKC** Fused-silica capillary: 60 cm × 75 μm i.d.; BGE: 100 mM phosphate buffer (pH 3.0) + 20% methanol + 20 mM SDS; separation voltage: 18 kV; temperature: 25°C; UV detection at 200 nm	UV	0.006–0.008 ng/mL [a]	–	–	[25]

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Table 1 (*continued*)

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Methamphetamine; ketamine; morphine; codeine	**CSEI-sweeping-MEKC** Fused-silica capillary: 40 cm × 50 μm i.d.; BGE: 25 mM phosphate buffer (pH 2.5) + 20% methanol + 100 mM SDS; separation voltage: –20 kV; temperature: 25°C; UV detection at 200 nm	UV	0.05–0.20 ng/mg [b]	Hair	Digestion with 100 mM hydrochloric acid (2 h at room temperature) and LLE with ethyl acetate	[26]
3,4-Methylenedioxymethamphetamine; lysergic acid diethylamide; phencyclidine	**FASI-CZE** Fused-silica capillary: 72 cm × 50 μm i.d.; BGE: 100 mM phosphate buffer (pH 6.0); separation voltage: 25 kV; temperature: 20°C; UV detection at 205 nm	UV	1.00–4.52 ng/mL [b]	Urine	DLLME	[27]
Amphetamine; ketamine; methamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxymethamphetamine	**FASI-CZE** Fused-silica capillary: 60 cm × 75 μm i.d.; BGE: 30 mM phosphate buffer (pH 2.0) + 15% v/v acetonitrile; separation voltage: 20 kV; temperature: 25°C; UV detection at 214 nm	UV	4.0–6.0 ng/mL [b]	Urine	MSPE with magnetite/silica/poly(methacrylic acid-*co*-vinylbenzyl chloride-*co*-divinylbenzene) magnetic microspheres	[28]
Amphetamine; ketamine; methamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxymethamphetamine; 6-acetylcodeine; codeine; heroin	**FASI-CZE** Fused-silica capillary: 60 cm × 75 μm i.d.; BGE: 30 mM phosphate buffer (pH 2.0) + 15% v/v acetonitrile; separation voltage: 20 kV; temperature: 25°C; UV detection at 214 nm	UV	15–105 ng/mL [b]	Urine	MSPE with magnetite/silica/poly(methacrylic acid-*co*-ethylene glycol dimethacrylate) magnetic microspheres	[29]
Amphetamine; *p*-chloroamphetamine; 2-amino-1,2-diphenylethanol	**CZE** Fused-silica capillary: 60 cm × 50 μm i.d.; BGE: 50 mM bis(2-hydroxyethyl)imino tris(hydroxymethyl)methane (pH 4.0) + 0.8 mM 18-crown-6-tetracarboxylic acid; separation voltage: 25 kV; temperature: 25°C; UV detection at 200 nm	UV	0.4–2.0 ng/mL [b]	Urine	SDME	[30]
Heroin; methamphetamine; 3, 4 -methylenedioxymethamphetamine; ketamine	**CZE** Fused-silica capillary: 37 cm × 50 μm i.d.; BGE: 100 mM phosphate buffer (pH 3.23) + 20 mM β-CD; separation voltage: 20 kV; temperature: 25°C; UV detection at 200 nm	UV	0.05–0.20 ng/mL [a]	Banknotes; Kraft paper; plastic bag; silver paper	DLLME	[31]
Ephedrine; norephedrine; pseudoephedrine	MEKC Fused-silica capillary: 64.5 cm × 75 μm i.d.; BGE: 25 mM borate buffer (pH 9.3) + 1.0 mM SDS; separation voltage: 15 kV; temperature: 25°C; UV detection at 194 nm	UV	12–33 ng/mL [b] 1.0–2.8 ng/mL [b]	Urine	DLLME or USAEME	[32]
Caffeine; theophylline; barbital; phenobarbital; morphine; O^6-monoacetylmorphine; O^3- monoacetylmorphine; codeine; ephedrine; heroin; amphetamine; 6-acetylcodeine; methamphetamine; thebaine; papaverine; narcotine	MEEKC Fused-silica capillary: 40 cm × 75 μm i.d.; BGE: 89.8% v/v 5 mM borate buffer (pH 9.5) + 3.3% v/v SDS + 6.0% v/v 1-butanol/0.9% octane; separation voltage: 20 kV; temperature: 25°C; UV detection at 200 nm	UV	1 000–1 500 ng/mL [a]	–	–	[33]

[a] Standard samples.
[b] Real samples.

Table 2
Overview list of capillary electrophoresis (CE) methodologies for cocaine determination

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Cocaine	**CZE** Fused-silica capillary: 48.5 cm × 75 μm i.d.; BGE: 60 mM Tris + 20 mM 2-hydroxyisobutyric acid (pH 8.4); separation voltage: 25 kV; temperature: 20°C; UV detection at 200 nm	UV	200 ng/mL [a]	Banknotes	Ultrasound extraction with acetonitrile	[34]
Amphetamine; methamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxymethamphetamine; 3,4-methylenedioxyethylamphetamine; ketamine; cocaine; cocaethylene; lidocaine; morphine; 6-monoacetylmorphine; heroin	**CZE** Fused-silica capillary: 48.5 cm × 75 μm i.d.; BGE: 95% v/v 20 mM Tris + 0.4% triethylamine (pH 2.5); separation voltage: 25 kV; temperature: 20°C; UV detection at 195 and 208 nm	UV	1.0–5.0 ng/mL [a]	Vitreous humor	LLE with ethyl acetate	[35]
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; cocaine; codeine; 6-acetylmorphine	**In-line SPE-CZE** Fused-silica capillary: 65.5 cm × 50 μm i.d.; BGE: 80 mM phosphate buffer (pH 3.0); separation voltage: 30 kV; temperature: 25°C; UV detection at 200 nm	UV	0.07–0.27 ng/mL [b]	Tap and river water	Filtration	[36]
Cocaine; benzoylecgonine	**In-line SPE-CZE** Fused-silica capillary: 65.5 cm × 50 μm i.d.; BGE: 55 mM phosphate buffer (pH 2.55); separation voltage: 30 kV; temperature: 25°C; UV detection at 200 nm	UV	0.02–0.10 ng/mg [b]	Hair	Digestion with 100 mM hydrochloric acid (overnight at 45°C)	[37]
Cocaine; cocaethylene; benzoylecgonine; norcocaine; ecgonine methyl ester	**FASI-CZE** Poly(vinyl alcohol)-coated capillary (homemade): 50 cm × 75 μm i.d.; BGE: 15 mM ammonium formate (pH 9.5); separation voltage: 25 kV; temperature: 20°C	ESI–MS (IT)	1.5–10 ng/mL [a]	Urine	SPE with Supelclean strong cation exchange cartridge	[38]
3,4-Methylenedioxyamphetamine; 4-methylenedioxymethamphetamine; methadone; cocaine; morphine; codeine; 6-monoacetylmorphine	**FASI-CZE** Fused-silica capillary: 100 cm × 75 μm i.d.; BGE: 50 mM ammonium phosphate (pH 6.5); separation voltage: –15 kV; temperature: 25°C	ESI–MS (TOF)	–	Hair	Digestion with 100 mM hydrochloric acid (overnight at 45°C) and LLE with Toxi-Tubes A	[39]
Morphine; codeine; 6-monoacetylmorphine; ethylmorphine; fentanyl; pethidine; buprenorphine; nalbuphine; dextromethorphan; methadone; 2-ethylidene-1,5-dimethyl-3,3 diphenylpyrrolidine; propoxyphene; amphetamine; methamphetamine; 3,4-methylenedioxyamphethamine; 3,4-methylenedioxymethamphethamine; 3,4-methylenedioxyethylamphethamine; N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; ephedrine; pseudoephedrine; norephedrine; methylphenidate; cocaine; cocaethylene; anhydroecgonine methyl ester; metoprolol; procaine; ketamine; trimipramine	**pH-mediated stacking-CZE** Fused-silica capillary: 80 cm × 50 μm i.d.; BGE: 20 mM ammonium formate (pH 2.5); separation voltage: 30 kV; temperature: 25°C	ESI–MS (TOF)	2–200 ng/mL [b]	Urine	Dilution	[40]

(*continued on next page*)

Table 2 (*continued*)

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Morphine; codeine; 6-monoacetylmorphine; ethylmorphine; fentanyl; pethidine; buprenorphine; nalbuphine; dextromethorphan; methadone; 2-ethylidene-1,5-dimethyl-3,3 diphenylpyrrolidine; propoxyphene; amphetamine; methamphetamine; 3,4-methylenedioxyamphethamine; 3,4-methylenedioxymethamphethamine; 3,4-methylenedioxyethylamphethamine; N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; ephedrine; pseudoephedrine; norephedrine; methylphenidate; cocaine; cocaethylene; anhydroecgonine methyl ester; metoprolol; procaine; ketamine; trimipramine	**CZE** Fused-silica capillary: 80 cm × 50 μm i.d.; BGE: 20 mM ammonium formate (pH 2.5); separation voltage: 30 kV; temperature: 25°C	ESI–MS(TOF)	0.1–10 ng/mL [b]	Urine	DLLME	[41]
Cocaine; benzoylecgonine; norcocaine; cocaethylene	**CSEI-sweeping-MEKC** Fused-silica capillary: 60 cm × 50 μm i.d.; BGE: 100 mM phosphoric acid (pH 1.8) + 75 mM SDS + 10% v/v 2-propanol + 10% v/v tetrahydrofuran; separation voltage: –20 kV; temperature: 25°C; UV detection at 230 nm	UV	0.03–0.24 ng/mL [a]	Urine	SPE with Oasis mixed-mode cation exchange cartridge	[42]
Amphetamine; methamphetamine; 3,4-methylenedioxyamphetamine; 3,4-methylenedioxymethamphetamine; 3,4-methylenedioxyethylamphetamine; cocaine; morphine; heroin; codeine	**CEC** Capillary: 30 cm × 100 μm i.d., packed 3 μm with cyano derivatized silica stationary phase; mobile phase: 25 mM ammonium formate (pH 3.0) + 30 % v/v acetonitrile; separation voltage: 12 kV; temperature: 20°C	ESI–MS (IT)	0.78–3.12 ng/mL [a]	Urine	SPE with strong cation exchange cartridge	[43]
Ephedrine; cocaine; strychnine; morphine; caffeine; theophylline; piroxicam	**Open-tubular CEC** Multi-wall carbon nanotubes immobilized fused-silica capillary: 57 cm × 50 μm i.d.; mobile phase: 20 mM borate buffer (pH 9.0); separation voltage: 15 kV; temperature: 25°C; UV detection at 214 nm	UV	0.94–17.64 ng/mL [a]	Horse urine	SDME	[44]

[a] Standard samples.
[b] Real samples.

gave better results in terms of reproducibility (standard deviations ≤0.05), proving to be an excellent tool for rapid routine screening of these illicit drugs, for example, in illegal drug laboratories and in drug seizures.

As mentioned, CE represents an attractive alternative to chromatographic approaches because only a few nL of sample need to be injected. However, it is well known that this advantage leads to relatively poor concentration limits of detection (LODs), and this is a problem, as many analytes are present at very low concentrations. In order to deal with the small amount of analytes injected in CE, various approaches have been developed [12,14], mostly using preconcentration procedures, such as sweeping [25,26] and field-amplified sample injection (FASI) [24,27–29]. For example, Airado-Rodríguez et al. [27] developed a CZE method with UV detection for the analysis of 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide and phencyclidine in urine. With the use of 100 mM phosphate at pH 6.0 as the BGE and FASI for on-line sample preconcentration, LODs of 1.0–4.5 ng/mL were reached. The method was successfully applied to the analysis of the three drugs in the abusers' urine, proving to be a promising strategy that should be further applied to assess other DOAs, since there is a common tendency towards polydrug consumption.

A combination of two on-line stacking strategies, cation-selective exhaustive injection (CSEI) with sweeping, with MEKC-UV, was established for the analysis of methamphetamine and other DOAs in hair by Lin et al. [26]. Using this strategy, the sensitivity enhancement was about 1000-fold compared to MEKC separation without electrophoretic preconcentration. Hair samples were analyzed using liquid-liquid extraction (LLE) for sample clean-up prior to analysis, reaching LODs (0.05–0.20 ng/mg) that are in accordance with the cut-offs recommended by the Society of Hair Testing {0.2 ng/mg [46]}. This method has been shown to be useful for detecting trace amounts of these illicit drugs in the addicts' hair, so one can conclude that electrophoretic preconcentration techniques are quite suitable for sensitivity improvement in the toxicological analysis of complex matrices, such as human hair.

Moreover, high sensitivity may also be obtained by including a clean-up and preconcentration process in the sample-preparation step. To date, classical techniques such as LLE [17,21,22,24,26] and solid-phase extraction (SPE) [18,19] have often been applied for this purpose, although, recently, magnetic SPE (MSPE), a novel mode of SPE based on the use of magnetic adsorbents, emerged as a powerful technique [28,29]. In this respect, an MSPE procedure combined with FASI-CZE-UV for the determination of ATS and ketamine in urine was presented by Gao et al. [28]. Using magnetite/silica/poly(methacrylic acid-*co*-vinylbenzyl chloride-*co*-divinylbenzene) magnetic microspheres, the extraction was completed within 3 min with recoveries in the range 84.0–123%. The proposed method was successfully applied to the analysis of abusers' urine with LODs in the range 4.0–6.0 ng/mL. However, these MSPE particles are not yet commercially available, and this may be considered a drawback of this approach, since the reproducibility of this method may be affected.

In recent years, greener pretreatment techniques were proposed, as they involve a reduction of organic-solvent volumes with the subsequent production of less toxic residues. In this respect, CE-based methods have been reported for ATS determination in different matrices using single-drop microextraction (SDME) [30], dispersive liquid-liquid microextraction (DLLME) [27,31,32] or ultrasound-assisted emulsification microextraction (USAEME) [32] for cleaning up the sample and preconcentration of the analytes.

For example, Choi et al. [30] successfully employed a rapid, efficient preconcentration strategy based on the use of SDME in a three-phase design coupled in-line to CZE for the chiral analysis of three primary-amine compounds, including amphetamine, in urine. The analytes were first extracted from the alkalized urine sample into a drop of *n*-octanol layered over the urine sample and then into a microdrop of the acceptor phase (120 mM bis-tris methane/citric acid at pH 4.0) suspended at the capillary-inlet tip. The enriched acceptor phase was directly injected into the capillary and then analyzed in a BGE composed of 50 mM bis-tris methane/citric acid with 0.8 mM 18-crown-6-tetracarboxylic acid at pH 4.0. The method achieved LODs of 0.4–2.0 ng/mL with a 1000-fold increase in UV-detection sensitivity. Its application to urine was shown through the analysis of some blank samples spiked with the analytes and no validation assay carried out. Nevertheless, the proposed in-line SDME procedure allows commercial CE instruments to handle complex matrices directly, facilitating the automation and miniaturization of CE methodologies.

Recently, the analytical performance of DLLME and USAEME were compared for the extraction of three ephedrines from urine prior to their determination by FASI-MEKC [32]. Under optimal extraction and stacking conditions, enrichment factors of up to 140 and 1750 were obtained compared to conventional CZE, resulting in LODs of 12–33 ng/mL and 1.0–2.8 ng/mL with DLLME/FASI-MEKC and USAEME/FASI-MEKC, respectively. With satisfactory recoveries (94.5–103.3%) and good values of intra-day relative standard deviations (RSDs) (≤8.2%), both methods would be appropriate as a routine tool for clean-up and sensitivity enhancement in the determination of substances of abuse, due to their simplicity, effectiveness, low cost and environmental friendliness.

2.2. Cocaine

Cocaine, an alkaloid derived from the *Erythroxylum coca* plant, is known to be a powerful addictive stimulant that directly affects the brain. For hundreds of years, it has been used as a local anesthetic during surgeries. However, in recent years, cocaine abuse became epidemic. Once ingested, it is primarily hydrolyzed in the body to benzoylecgonine and ecgonine methyl ester, but it is also metabolized to norcocaine and several other metabolites. As a result, cocaine abuse can be proved through detection of its metabolites, which are indicative of ingestion of the drug [16].

In 2010, Janicka et al. [47] reviewed analytical protocols proposed within the past 10 years for analysis of cocaine and its metabolites in biological samples, including CE-based methods. With this in mind, this section focuses on papers published since 2010 up to the present related to the determination of these compounds by CE in biological samples, as well as in other matrices, such as environmental samples and banknotes, as these have also been analyzed for this purpose.

As shown in Table 2, procedures using different CE modes have been reported, such as detectors using CZE [34–41], MEKC [42] and CEC [43,44] with MS [38–41,43] and UV [34–37,42,44] for determination of several DOAs, including cocaine and its metabolites.

As is well known, paper money can become contaminated with cocaine during drug deals and/or directly through drug use with users snorting the drug through rolled bills. In this context, a fast CZE method with UV detection was proposed for the determination of cocaine on Brazilian banknotes [34]. Cocaine was extracted from banknotes by sonication for 8 min with acetonitrile and then directly loaded into the CZE-UV instrument without a preconcentration procedure, achieving a limit of quantification (LOQ) of 800 ng/mL. The method developed was successfully applied to determine cocaine on banknotes obtained directly from general circulation, proving to be a useful tool for identifying the presence of this drug, for example, in forensic criminology analysis of this type of sample.

Some authors have developed strategies to increase the sensitivity of CE for the determination of cocaine and its metabolites in environmental samples [36], as well as biological samples [35,37–44], using different preconcentration techniques, such as in-line SPE

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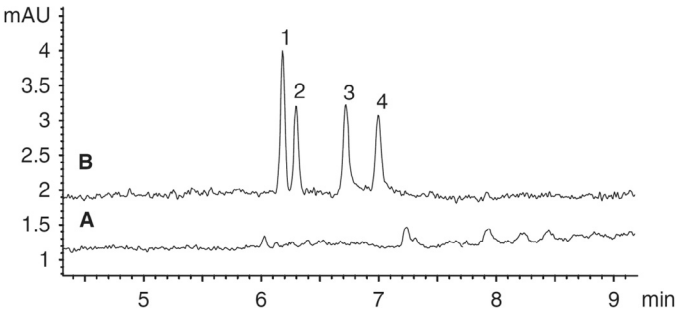

Fig. 1. (A) Electropherogram of blank river water. (B) Electropherogram of river water spiked with the analytes at a concentration of 1.0 ng/mL analyzed by in-line SPE-CZE-UV. Peak identification: (1) 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, (2) cocaine, (3) codeine and (4) 6-acetylmorphine. {Reproduced from [36] with the permission of Elsevier, ©2010}.

[36,37], FASI [38,39], pH-mediated stacking [40], DLLME [41], sweeping [42] and SDME [44].

Botello et al. [36] described the use of in-line SPE, as a clean-up step and enrichment technique, in combination with CZE for determining cocaine, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, codeine and 6-acetylmorphine in environmental waters. The SPE-CE device consisted of a short length of capillary of 2 mm filled with a hydrophilic-lipophilic-balance sorbent and inserted near to the inlet end of the separation capillary. With UV detection and simple filtration of the urine samples prior to in-line SPE-CE analysis, LODs of 0.07–0.27 ng/mL were achieved. Compared with CZE without preconcentration, this method improved sensitivity up to 5300-fold. With high recoveries (85–97%) and good values of RSDs (<11% in both intra-day and day-to-day experiments), as well as consumption of very low quantities of organic solvent, the developed method has proved to be very environmentally friendly, simple and cheap. Fig. 1 shows the electropherogram obtained from the analysis of river water following the proposed in-line SPE-CZE-UV procedure.

Later, the same group developed and validated a method for the simultaneous determination of cocaine and its major metabolite, benzoylecgonine, in hair [37]. An in-line SPE-CZE approach similar to that previously described was applied in conjunction with an extraction process of the analytes based on a digestion procedure in an acidic medium of the hair matrix prior to the in-line SPE-CZE-UV analysis. LOD values were 0.02 ng/mg for cocaine and 0.10 ng/mg for benzoylecgonine, which are reasonably suitable for therapeutic drug monitoring, forensic determinations and doping control. The method was successfully applied to hair samples of cocaine abusers who were undergoing a drug-rehabilitation program on the basis of segmental analysis to determine their compliance with the therapy. The major disadvantage of these methods [36,37] is that the in-line SPE-CE devices are not yet commercially available and this fact may have an influence on the reproducibility of the method.

A simple FASI method was described for the analysis of cocaine and its metabolites in urine by CZE coupled to electrospray ionization (ESI)-ion-trap (IT)MS via a pressurized nano-liquid-junction interface [38]. The use of this device combined with the on-line preconcentration technique increased the detection sensitivity of the method, reaching LODs of 1.5–10.0 ng/mL. The analytical method developed operated under nano-flow conditions and provided high sensitivity. However, its application to urine was shown only through the analysis of some blank samples spiked with the analytes and no complete method validation carried out.

Recently, Kohler et al. [40] used pH-mediated stacking in a two-step CE-MS method for the analysis of 30 toxicological compounds (amphetamines and their derivatives, opiates, cocaine and its metabolites, and pharmaceuticals) in urine. The screening step was performed by CZE-ESI-time-of-flight (TOF)MS. pH-mediated stacking was applied with a short plug of 7% w/v ammonium hydroxide injected prior to the urine sample being diluted with BGE and water (1:1:8), reaching LODs as low as 2.0 ng/mL. Compound quantitation for positive samples was performed by CZE-ESI-triple quadrupole (QqQ)-MS/MS, achieving LOQs as low as 10.0 ng/mL in real samples, which are far below the cut-off levels in urine {300 ng/mL [48]}. The same group also presented a different approach using DLLME as the preconcentration technique with CZE-ESI-(TOF)MS for the toxicological screening of the same illicit substances in urine with LODs at the *sub*-ng/mL level [41]. Both CZE-ESI-(TOF)MS methods [40,41] were successfully applied to the analysis of real cases. Compared to other mass spectrometers, such as (IT)MS [38], CZE coupled to (TOF)MS allowed identification of a larger number of different kinds of DOA with better sensitivity, which is very useful in therapeutic drug monitoring, drug-rehabilitation programs, doping control and forensic analysis. However, the use of (QqQ)-MS/MS can greatly enhance selectivity using the selected reaction monitoring mode that ensures more accurate measurements and lower LOQs by reducing the chemical noise, providing, in this way, a high level of certainty in identification.

With respect to the use of CEC, Aturki et al. [43], who described a method for the determination of nine DOAs, including cocaine, in urine using columns packed with cyano as the stationary phase coupled to ESI-(IT)MS. The coupling was achieved using a liquid-junction interface, providing several advantages, such as high sensitivity (LODs of 0.78–3.12 ng/mL) and short analysis time (13 min), as well as unambiguous identification and confirmation using MS/MS.

Open-tubular CEC has also been proposed for the analysis of illicit drugs, including cocaine, using multi-wall carbon nanotubes (MWCNTs) immobilized into a fused-silica capillary as the stationary phase [44]. Good run-to-run (≤2.83%), day-to-day (≤4.32%) and capillary-to-capillary (≤2.35%) reproducibility have been obtained. Moreover, the regeneration of the functionalized capillary allows its reusability for at least six months. An off-line SDME procedure was used for the preconcentration of the analytes with enrichment factors of 38–102-fold. Both CEC-based methods [43,44] may be advantageously employed in doping control and forensic analysis.

3. Depressants

Depressants, also referred to as sedatives and tranquilizers, are chemicals that can slow brain activity. This property makes them suitable for treating anxiety and sleep disorders. Among the medications commonly prescribed for these purposes are barbiturates and BZDs. Despite their beneficial effects, these drugs have the potential for abuse and should be used only as prescribed [49]. With this in mind, this section consists of two parts, the first focusing on barbiturates and the second on BZDs, with the CE-based analytical methods currently used being reviewed and summarized in Table 3 [50–61].

3.1. Barbiturates

Barbiturates are chemical derivatives of barbituric acid, and are used as sedatives, hypnotics, anticonvulsants, anesthetics and tranquilizers. In recent years, their use decreased markedly, as BZDs replaced barbiturates for the majority of clinical indications. However, the illegal abuse of barbiturates is still widespread, being implicated in cases of murder, suicide and accidents. Commonly-abused barbiturates include secobarbital, pentobarbital and amobarbital [49], so analytical methods for their efficient and accurate determination are required.

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Table 3
Overview list of capillary electrophoresis (CE) methodologies for determination of depressants

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Barbital acid; Barbital; phenobarbital; pentobarbital; amobarbital; thiobarbituric acid; butobarbital; N-methyl-5-phenyl-ethyl barbital acid; 5-cyclohexenyl-5-ethyl barbital acid	**CZE** Fused-silica capillary: 42 cm × 50 μm i.d.; BGE: 20 mM borate buffer (pH 10.0) + 4% w/v hexadimethrine bromide + 2.06 mM α-CD; separation voltage: –25 kV; temperature: 25°C; UV detection at 200 nm	UV	870–3 500 ng/mL [a]	Urine	Filtration	[50]
Barbital; amobarbital; phenobarbital; secobarbital	**LVSS-CZE** Fused-silica capillary: 60.5 cm × 75 μm i.d.; BGE: 40 mM borate buffer (pH 8) + 20% v/v methanol; separation voltage: 20 kV; temperature: 25°C; UV detection at 214 nm	UV	15–57 ng/mL [a]	Plasma	LLE with dichloromethane	[51]
Barbital; phenobarbital; secobarbital	**EKS-CZE** Fused-silica capillary: 100 cm × 75 μm i.d.; BGE: 20 mM borate buffer (pH 9.15); terminating electrolyte: 100 mM CHES; leading electrolyte: 50 mM sodium chloride; separation voltage: –30 kV; temperature: 25°C; UV detection at 214 nm	UV	8–15 ng/mL [b]	Urine	LLE with ethyl acetate/*n*-hexane 40:60 v/v	[52]
Phenobarbital; *p*-hydroxyphenobarbital	**Reverse migrating pseudostationary phase-MEEKC** Fused-silica capillary: 50 cm × 75 μm i.d.; BGE: 0.8% v/v ethyl acetate + 6.6% v/v butan-2-ol + 1.0% v/v acetonitrile + 2.0% w/v SDS + 89.6% v/v 7.5 mM ammonium formate (pH 8.0); separation voltage: 25 kV; temperature: 25°C; UV detection at 214 nm	UV	16.8 ng/mL [b]	Rat urine	SPE	[53]
Nitrazepam; oxazepam; alprazolam; flunitrazepam; temazepam; diazepam; 7-aminoflunitrazepam; 7-aminonitrazepam; 7-aminoclonazepam	**CZE** Poly(diallyldimethylammonium chloride) and dextran sulfate-coated capillary: 69 cm × 50 μm i.d.; BGE: 100 mM ammonium phosphate (pH 2.5); separation voltage: 28 kV; temperature: 25°C; UV detection at 200 nm	UV	2 700–41 500 ng/mL [a]	Beverages	–	[54]
Diazepam; clorazepate; chlordiazepoxide; bromazepam; nitrazepam; alprazeolam; flunitrazepam	**MEKC** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 20 mM phosphate buffer (pH 7.0) + 400 mM lithium bis(trifluoromethanesulfonyl)imide; separation voltage: 15 kV; temperature: 25°C; UV detection at 230 nm	UV	–	–	–	[55]
Lorazepam; bromazepam; clorazepate; nitrazepam; diazepam; alprazolam; flunitrazepam	**MEKC** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 20 mM phosphate buffer (pH 7.0) + 55% v/v methanol + 170 mM 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide + 10 mM SDS; separation voltage: 20 kV; temperature: 25°C; UV detection at 230 nm	UV	2 740–4 420 ng/mL [a]	Urine	SPE with Oasis mixed-mode cation exchange cartridge	[56]

(*continued on next page*)

Table 3 (*continued*)

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Alprazolam; bromazepam; medazepam; nitrazepam; chlorazepate; chlordiazepoxide; diazepam; oxazepam	**MEKC** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 25 mM borate buffer (pH 9.3) + 50 mM SDS + 15 mM β-CD + 2 M urea; separation voltage: 25 kV; temperature: 25°C; UV detection at 214 nm	UV	580–1 510 ng/mL [a]	–	–	[57]
Diazepam; clorazepate; Chlordiazepoxide; bromazepam; nitrazepam; alprazeolam; flunitrazepam	**Sweeping-MEKC** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 15 mM borate buffer (pH 9.0) + 20 mM *N*-cetyl-*N*-methylpyrrolidinium bromide + 30% v/v methanol; separation voltage: –25 kV; temperature: 25°C; UV detection at 230 nm	UV	4.7–9.8 ng/mL [a]	Urine	SPE with Oasis mixed-mode cation exchange cartridge	[58]
Alprazolam; clonazepam; diazepam; flunitrazepam; oxazepam; α-hydroxyalprazolam; 7-aminoclonazepam; nordiazepam; 7-aminoflunitrazepam; N-demethylflunitrazepam; ketamine; codeine; morphine; methamphetamine	**Reverse migrating micelles-Sweeping-MEKC** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 75 mM phosphate buffer (pH 2.5) + 30% v/v methanol; sweeping buffer: 75 mM phosphate buffer (pH 2.5) + 10% v/v methanol + 65 mM SDS; separation voltage: –15 kV; temperature: 25°C; UV detection at 200 nm	UV	20–50 ng/mL [b]	Urine	LLE with dichloromethane	[59]
Codeine; morphine; methamphetamine; ketamine; alprazolam; clonazepam; diazepam; flunitrazepam; nitrazepam; oxazepam	**LVSS-sweeping-MEKC** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 50 mM phosphate buffer (pH 2.3) + 10% methanol + 150 mM SDS; separation voltage:-15 kV; temperature: 25°C; UV detection at 200 nm	UV	7.5–30 ng/mL [b]	Urine	LLE with ethyl acetate	[60]
Alprazolam; chlordiazepoxide; nitrazepam; triazolam; lorazepam; clonazepam; flunitrazepam; clorazepate; diazepam; prazepam	**CEC** Capillary: 50 cm × 100 μm i.d., 30 cm packed with hexyl acrylate-based porous monolith; mobile phase: 5 mM ammonium acetate (pH 7.0) + 70% v/v acetonitrile; separation voltage: 20 kV; temperature: 25°C	ESI–MS (TOF)	0.6–1.8 ng/mL [a]	Urine	LLE with ethyl acetate	[61]

[a] Standard samples.
[b] Real samples.

As shown in Table 3, the separation of barbiturate drugs by CE has been performed by CZE [50–52] and MEEKC [53] using UV as the detection system. In most of these papers, the methods developed have been applied to the analysis of human biological samples. For example, a simple, rapid CZE-based method has been developed for the determination of nine barbiturates in urine, using hexadimethrine bromide as electroosmotic-flow modifier [50]. Good intra-day RSDs of the migration times (≤3.84%) and peak areas (≤5.45%), and LODs in the range 0.87–3.5 μg/mL were obtained, without prior sample preparation. Although the authors claimed that the established sensitivity was enough to quantify urine samples at therapeutic levels, the standard cut-offs used in hospitals for barbiturates in urine are 0.3 μg/mL [48]. This means that, in some cases, false-negative results could be reported. As a result, this method might have limited use in analytical laboratories and, in this regard, a number of authors have established different strategies to increase the concentration sensitivity of barbiturates.

For example, various preconcentration methodologies have been developed, such as large-volume sample stacking (LVSS) and electrokinetic supercharging (EKS) both in CZE [51,52], and sample stacking induced by reverse migrating pseudostationary phase in MEEKC [53]. Fan et al. [51] developed an LVSS-CZE method with polarity switching for the determination of barbiturates in plasma. This technique involves injecting a large volume of sample into the capillary and removing the sample matrix plug out of the capillary by reversing the polarity. Combined with off-line LLE, enrichment factors of up to 203-fold were obtained with UV detection, compared to conventional CZE, resulting in LOQs of 0.15–0.57 ng/mL. The method was successfully applied to the analysis of barbiturates in two kinds of forensic specimen (blood and urine), so it can be concluded that this stacking method may be an efficient and useful way for identifying and quantifying these DOAs at very low levels.

EKS in combination with CZE was investigated for the determination of three barbiturates in urine by Botello et al. [52]. A 1050-fold enhancement in detection sensitivity was obtained when the sample was injected at –8.5 kV for 300 s. Sodium chloride (50 mM) was used as leading electrolyte and CHES (100 mM) was used as terminating electrolyte. Blank urine spiked with the drugs were analyzed using LLE as a clean-up step prior to analysis, achieving LODs (8–15 ng/mL) with UV detection far below the cut-off values in urine {300 ng/mL [48]}. This method proved suitable for determining trace amounts of these illicit drugs in forensic analysis.

Kadi et al. [53] also investigated stacking induced by reverse migrating pseudostationary phase in MEEKC for the determination of phenobarbital and its *p*-hydroxyphenobarbital metabolite in rat urine. When this preconcentration technique is used, the sample stacking and the separation processes take place successively by changing the voltage with an intermediate polarity-switching step. Blank rat urine spiked with the drugs was analyzed using SPE as a clean-up step prior to analysis, achieving LODs of 16.8 ng/mL for both analytes under study. The proposed method seems appropriate for conducting pharmacokinetic studies.

3.2. Benzodiazepines

BZDs are currently among the most frequently prescribed drugs worldwide. They act as anxiolytics, sedatives, hypnotics, amnesics, antiepileptics and muscle relaxants. They are also often used for the treatment of epilepsy, convulsions and many psychiatric disorders. Overdoses of these substances can cause acute toxicity with symptoms, such as drowsiness, dizziness, blurred vision, slurred speech, difficulty breathing, and coma, so it is important to monitor their concentrations in biological samples [49].

However, the physicochemical nature of BZDs may give rise to some problems when using CE as the analytical technique, as these compounds are difficult to ionize, due to their fairly low-pK_a values, which makes MEKC the preferred CE separation mode [55–60]. But, these have also been successfully separated by CZE using coated capillaries [54] and CEC [61], as shown in Table 3.

With respect to the detection system, UV has been more widely employed [54–60], although the use of MS has also been reported [61]. With respect to application to biological samples, most of the papers describe the determination of BZDs in urine, using SPE [56,58] or LLE [59–61] as a sample pretreatment. As can be seen in Table 3, analysis of BZDs has also been reported together with other drugs, such as the opiate-related compounds, hallucinogens and ATSs [59,60].

However, the determination of BZDs in beverages is also of great interest, since these constitute one of the groups of substances most commonly associated with drug-facilitated crimes, being secretly administered to the victim prior to the assault, usually in alcoholic drinks. In this respect, a rapid CZE method has been developed for the simultaneous determination of nine BZDs in spiked beverages [54]. Because these analytes have very similar hydrophobicity, making them difficult to analyze by CZE, a double-coated capillary with poly(diallyldimethylammonium chloride) and then dextran sulfate was employed, which allowed a good baseline resolution between consecutive peaks in a run time of <6.5 min. Peak area repeatability was in the order of 0.9–11% RSD and the migration time repeatability was 0.3–0.8% RSD, making the proposed method useful when a general profile of BZDs in drinks is desired. However, the gradual removal of dextran sulfate from the capillary wall during analyses led to irreproducible electroosmotic flow, which may be considered a drawback of this strategy. In this work, flushing with dextran sulfate was required approximately every 20 injections in order to overcome this issue.

A somewhat different approach when using MEKC-UV was given by Su et al. [55] with the purpose of improving the resolution in the simultaneous analysis of several BZDs. The authors used a chaotropic salt, lithium bis(trifluoromethanesulfonyl)imide, as the single modifier of the BGE, achieving complete resolution of the BZDs within 16 min without the need to add an organic solvent, in contrast to the need for both surfactant and organic solvent in the conventional MEKC separation. According to the authors, the resolution was improved because the chaotropic anions strongly disrupted hydration of analytes, increasing their hydrophobicity and strengthening their interactions with them. Using 20 mM phosphate with 300 mM lithium bis(trifluoromethanesulfonyl)imide at pH 7.0 as the BGE, the separation was very reproducible with RSDs of the migration times for all the analytes <0.38%, making the proposed method a reliable, simple alternative for improving the separation of this kind of compound.

The same research group also reported enhancement in the separation of BZDs using 1-butyl-3-methylimidazolium-based ionic liquids (ILs) and sodium dodecyl sulfate (SDS) as modifiers in the BGE [56]. In particular, 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide was the best IL additive for the separation because its anionic moiety interacted favorably with the BZDs. The optimal BGE of 20 mM phosphate at pH 7.0 with 170 mM 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide and 10 mM SDS in 55% methanol provided good reproducibility (RSDs of the migration times and peak areas <0.3% and <10%, respectively), and high efficiency (up to 352,000 plates/m), as well as satisfactory peak shapes for all of the analytes. The LODs were 2.74–4.42 μg/mL. Application to biological samples was performed (blank urine spiked with the analytes) after off-line SPE. However, poor sensitivity was achieved and this means that, using the proposed method, single-drug intake cannot be detected, which is especially significant if there are legal implications of drug consumption (e.g., in drug-facilitated crimes).

In order to increase the sensitivity of CE for the determination of BZDs, some authors also developed methodologies in which

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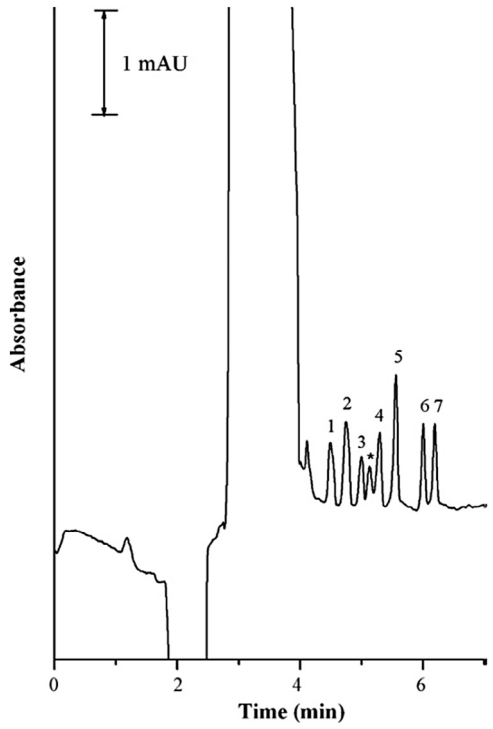

Fig. 2. Electropherogram of urine spiked with the analytes at a concentration of 1 μg/mL analyzed by sweeping-MEKC-UV after SPE. Peak identification: (1) bromazepam, (2) alprazeolam, (3) flunitrazepam, (*) unknown peak, (4) chlordiazepoxide, (5) diazepam, (6) clorazepate and (7) nitrazepam. (Reproduced from [58] with the permission of Elsevier, ©2010).

different preconcentration strategies based on sweeping were used [58,59]. For example, Chiang et al. [59] reported a sweeping-MEKC method for the simultaneous determination of different BZDs and ketamine in abusers' urine. For sensitivity enhancement, hydrodynamic sample loading was carried out in this study, combined with stacking with reverse migrating micelles. The capillary was filled with a high-conductivity buffer (75 mM phosphate at pH 2.5 in 30% methanol), followed by a large volume of sample in a low-concentration buffer (15 mM phosphate at pH 5.0). Simultaneous sweeping and separation was performed in a sweeping BGE (75 mM phosphate with 64 mM SDS at pH 2.5 in 10% methanol). At application of voltage, SDS started to sweep the analytes to the outlet; meanwhile, the analytes decelerated at the boundary between the low-conductivity buffer and the high-conductivity buffer and formed a narrow zone. The LODs were 20–50 ng/mL.

Alternatively, given that ILs with surfactant properties can form micelles and hence be useful in MEKC, the use of IL-type surfactants as sweeping carriers was proposed for the preconcentration of BZDs during the sweeping-MEKC process [58]. The authors investigated 1-cetyl-3-methylimidazolium bromide and *N*-cetyl-*N*-methylpyrrolidinium bromide as sweeping cationic surfactants and compared them with the most commonly employed cationic surfactant, cetyltrimethylammonium bromide. The experimental results showed that cationic surfactant *N*-cetyl-*N*-methylpyrrolidinium bromide exhibited superior sweeping power compared to 1-cetyl-3-methylimidazolium bromide and cetyltrimethylammonium bromide. Using SDS consisting of 15 mM borate with 20 mM *N*-cetyl-*N*-methylpyrrolidinium bromide at pH 9.0 in 30% methanol, enrichment factors up to 165-fold were obtained with UV detection, compared to conventional MEKC. The LODs achieved were of the order of 4.7–9.8 ng/mL. The applicability of the method for the analysis of urine samples (blank urine spiked with the analytes) was demonstrated, employing SPE as a clean-up step before the analysis. The sweeping-MEKC electropherogram of spiked urine after the SPE is shown in Fig. 2.

CEC using a hexyl acrylate-based monolithic column coupled to ESI-(TOF)MS, which offers the advantage of accurate-mass measurements, has also been demonstrated to be effective for separation and determination of BZDs in urine [61]. Using a mobile phase of 5 mM ammonium acetate at pH 7.0 in 70% acetonitrile, RSD values were obtained of 1.4–2.3% for retention times and 1.1% and 9.2% for relative areas. Stacking of the analytes at the head of the stationary phase was performed, consisting of the injection of a large volume of sample present in aqueous solution, enabling an improvement in sensitivity of 75–140-fold. This strategy enabled the quantification of these drugs down to 1 ng/mL after off-line LLE, which is far below the cut-off levels in urine {300 ng/mL [48]}. Although these were probably preliminary results, as complete method validation was not carried out, this work shows the potential of coupling CEC with ESI-(TOF)MS for determining very low levels of benzodiazepines in urine, which could allow quantification of single-drug intake useful in cases involving drug-facilitated crimes.

4. Opium-related compounds

Derived from the poppy plant, opiates are powerful drugs that have been used for centuries to relieve pain. Also known as narcotics, opiates can be natural or synthetic. Natural opiates include morphine, codeine and thebaine. Other substances, called opioids, are man-made and are also used to treat chronic or severe pain. These substances include pethidine, oxycodone, hydrocodone, fentanyl, buprenorphine, and methadone. Heroin is a manufactured opioid and has no medicinal uses. It is mainly used for its ability to give the user a feeling of euphoria [16]. Opium-related substances are highly addictive and overuse can cause overdose and potentially death. Since their abuse has risen substantially worldwide, methodologies able to analyze opioids need to be developed. Table 4 reviews and summarizes the CE-based analytical methods currently used for this purpose [62–75], two of which involve the determination of the opium-related compounds together with ATSs [64,72].

As shown in Table 4, toxicological analysis of opiate-related compounds has been performed using CZE [62–65,74,75], CEC [66–68], MEKC [69,71–73] and non-aqueous CE [70] with detection by UV [63,66,68,69,71–73,75] and MS [64,65,67,70,74]. For example, Zhang and co-workers [65] reported an approach for the analysis of heroin and its related alkaloids using charged polymer-protected gold nanoparticle (AuNP)-coated capillaries with CZE-ESI-(IT)MS. The use of AuNP-coated capillaries exhibited good efficiency in separation (up to 498,052 plates/m), peak shape and analysis time with satisfactory run-to-run and capillary-to-capillary repeatabilities, obtaining RSDs of migration time in the range 0.43–0.62% and RSDs of peak area in the range 1.49–4.68%. The capillary-coating procedure proved to be simple and fast (30 min) without requiring heating. This method seems to be reasonably suitable for the identification of the geographical origin of illicit samples.

The application of a pressure via a micro-HPLC pump (a pressure of 1000 psi and a flow rate of 0.05 mL/min) also allowed high resolution combined with a considerable reduction of the analysis time in the determination of five opium alkaloids in *Pericarpium papaveris* samples by CEC using a poly(glycidyl methacrylate-*co*-ethylene dimethacrylate-*co*-3-sulfopropyl methacrylate potassium salt) monolithic column prepared in-house [66]. With UV detection, the LODs were in the range 1.5–6.0 μg/mL, which are sufficient for detection of these compounds in cases involving drug possession and seized drugs. Good intra-day RSDs (≤1.94%), inter-day RSDs (≤3.49%) and column-to-column RSDs (≤5.24%) for the retention time were obtained. However, the preparation procedure of the monolithic

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Table 4
Overview list of capillary electrophoresis (CE) methodologies for determination of opium-related compounds

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Pethidine; methadone	**CZE** Fused-silica capillary: 67.5 cm × 25 μm i.d.; BGE: 30 mM phosphate buffer (pH 6.0); separation voltage: 14 kV; temperature: 25°C	ECL	0.5 μM [a]	–	–	[62]
Morphine; codeine; oripavine; thebaine	**CZE** Fused-silica capillary: 50 cm × 50 μm i.d.; BGE: 100 mM Tris (pH 2.8) + 30 mM hydroxypropyl-β-CD; separation voltage: 25 kV; temperature: 25°C; UV detection at 214 nm	UV	2.5 10^{-6} M [a]	Process liquor	–	[63]
Amphetamine; ephedrine; methadone; pethidine; tetracaine; codeine; heroin	**CZE** Fused-silica capillary: 70 cm × 50 μm i.d.; BGE: 20 mM ammonium acetate (pH 9.0); separation voltage: 22 kV; temperature: 20°C	ESI–MS (Q)	0.40–1.0 ng/mL [a]	Urine	Protein precipitation	[64]
Morphine; codeine; 6-acetylmorphine; thebaine; 6-acetylcodeine; heroin; papaverine; narcotine	**CZE** Charged polymer-protected gold nanoparticles-coated capillary: 68 cm × 50 μm i.d.; BGE: 120 mM ammonium acetate (pH 5.2) + 13% v/v methanol; separation voltage: –20 kV; temperature: 20°C	ESI–MS (IT)	–	–	–	[65]
Narcotine; papaverine; thebaine; codeine; morphine	**Pressure-assisted CEC** Capillary: 55 cm × 100 μm i.d., 30 cm packed with polymeric monolith; mobile phase: 5 mM phosphate buffer (pH 4.0) + 90% v/v acetonitrile; separation voltage: –13 kV; temperature: 25°C; UV detection at 224	UV	1 500–6 000 ng/mL [b]	Pericarpium papaveris	Ultrasound extraction with methanol for 1 h and then soaked for 24 h	[66]
Methadone; pethidine; fentanyl; morphine; diamorphine	**Pressure-assisted CEC** Silica-based monolithic capillary: 56 cm × 100 μm i.d.; mobile phase: 20 mM ammonium acetate (pH 6.0) + 65.5% v/v acetonitrile; separation voltage: 25 kV; temperature: 25°C	ESI–MS (Q)	2.0–80 nmol/L [a]	Urine	Centrifugation with methanol at 4 500 rpm for 20 min	[67]
Morphine; thebaine; narcotine; papaverine	**Open-tubular CEC** Capillary: 60 cm × 50 μm i.d., covalently modified with hydrophilic polysaccharide; mobile phase: 50 mM phosphate buffer (pH 6.0); separation voltage: –15 kV; temperature: 25°C; UV detection at 214 nm	UV	–	–	–	[68]
Paclitaxel; morphine; codeine	**MEKC** Fused-silica capillary: 57 cm × 75 μm i.d.; BGE: 20 mM borate buffer (pH 9.2) + 60 mM SDS + 5% v/v methanol; separation voltage: –25 kV; temperature: 20°C; UV detection at 212 nm	UV	30–90 ng/mL [a]	Urine	Centrifugation at 5 000 rpm for 5 min	[69]

(*continued on next page*)

Table 4 (*continued*)

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Fentanyl derivatives	**Non-aqueous CE** Fused-silica capillary: 80 cm × 50 μm i.d.; BGE: non-aqueous solution of ammonium acetate 200 mM + 90% v/v acetonitrile; separation voltage: 28 kV; temperature: 20°C	ESI–MS^2 (IT)	0.5 ng/mL [a]	Seized samples	Dilution	[70]
Morphine; codeine; normorphine; morphine-3-glucuronide; morphine-6-glucuronide	**CSEI-Sweeping-MEKC** Fused-silica capillary: 40 cm × 50 μm i.d.; BGE: 25 mM phosphate buffer (pH 2.5) + 22% v/v methanol + 100 mM SDS; separation voltage: –20 kV; temperature: 25°C; UV detection at 200 nm	UV	10–35 ng/mL [a]	Urine	SPE with BondElut Certify cartridges	[71]
Methadone; 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline; amphetamine; methamphetamine; morphine	**CSEI-Sweeping-MEKC** Fused-silica capillary: 30 cm × 50 μm i.d.; BGE: 100 mM phosphoric acid (pH 4.0) + 100 mM SDS + 20% v/v tetrahydrofuran; separation voltage: –15 kV; temperature: 25°C; UV detection at 214 nm	UV	0.2–0.4 ng /mL [b]	Serum	LLE with ethyl ether	[72]
Heroin; morphine; codeine; 6-acetylmorphine	**CSEI-sweeping-MEKC** Fused-silica capillary: 38 cm × 50 μm i.d.; BGE: 20 mM phosphate buffer (pH 2.5) + 80 mM SDS; separation voltage: 20 kV; temperature: 25°C; UV detection at 214 nm	UV	10 ng/mL [b]	Urine	LLE with dichloromethane:*n*-propanol, 85:15 v/v	[73]
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline; 6-acetylmorpine; codeine; dihydrocodeine	**In-line SPE-CZE** Fused-silica capillary: 100 cm × 50 μm i.d.; BGE: 60 mM ammonium acetate (pH 3.8); separation voltage: 30 kV; temperature: 25°C	ESI–MS (IT)	13–210 ng/mL [b]	Urine	Dilution	[74]
Morphine; codeine; 6-acetylmorphine	**pH-mediated stacking-CZE** Fused-silica capillary: 50 cm × 75 μm i.d.; BGE: 100 mM phosphate buffer (pH 3.0) + 20% v/v methanol + 5% v/v *n*-propanol; separation voltage: 25 kV; temperature: 25°C; UV detection at 214 nm	UV	7 ng/mL [a]	Saliva	Dilution or LLE	[75]

[a] Standard samples.
[b] Real samples.

Table 5
Overview list of capillary electrophoresis (CE) methodologies for determination of cannabinoids

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
Δ^9-tetrahydrocannabinol; 11-hydroxy-Δ^9-tetrahydrocannabinol; 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol	**Sweeping-MEKC** Fused-silica capillary: 60 cm × 50 μm i.d.; BGE: 25 mM phosphate buffer (pH 2.6) + 40% v/v methanol + 75 mM SDS; separation voltage: –20 kV; temperature: 25°C; UV detection at 210 nm	UV	17.2–23.3 ng/mL [b]	Urine	SPE with Bond Elut Certify cartridge	[76]
Δ^9-tetrahydrocannabinol; 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid; 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid glucuronide	**CZE** Fused-silica capillary: 85 cm × 50 μm i.d.; BGE: 40 mM ammonium formate (pH 6.4); separation voltage: 30 kV; temperature: 25°C	ESI–MS (QqQ)	50 ng/mL [b]	Urine	Dilution	[77]

[a] Standard samples.
[b] Real samples.

column is quite time-consuming, which may be considered a drawback of this method.

The modification of the inner surface of the capillary in open-tubular CEC with carboxymethyl chitosan showed that it counteracts adsorption of the analytes and, most importantly, stabilizes the electroosmotic flow when four opium alkaloids (morphine, thebaine, papaverine and narcotine) were baseline separated in phosphate buffer (50 mM, pH 6.0) with column efficiencies up to 132,000 plates/m [68]. Although good repeatability was gained with RSDs of the migration time less than 1.3% for run-to-run and less than 3.2% for day-to-day, and RSDs of peak area less than 5.6% for run-to-run and less than 8.8% for day-to-day, no application to real samples was proposed.

In recent years, many illegal drug laboratories started to produce synthetic derivatives of fentanyl, which are more potent that morphine, and, as a result, abuse of these drugs is rapidly growing. With this in mind, a non-aqueous CE-MS^2 method was proposed for the trace identification of these illicit drugs, which were detected down to the nanomolar level (0.5 ng/mL for fentanyl) [70]. The method was successfully applied for the analysis of three samples from forensic casework. From an electrophoretic point of view, the system is quite straightforward and, although it is not particularly innovative, its performance seems to be suitable for the stated purpose.

In order to increase the sensitivity in CE and, consequently, decrease LODs for the determination of opiates/opioids, some authors have developed methodologies using different preconcentration techniques, such as sweeping [71–73], in-line SPE [74] and pH-mediated staking [75]. For example, Meng et al. [75] used both acid-mediated and base-mediated stacking in CZE-UV for the analysis of codeine, morphine and 6-acetylmorphine in saliva. 100 mM hydrochloric acid and 12.5% ammonium hydroxide in 50% methanol were used to create the respective pH pulses in acid- and base-mediated stacking, respectively. The methods resulted in a 1000-fold sensitivity increase compared with normal hydrodynamic injection, with LODs ≤7 ng/mL. In acid-mediated stacking, the sample preparation involved dilution with methanol while, in basic-mediated stacking, an LLE step was necessary to make the analytes go into the acid matrix. Unfortunately, acid-mediated stacking displayed a considerable background noise and, in the case of basic-mediated stacking, although cleaner electropherograms were obtained, the separation efficiency was lower in comparison to acid-mediated stacking. Despite this, preconcentration factors were higher than those in the analysis of the same substances of abuse in urine samples by CSEI-sweeping-MEKC [73].

Methadone is an opioid that is primarily used therapeutically in maintenance treatment of heroin addicts, although, in recent years, there has been abuse of this compound on the drug scene. With this in mind, Wang et al. [72] developed a sweeping-MEKC method for the determination of methadone and its metabolites in serum using off-line LLE and injection under CSEI conditions for the sample clean-up and preconcentration of the analytes, providing LODs in the range 0.2–0.4 ng/mL with UV detection. Analytical precision and accuracy were fairly acceptable, with RSDs and relative errors<19% in real samples in both intra-day and day-to-day experiments. The method was successfully applied for monitoring the studied drugs in heroin addicts, proving to be a valuable, reliable tool for toxicological analysis, therapeutic drug monitoring, drug-rehabilitation programs and doping control.

Since methadone and its metabolites are chiral compounds, enantiomeric separation of these drugs plays a fundamental role in the determination of synthetic pathways and for impurity profiling of the drug. In this respect, a review was published describing the most recent papers on the enantioseparation of methadone and its metabolites [45]. In short, native CDs and CD derivatives (sub-section 2.1) are the most popular chiral selectors for the enantiomeric analysis of methadone and its metabolites in biological samples. The number of newly synthesized CD derivatives is increasing and their application is continually being investigated. However, none of these investigations has yet clearly proved to offer significant advantages over the others.

5. Cannabinoids

Cannabinoids are compounds derived from *Cannabis sativa* (marijuana). The primary psychoactive component of marijuana is Δ^9-tetrahydrocannabinol that is found in the flowering tops, leaves and resin of the plant. Once ingested, it is primarily metabolized in the body to 11-hydroxy-Δ^9-tetrahydrocannabinol, which is further oxidized to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. Other cannabinoids are also present in significant amounts, including cannabidiol and cannabinol. Marijuana consumption can cause euphoria, hallucination, difficulties in concentration and impairment of memory, among other symptoms. Because of its increasingly widespread abuse worldwide, there is a need for rapid, sensitive and accurate analytical methods for the determination of cannabinoids.

To the best of our knowledge, in the period covered by this review, only two papers were reported on the application of CE for the determination of these DOAs {Table 5 [76,77]}. Su et al. [76] described a sensitive method that utilizes both off-line and on-line procedures in MEKC for the simultaneous determination of Δ^9-tetrahydrocannabinol and its metabolites in urine. In particular, the authors used SPE for off-line preconcentration and clean-up of the sample and on-line preconcentration based on sweeping, resulting in enhancement factors of up to 200-fold compared to

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Table 6
Overview list of capillary electrophoresis (CE) methodologies for determination of designer drugs

Substance	CE conditions	Detection	LOD	Sample	Sample pretreatment	Ref.
1-(2-Chlorophenyl)piperazine; 1-(3-chlorophenyl)piperazine; 1-(4-chlorophenyl)piperazine	**CZE** Fused-silica capillary: 60 cm × 50 μm i.d.; BGE: 20 mM phosphoric acid (pH 2.5) + 10 mM α-CD; separation voltage: 25 kV; temperature: 25°C; UV detection at 236 nm	UV	2 000–3 500 ng/mL [a]	Confiscated pills	Ultrasound extraction with methanol for 15 min	[80]
7 Benzofurys; 4 synthetic cathinones; 2 diphenidines; ethylphenidate; methiopropamine; thiothinone	**CZE** Fused-silica capillary: 60 cm × 50 μm i.d.; BGE: 50 mM ammonium acetate (pH 4.5) + 15 mM sulfobutylether-β-CD + 10% v/v acetonitrile; separation voltage: 25 kV; temperature: 25°C; UV detection at 230 and 280 nm	UV	–	–	–	[81]
12 Synthetic cathinones derivatives	**CZE** Fused-silica capillary: 57.5 cm × 50 μm i.d.; BGE: 100 mM phosphate buffer (pH 2.5) + 10 mM β-CD; separation voltage: 25 kV; temperature: 25°C; UV detection at 206 nm	UV	4.2–7.0 ng/mL [a]	–	–	[82]
	Fused-silica capillary: 57.5 cm × 50 μm i.d.; BGE: 50 mM phosphate buffer (pH 2.5) + 0.6% v/v highly sulfated-β-CD; separation voltage: 25 kV; temperature: 25°C	ESI–MS (TOF)	1.0–11.0 ng/mL [a]	–	–	
6 Synthetic cathinones derivatives; amphetamine; methamphetamine	**CZE** Fused-silica capillary: 90 cm × 20 μm i.d.; BGE: 15 mM 18-crown-6-tetracarboxylic acid + 0.125% v/v highly sulfated-γ-CD; separation voltage: 25 kV; temperature: 25°C	Sheathless CE–MS (IT)	–	–	–	[83]
9 Synthetic cannabinoids	**MEKC** Fused-silica capillary: 40 cm × 30 μm i.d.; BGE: 25 mM borate buffer (pH 8.0) + 30 mM SDS + 20% v/v *n*-propanol; separation voltage: 30 kV; temperature: 25°C; UV detection at 220 nm	UV	1 000–1 500 ng/mL [a]	Herbal blends	Ultrasound extraction with methanol for 15 min and centrifugation at 3 500 rpm for 10 min	[84]
12 Synthetic cathinones derivatives	**MEKC** Fused-silica capillary: 90 cm × 50 μm i.d.; BGE: 100 mM perfluooctanoic acid + 200 mM ammonium hydroxide; separation voltage: 22 kV; temperature: 25°C	ESI–MS/MS (QqQ)	10–78 ng/mL [b]	Urine	SPE with Supel-Select strong cation exchange cartridge	[85]
12 Synthetic cannabinoids	**MEKC** Fused-silica capillary: 90 cm × 50 μm i.d.; BGE: 50 mM perfluooctanoic acid (pH 9.0) + 20% v/v acetonitrile/water; separation voltage: 30 kV; temperature: 25°C	ESI–MS/MS (QqQ)	6 500–30 200 ng/g [a]	Herbal blends	Ultrasound extraction with methanol for 10 min	[86]
10 Synthetic cathinones derivatives	**FASI-CEC** Capillary: 100 μm i.d., packed with amylose *tris*(5-chloro-2-methylphenylcarbamate); mobile phase: 250 mM sodium acetate (pH 9.0) + 89% v/v acetonitrile + 10% v/v methanol; separation voltage: 10 kV; temperature: 20°C; UV detection at 206 nm	UV	25–100 ng/mL [a]	–	–	[87]

[a] Standard samples.
[b] Real samples.

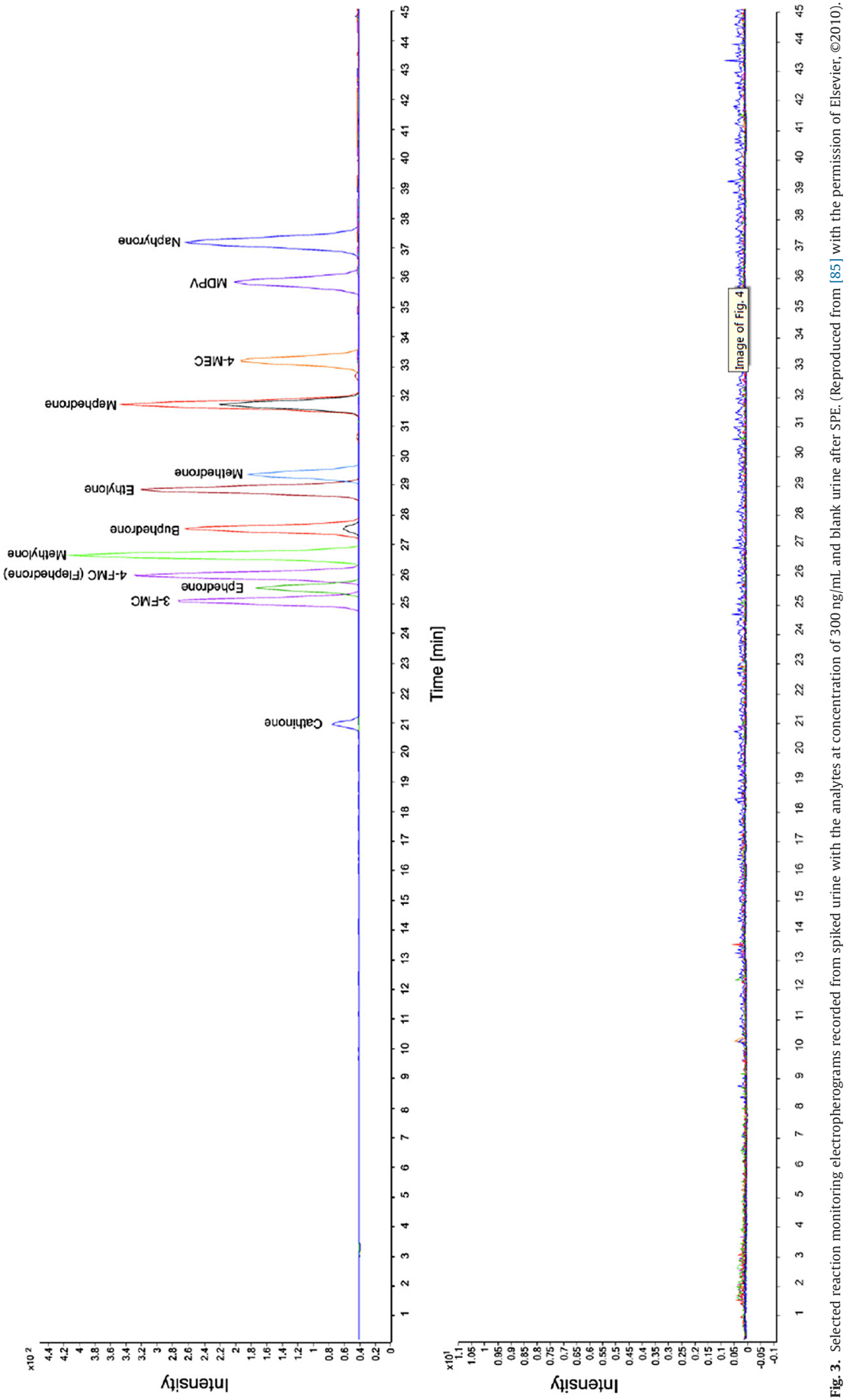

Fig. 3. Selected reaction monitoring electropherograms recorded from spiked urine with the analytes at concentration of 300 ng/mL and blank urine after SPE. (Reproduced from [85] with the permission of Elsevier, ©2010).

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conventional MEKC with UV detection. The LODs for urine samples were in the range 17.2–23.3 ng/mL, which are below the cut-off levels in urine {50 ng/mL [48]}. However, the overall analysis time including sample preparation (off-line SPE) was ~80 min, which makes the method less appealing for routine analysis.

Recently, a faster (approx. 30 min), simpler method was developed for the direct analysis of Δ^9-tetrahydrocannabinol's metabolites in urine by CZE-ESI-(QqQ)MS [77]. Due to the use of a highly-selective detector, such as (QqQ)MS, the only pretreatment needed for urine sample was dilution with methanol and centrifugation achieving LODs of 50 ng/mL, which proved once more the potential of the coupling between CE and (QqQ)MS for the analysis of low levels of DOAs. Analytical precision was reasonably acceptable with RSDs ≤0.36% for migration times and ≤25.0% for areas in real samples, in both intra-day and day-to-day experiments. The proposed method was successfully applied to the analysis of urine samples collected from cannabis users.

6. New designer drugs

Recent abuse of new designer drugs (synthetic cathinones, synthetic cannabinoids, piperazines, benzofurans, thiophenes and structural analogues of methylphenidate) gave rise to the need for sensitive, reliable, reproducible analytical methods for their determination in different kinds of matrix [78]. In 2010, Wohlfarth et al. [79] reviewed analytical protocols proposed in the past 10 years for the analysis of these compounds in biological matrices, including CE-based methods. With this in mind, this section will focus on papers {Table 6 [80–87]} related to the determination of new designer drugs by CE published after 2010 up to the present in different types of samples.

CZE-UV with CDs as chiral selectors for enantioseparations [80–82] and MEKC-UV with SDS as surfactant to enhance the separation [84] have been successfully applied for the analysis of new designer drugs in confiscated pills [80] and herbal blends [84]. However, in conjunction with ESI–MS, CDs and SDS may cause significant signal suppression. In order to overcome this issue, the application of a novel interface was proposed, referred to as sheathless CE, allowing the coupling of CE with ESI-MS in a single dynamic process within the same device for the MEKC-MS analysis of various cathinones [83]. A detailed description of this interface has been given by Haselberg et al. [88]. The approach proved to be suitable for reducing the ion suppression due to the presence of highly sulfated-γ-CD and 18-crown-6-tetracarboxylic acid used in this work as the BGE with good chiral resolution for the studied compounds within reasonable analysis times (20 min). However, further analysis and validation are required before being adopted by analytical laboratories to carry out enantioselective determinations on a routine basis.

As an alternative to the use of these conventional surfactants, salts of perfluorocarboylic acid have been used as the BGE in MEKC-ESI-MS for the determination of several cathinones in urine [85]. According to that work, the BGE used to form micelles did not affect the electron-ionization efficiency of MS, in contrast to SDS or CD micelles. Blank urine spiked with the drugs was analyzed using SPE as clean-up prior to analysis, reaching LODs of 10–78 ng/mL. Fig. 3 shows the reconstructed selected reaction monitoring electropherograms of the blank and spiked urine after the SPE. A similar method for the analysis of various synthetic cannabinoids in herbal blends was also presented with satisfactory results [86]. Nevertheless, the widespread application of these approaches might be limited because the salts of perfluorocarboylic acid are toxic surfactants, but, in any case, the developed methods represent a good tool for a rapid screening of new psychoactive drugs in illegal drug laboratories as well as in drug seizures.

CEC with UV [87] was also proposed for the analysis of cathinones using a fused-silica capillary column packed with amylose *tris*(5-chloro-2-methylphenylcarbamate), also called Sepapak 3 or Lux Amylose-2. To obtain enhanced sensitivity, FASI for on-line sample preconcentration was performed, achieving LODs of 25–100 ng/mL. Good intra-day RSDs (≤1.6%), inter-day RSDs (≤2.3%) and column-to-column RSDs (≤2.9%) for the retention time were obtained. However, no complete method validation or application on real samples was carried out.

7. Concluding remarks

This review compiles and summarizes the most recent developments in determining DOAs and their metabolites using CE. Although CE still has some important drawbacks, mostly due to its low sensitivity, CE-based techniques have often been successfully used for the analysis of these substances in a variety of matrices, shown by the large number of applications developed in recent years.

The lack of sensitivity has been overcome in several ways, using chromatographic and electrophoretic preconcentration techniques or a combination of these two procedures. However, these steps also involve certain disadvantages, such as more complicated and time-consuming procedures, lower reproducibility or, in some cases, environmental damage. Therefore, in this respect, future trends should focus on the study and the application of more sophisticated and more efficient on-line preconcentration strategies in order to achieve better analytical performance.

With respect to the samples tested to determine substances of abuse, existing literature mainly focuses on biological samples, with urine being the most commonly used matrix. In this sense, further work is needed in developing CE-based tools suitable for analyzing other biological fluids, as well as other different types of matrix, such as environmental.

As is well known, MS offers an alternative to the sensitivity enhancement in CE, so the importance of coupling this detection system with CE will continue to grow in toxicological analysis, so further improvements are needed in terms of combining on-line preconcentration strategies and CE separation with MS detection, as are considerable developments in terms of instrumentation and methodology of CE-MS interfacing.

Lastly, very few of the reported papers include complete method validation in accordance with commonly accepted international guidelines [89], which is a major drawback, since it impacts negatively on the reliability of the methods themselves. With this in mind, the future challenge is the development of CE-based procedures that allow method application with sensitivity, precision and reliability compared to those commonly obtained by chromatographic techniques.

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