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Capillary electrophoresis analysis of a wide variety of seized drugs using the same capillary with dynamic coatings

Capillary electrophoresis methodology is presented for the routine analysis of a wide variety of seized drugs using the same capillary with dynamic coatings and multiple run buffers. The types of exhibits analyzed using diode array UV detection include phenethylamines, cocaine, oxycodone, heroin, lysergic acid diethylamide (LSD), opium, hallucinogenic mushrooms, and γ-hydroxybutyrate-γ-butyrolactone (GHB-GBL). Both qualitative and quantitative analyses are achieved using run buffers that contain additives that provide for secondary equilibrium and/or dynamic coating of the capillary. Dynamic coating of the capillary surface is accomplished by rapid flushes of 0.1 N sodium hydroxide, water, buffer containing polycation coating reagent, and a buffer containing a polyanionic coating reagent (with or without cyclodextrin(s)) or a micelle coating reagent. Dynamic coating with a polyanionic coating reagent is used for the analysis of moderately basic seized drugs and adulterants. The use of cyclodextrin in the run buffer not only allows for chiral analysis but also greatly enhances separation selectivity for achiral solutes. A capillary dynamically coated with a micelle allows for the analysis of neutral, acidic, and weakly basic drugs (GHB, GBL and neutral, acidic, and weakly basic adulterants). Dynamic coating, which gives rise to a relatively high and robust electroosmotic flow at pH < 7, allows for rapid, precise and reproducible separations. For a wide variety of drugs, excellent linearity and migration time precision and good peak area precision (external and internal standard) is obtained. Quantitative results for synthetic mixtures are in good agreement with actual values. Screening for adulterants is greatly enhanced by the use of automated library searches.

Keywords: Capillary electrophoresis / Dynamically coated capillaries / Seized drugs
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1 Introduction

The analysis of seized drugs is important for legal and intelligence purposes. Rapid, precise, and reproducible methodology is required for the quantitative as well as qualitative determination of drugs of forensic interest and related materials. Gas chromatography (GC) [1, 2], gas chromatography-mass spectrometry (GC-MS) [3, 4], high-performance liquid chromatography (HPLC) [5–8], HPLC-MS [9, 10], nuclear magnetic resonance (NMR) [11, 12], and capillary electrophoresis (CE) [13–18] have been used for this purpose. Although GC offers the highest resolving power for achiral solutes, limitations exist for the analysis of highly polar (*e.g.*, amphetamines, morphine), thermally labile (*e.g.*, lysergic acid diethylamide (LSD), psilocybin, γ-hydroxybutyrate (GHB)) and nonvolatile solutes (*e.g.*, sugars and polyhydric alcohols) [8]. For these solutes, derivatizations and/or prior extractions are required. HPLC, which allows for the direct analysis of the above compounds, inherently lacks resolution. Although NMR identifies compounds in simple mixtures, and can perform quantitation without a primary reference drug standard, complex samples can be difficult to identify and quantitate. CE, which also allows for the direct analysis of the above solutes, has significantly greater resolving power than HPLC. In addition, for the chiral GC or HPLC analysis of chiral solutes, expensive chiral columns (usually specific for a class of compounds) and/or derivatization are required [19]. Even with the above, HPLC can

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Abbreviations: DM-β-CD, dimethyl-β-cyclodextrin; **GBL**, γ-butyrolactone; **GHB**, γ-hydroxybutyrate; **HP-β-CD**, hydroxypropyl-β-CD; **LAMPA**, lysergic acid methylpropylamide; **LSD**, lysergic acid diethylamide; **MDA**, methylenedioxyamphetamine; **MDEA**, methylenedioxyethylamphetamine; **MDMA**, methylenedioxymethamphetamine

suffer from poor resolution and/or long analysis time [19, 20]. CE, which uses chiral additives such as cyclodextrins (CDs) in the run buffer, allows for the direct chiral determination of seized drugs using conventional capillaries [21].

Numerous methods using uncoated capillaries have been reported for the routine CE analysis (achiral and/or chiral) of seized drugs including phenethylamines [21–32], cocaine [33–35], propoxyphene [36], heroin [25, 27, 37–39], LSD [27], opium [40–44], psilocybin [45, 46], and GHB-γ-butyrolactone (GBL) [47]. These methods involve capillary zone electrophoresis (CZE) [24, 26–32, 34, 36, 39, 41, 44–46] with or without secondary equilibrium, micellar electrokinetic chromatography (MEKC) [22, 23, 33, 35, 37, 38, 40, 42, 47], and electrokinetic chromatography (ECC) [21, 43]. For methods using CZE, MEKC or ECC, separate capillaries would be recommended. "Memory effects" lead to nonreproducible separations unless tedious reconditioning steps are carried out. Therefore, for a forensic lab wishing to perform CE analysis on a wide variety of seized drugs, either multiple instruments, or a single instrument with multiple capillaries would be recommended. In the latter case, the types of unattended analysis would be limited, the complexity of operation would increase and the frequent swapping of capillaries greatly increases the chances of broken capillaries.

Dynamically coated capillaries provide significant improvement in separation times, precision and selectivity. Dynamically coated capillaries, using an initial coating with a polymeric cation and subsequent coating with a polymeric anion or micelle, offer faster separation times, improved precision, and increased selectivity for the analysis of basic, acidic, and neutral drugs of forensic interest. Phenethylamines and cocaine exhibits have been analyzed using this dual coating procedure with a polymeric anion [12]. Opium preparations [48] and heroin samples [49] have been analyzed using this latter coating methodology with CD(s) added to the buffer containing the polymeric anion. The same methodology can be used for LSD exhibits [48]. A coating procedure developed by Chevigne and Janssens [50] is the basis for above separations. This methodology, carried out during every run, consists of a two-step process whereby the capillary (after base hydrolysis) is first coated with a polycation (an initiator), then coated with a polyanion (an accelerator). The run buffer contains the latter coating reagent. This process gives rise to a highly precise EOF over a wide pH range and to a capillary surface with more favorable kinetics. For basic solutes, it is desirable to perform dynamic coating at a low pH such as 2.5. At this pH most basic solutes (moderately basic compounds) are fully ionized ($pK_a > 5$) and therefore their mobilities will not change with small differences in run buffer pH. In addition, at pH 2.5 this CE system is highly selective for the analysis of moderately basic solutes since most weakly basic, acidic, and neutral compounds exhibit smaller positive mobilities, negative mobilities or no mobilities, respectively. For separations (achiral or chiral) requiring additional selectivity, CDs are added to the pH 2.5 dynamically coated capillary system [48, 49, 51]. The use of dynamically coated capillaries in the normal polarity MEKC mode allows for the analysis of neutral, acidic, and weakly basic solutes over a wide pH range, even at low pH. For dynamically coated MEKC, several approaches have been reported including using dextran sulfate [52], polyvinylsulfonate [53] or SDS [54] as the anionic coating with sodium dodecyl sulfate (SDS) in the run buffer. At pH $\leq$ 6.5, excellent selectivity has been demonstrated for the determination of neutral, acidic, and weakly basic adulterants present in heroin in the presence of moderately basic compounds [49]. At this pH range, most basic solutes are significantly ionized and will ion-pair with SDS (either on stationary phase or run buffer) and therefore will migrate after the acidic and neutral solutes.

In this report, the routine analysis of a wide variety of seized drugs using a single dynamically coated capillary with run multiple buffers is demonstrated. A dual coating procedure is used consisting of initial coating with a proprietary polymeric cation followed by coating with either a proprietary polymeric anion (with or without CD(s)) or SDS.

2 Materials and methods

2.1 Chemicals

Drug standards were obtained from the reference collection of the Drug Enforcement Administration Special Testing and Research Laboratory (Dulles, VA, USA). CElixir Reagent A (pH 2.5), CElixir Reagent B (pH 2.5), 50 mM phosphate-borate (pH 6.5), 50 mM phosphate (pH 6.5), and 0.1 N sodium hydroxide were acquired from MicroSolv Technology (Eatontown, NJ, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD) dimethyl-β-cyclodextrin (DM-β-CD), and sodium dodecyl sulfate (SDS) were obtained from Sigma (St. Louis, MO, USA). Sodium phosphate (monobasic), phosphoric acid, and sodium hydroxide were reagent grade. HPLC-grade methanol was acquired from Burdick and Jackson (Muskegon, MI, USA). Deionized and high purity water (HPLC-grade water) were obtained from a Millipore Synergy 185 water system (Bedford, MA, USA).

2.2 Instrumentation and procedures

An Agilent Model HP^{3D}CE Capillary Electrophoresis System equipped with a diode array detector (Waldbronn, Germany) was used for CE experiments. Prior to first

use, new bare silica capillaries were conditioned following the same procedure used for regular analysis using CElixir Reagent B (pH 2.5). The capillaries were first flushed with 0.1 N sodium hydroxide for 1 min, then water for 1 min, followed by CElixir Reagent A for 1 min, and finally run buffer for 2 min. For conditioning new dynamically coated capillaries for use with run buffers containing SDS, the capillaries were first flushed with 0.1 N sodium hydroxide for 1 min, then water for 1 min, followed by CElixir Reagent A for 1 min, then either 50 mM phosphate-borate (pH 6.5) or 50 mM phosphate (pH 6.5) for 1 min, and finally the run buffer for 6 min. For subsequent injections only 2 min flushes with run buffer were required. When switching between a CE method using CElixir Regent B and a method using SDS, the new capillary coating procedure (for an SDS run buffer) was employed for the first injection. For overnight or prolonged storage, the capillary was flushed with water for 10 min and then stored with the inlet and outlet dipped in water. The method used either 2.0 mL CE glass vials or 1.0 mL CE polypropylene vials as electrolyte reservoirs. When using glass vials, waste vials were filled with 500 μL of water, while flush vials, run buffer, standard and sample vials were filled with 1000 μL of liquid (for 0.1 N sodium hydroxide add 500 μL to polypropylene vial). For polypropylene vials, waste vials were filled with 250 μL of water, while all others were filled to 500 μL of liquid.

2.3 Preparation of standards and samples

The preparation of the internal standard, standard and sample solutions is shown in Tables 1 and 2, respectively. Unless indicated otherwise, samples are sonicated for 15 min. For the above standard and sample prepara-

Table 1. Preparation of internal standards

No.	Internal standard (IS)	Concentration (mg/mL)	Dilution solvent No.
1	*N*-Butylamphetamine HCl	1.0	1[a)]
2	Phenyltoloxamine citrate	1.0	1[a)]
3	Procaine HCl	1.0	1[a)]
4	Tetracaine HCl	0.3	1[a)]
5	Tetracaine HCl	0.3	2[b)]
6	Resorcinol	1.0	3[c)]

a) 75 mM sodium phosphate monobasic, adjusted to pH 2.6 with phosphoric acid and diluted 1:20 with HPLC-grade water; alternatively, injection solvent concentrate (which can be purchased from MicroSolv) diluted 1:20 with HPLC-grade water
b) 1:11 mixture of methanol and dilution solvent 1
c) 50 mM sodium phosphate monobasic, adjusted to pH 6.5 with sodium hydroxide and diluted 1:10 with HPLC-grade water

Table 2. Preparation of standard and sample solutions

Analysis (standard solute(s) used)	Target standard (Std) and sample concentration (mg/mL)	IS No.[a)]	IS Std and IS sample concentration (mg/mL)	Std and sample dilution solvent No.[a)]
Phenethylamines (racemic HCl solutes)	0.08	1	0.1	1
Cocaine (cocaine HCl)	0.1	2	0.1	1
Oxycodone (oxycodone HCl)	0.1	3	0.1	1
Propoxyphene (d-propoxyphene HCl, l-propoxyphene napsylate)	0.05			1
LSD (LSD tartrate)	0.008[b)]	4	0.025	[b)]
Opium and latex	[c)]	5	0.025	[c)]
GHB, GBL	3.0, 7.0	6	0.1	3
Heroin (heroin HCl)	0.4			4
Psilocybin mushrooms	[d)]			[d)]

a) See Table 1
b) 0.1 mg/mL standard in methanol (sample extracted mechanically shaken for 30 min); combine 1.0 mL methanol solution with 1.0 mL IS solution and 10.0 mL of dilution solvent 1
c) 0.025 mg/mL each of standard morphine HCl, codeine HCl, thebaine base, noscapine base, and papaverine HCl dissolved in dilution solvent 2. For opium sample, weigh 100 mg opium into 50 mL volumetric flask, add 25 mL methanol, sonicate 30 min at 50–60°C, dilute to volume with dilution solvent 1. For opium latex, after thoroughly mixing, weigh 250 mg into 100 mL volumetric flask, add 50 mL methanol, vortex 1 min, dilute to volume with dilution solvent 1. For both sample types, pipette 200 μL of sample into a 2.0 mL vial and combine with 1.0 mL of tetracaine HCl (0.03 mg/mL with dilution solvent 1)
d) 0.5 mg/mL and 0.6 mg/mL standard psilocin and psilocybin, respectively, in methanol (sonicated for 5 min). For Psilocybe mushroom exhibits, psilocybin concentration in methanol (after sonicating for 50 min) should equal approximately the standard concentration. Combine 1.0 mL of standard and sample solution with 11.0 mL of dilution solvent 1

tions, filter approximately 500 μL or 1.0 mL of solution with 0.5 μm nylon filter (SRI) into either a 1.0 mL CE polypropylene plastic vial, or a 2.0 mL CE glass vial, respectively (Agilent).

2.4 Capillary electrophoretic conditions

All experiments were carried out with either a 50 μm ID 32 cm (24 cm to the detector) fused-silica capillary obtained from Polymicro Technologies (Phoenix, AZ, USA) or a 50 μm ID 33 cm (24.5 cm to the detector) pre-made capillary (Agilent) operated at 15°C. 50 mbar pressure injections of 2–10 s durations were used followed by a 35 mbar pressure injection of water for 1 s. For electrophoresis, an initial 0.5 min linear voltage ramp from 0 V to the final voltage was used for most analyses (heroin analysis 1.0 min ramp). All run buffers (which can be purchased from MicroSolv) were filtered into a 22 mL Teflon PVA vial (Cole Parmer) using a 0.5 μm nylon filter (SRI) and refiltered weekly. CE conditions for the various analyses are given in Table 3.

3 Results and discussion

For the analysis of seized drugs, dynamically coated capillaries have been used in both the CZE mode [12, 48, 49] (moderately basic solutes) and MEKC mode [49] (weakly basic, acidic, and neutral). In order for a single capillary to be used for a wide variety of seized drugs, these coating procedures should be compatible. Five injections of moderately basic solutes (phenethylamines) followed by five injections of weakly basic, acidic, and neutral compounds (acetaminophen, theophylline, caffeine, aspirin, salicylic acid, antipyrene, phenobarbital, phenacetin, and benzocaine) and subsequently five injections of moderately basic solutes were performed. Both systems are highly compatible and precise (run-to-run migration time RSDs $\leq 0.12\%$, $\leq 0.61\%$, and $\leq 0.04\%$, respectively, for the three experiments). For CZE analysis, a dual coating procedure is required between every injection for good CE performance. However, for MEKC separations a dual coating procedure was only required for the first separation. For subsequent injections using this technique, the capillary was flushed with run buffer.

Table 3. CE conditions for the analysis of a wide variety of seized drugs using dynamically coated capillaries

Analysis	Run buffer	Voltage	Injection
Phenethylamines, cocaine, and oxycodone	CElixir Reagent B (pH 2.5) [12]	10 kV	100 mbar·s
Chiral	CElixir Reagent B (pH 2.5) + 7.88%[a] HP-B-CD	20 kV	100 mbar·s; followed by co-injection 100 mbar·s sample + 30 mbar·s standard
LSD	CElixir Reagent B (pH 2.5) + 3.94%[a] HP-B-CD + 9.98%[a] DM-B-CD [48]	20 kV	500 mbar·s
Opium	Celixir, Reagent B (pH 2.5) + 3.94%[a] HP-B-CD + 9.98%[a] DM-B-CD [48]	20 kV	250 mbar·s
Heroin	CElixir Reagent B (pH 2.5) + 13.30%[a] DM-B-CD [49]	13 kV	250 mbar·s
Hallucinogenic mushrooms	CElixir Reagent B (pH 1.8)[b]	10 kV	100 mbar·s
Neutral, acidic, and weakly basic adulterants	50 mM phosphate-borate (pH 6.5) + 3.00%[a] SDS [49]	8.5 kV	100 mbar·s
GHB, GBL	50 mM phosphate (pH 6.5) + 3.00%[a] SDS	8.5 kV	100 mbar·s

a) % w/v
b) pH adjusted using phosphoric acid

3.1 Quantitative analysis of phenethylamines, cocaine, and oxycodone and identification of moderately basic adulterants

For the quantitative analysis of phenethylamine and cocaine exhibits, updated coating methodology and sample preparation procedures over reported methodology [12] are presented (see Section 2.2). The same conditioning steps used between injections are now used for a new capillary. It is not necessary, as previously reported [12], to use a longer base wash for the first injection on a new capillary than for subsequent injections. In addition, 0.1 N sodium hydroxide is used instead of 1 N sodium hydroxide to improve longevity of the capillary. Figures of merit for amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), cocaine, and oxycodone are shown in Table 4. Excellent linearity, good run-to-run relative area precision and good quantitative accuracy are obtained for these solutes. Relative migration time data for phenethylamines and related compounds (internal standard, impurities and adulterants) and cocaine, oxycodone and related compounds are shown in Tables 5 and 6, respectively. Detection at 235 nm provides increased selectivity (de-creased sensitivity) for cocaine analysis. Screening for basic adulterants is facilitated by the use of automated library searches.

Table 4. Figures of merit[a)] for seized drugs using dynamically coated capillary systems

Solute	Linearity range (mg/mL)	R^2	Peak area precision $n = 5$ (RSD)	Accuracy[b)] (E%)
Amphetamine	0.00318–0.10	0.9998	$< 2.0\%$[c)]	< 2.4
Methamphetamine	0.00316–0.10	0.9999	$< 2.0\%$[c)]	< 1.2
MDA	0.00322–0.10	1.0000	$< 2.6\%$[c)]	< 2.4
MDMA	0.00318–0.10	1.0000	$< 1.6\%$[c)]	< 3.2
MDEA	0.00316–0.10	1.0000	$< 2.7\%$[c)]	< 1.5
Cocaine	0.00314–0.40	0.9999	$< 2.2\%$[c)]	< 3.7
Oxycodone	0.0317–0.50	0.9999	$< 1.5\%$[d)]	< 4.5
Heroin	0.025–0.802	0.9999	$< 0.4\%$[e)]	< 2.3
LSD	0.000797–0.0255	1.0000	$< 2.5\%$[c)]	< 0.7
GHB	0.304–9.73	0.9999	$< 1.3\%$[d)]	< 2.7
GBL	0.606–9.69	1.0000	$< 1.5\%$[d)]	< 3.3

a) Linearity and accuracy data for amphetamine, methamphetamine, MDA, MDMA, MDEA, and cocaine obtained from [12]
b) For basic solutes analysis was performed on known mixtures of the seized drug and mannitol, inositol or lactose. For GHB and GBL analysis was performed on Gatorade spiked with known amount of seized drug.
c) Area of solute/area of internal standard
d) Corrected area (area/migration time) of solute/corrected area of internal standard
e) Corrected area

3.2 Identification of enantiomers of phenethylamines and propoxyphene

The identification of enantiomers of solutes such as phenethylamines (controlled and noncontrolled) and propoxyphene is easily accomplished by adding the chiral selector HP-β-CD to CElixir Reagent B (pH 2.5). As shown in Fig. 1, an excellent simultaneous separation of six racemic phenethylamines is obtained in < 4 min. d,l-Propoxyphene is baseline-resolved in < 5 min. Excellent run-to-run precision is obtained for the phenethylamines (RSD $\leq 0.12\%$) and propoxyphene (RSDs $\leq 0.04\%$). Enantiomer identification is facilitated by peak enhancement accomplished by subsequentially co-injecting a mixture of standard and sample. Relative migration data for phenethylamine and propoxyphene enantiomers is presented in Table 7.

3.3 Quantitative analysis of LSD

d-LSD and d-lysergic acid methylpropylamide (d-LAMPA) comigrate, while d-LSD and d-iso-LSD are resolved using CElixir Reagent B (pH 2.5). The addition of a mixture of CDs to the CElixir Reagent B (pH 2.5) run buffer provided,

Table 5. Relative migration times for phenethylamines and related compounds using a dynamically coated capillary system

Solute	Relative migration time
Doxylamine	0.765
Chlorpheniramine	0.784
Quinine	0.804
β-Phenethylamine	0.807
Chloroquine	0.812
Nicotinimide	0.836
Amphetamine	0.868
Methamphetamine	0.883
Procaine	0.883
MDA	0.900
Norpseudophedrine	0.906
MDMA	0.914
Norephedrine	0.917
Pseudoephedrine	0.919
Tetracaine	0.927
Ephedrine	0.932
Phenylephrine	0.951
MDEA	0.961
Ketamine	0.962
Phenyltoxylamine	0.971
n-Butylamphetamine	1.000 (4.6 min)
Methorphan	1.000
Lidocaine	1.030
Benzocaine	1.250

Table 6. Relative migration times for cocaine, oxycodone, and related compounds using a dynamically coated capillary system

Solute	Relative migration time
Procaine	0.909
Tetracaine	0.955
Phenyltoxylamine	1.000 (4.5 min)
Cocaine	1.040
Lidocaine	1.060
cis-Cinnamoylcocaine	1.070
trans-Cinnamoylcocaine	1.090
Oxycodone	1.120
Benzocaine	1.290
Benzoylecgonine	1.730

via secondary equilibria, the selectivity to fully resolve the above solutes (Fig. 2). It is unclear as to why LAMPA (not present in actual LSD samples) exhibits a broader peak than LSD. Figures of merit for LSD are shown in Table 4. Excellent linearity, good run-to-run area precision and excellent quantitative accuracy are obtained for these solutes. Good run-to-run precision is also obtained for

Table 7. Relative migration times for phenethylamine and propoxyphene enantiomers using a dynamically coated capillary system

Solute	Relative migration time
l-Norpseudophedrine	0.814
d-Norephedrine	0.832
l-Norephedrine	0.832
l-Pseudophedrine	0.833
l-Amphetamine	0.850
d-Ephedrine	0.858
d-Amphetamine	0.861
l-Ephedrine	0.866
l-Methamphetamine	0.874
d-Norpseudoephedrine	0.876
d-Methamphetamine	0.889
d-Pseudoephedrine	0.900
n-Butylamphetamine[a)]	1.000 (3.75 min)
n-Butylamphetamine[a)]	1.020
MDA[a)]	1.030
MDA[a)]	1.040
MDMA[a)]	1.050
MDMA[a)]	1.070
MDEA[a)]	1.100
MDEA[a)]	1.120
d-Propoxyphene	1.140
l-Propoxyphene	1.160

a) d- or l-enantiomer

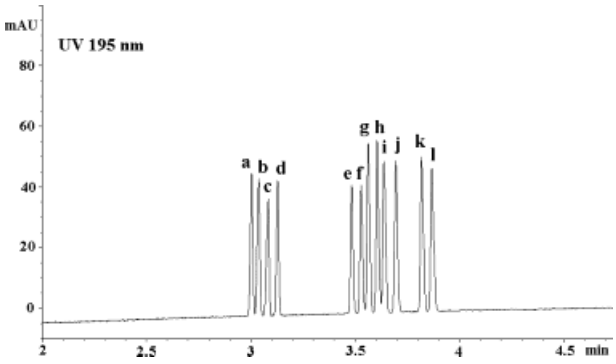

Figure 1. Dynamically coated CE separations using a 33 cm (24.5 cm to the detector window) × 50 μm ID fused-silica capillary. Solute concentrations were approximately 0.05 mg/mL with CE conditions as described in Section 2. Electropherogram of a standard mixture of (a) l-amphetamine, (b) d-amphetamine, (c) l-methamphetamine, (d) d-methamphetamine, (e) l- or d-*n*-butylamphetamine, (f) l- or d-*n*-butylamphetamine, (g) l- or d-MDA, (h) l- or d-MDA, (i) l- or d-MDMA, (j) l- or d-MDMA, (k) l- or d-MDEA, and (l) l- or d-MDEA.

LSD and tetracaine (IS) (RSD ≤ 0.76%) [48]. Relative migration time data for LSD and related compounds is shown in Table 8.

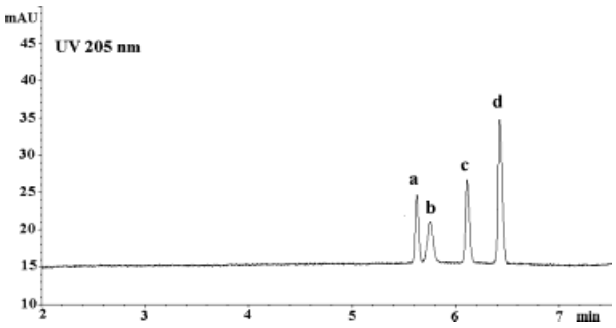

Figure 2. Electropherogram of a standard mixture of (a) d-LSD, (b) d-LAMPA, (c) d-iso-LSD, and (d) tetracaine (internal standard). A 32 cm (23.5 cm to the detector window) × 50 μm ID fused-silica capillary was used. Solute concentrations were approximately 0.008 mg/mL with CE conditions as described in Section 2.

Table 8. Relative migration times for LSD and related compounds using a dynamically coated capillary system

Solute	Relative migration time
Lysergic acid amide	0.808
LSD	0.873
LAMPA	0.896
Iso-LSD	0.949
Lysergic acid	0.979
Tetracaine	1.000 (6.4 min)
Ergotamine	1.070

3.4 Identification of major alkaloids in opium

Five major alkaloids in opium, including morphine, papaverine, codeine, noscapine, and thebaine, are well resolved using the same run buffer used for LSD (Fig. 3).

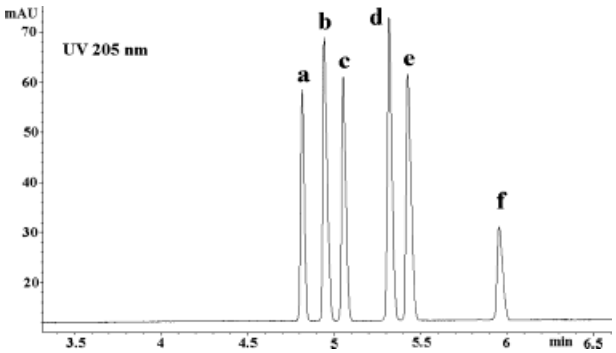

Figure 3. Electropherogram of a standard mixture of (a) morphine, (b) papaverine, (c) codeine, (d) noscapine, (e) thebaine, and (f) tetracaine (internal standard). A 32 cm (23.5 cm to the detector window) × 50 μm ID fused-silica capillary was used. Solute concentrations were approximately 0.02 mg/mL with CE conditions as described in Section 2.

Excellent run-to-run migration time precision is obtained for these solutes (RSD $\leq$ 0.12%) [48]. The same CE conditions are applicable for special analyses, *i.e.*, the quantitation of opium preparations [48]. Identification of the major opium alkaloids is facilitated by the use of automated library searches.

3.5 Quantitative analysis of heroin and identification of moderately basic adulterants

Methodology has been previously reported for the quantitative analysis of heroin, basic impurities, and basic adulterants using dynamically coated capillaries [49]. It was necessary to add dimethyl-β-cyclodextrin (DM)-β-CD to CElixir Reagent B (pH 2.5) not only to resolve solutes from heroin, but also to improve the separation of the basic impurities. In order to fully resolve these compounds, a longer capillary (relative to the capillary length in this present study) was required (total length, 64 cm) with a higher temperature [49]. For this study since we are only concerned with the quantitation of heroin and the screening of adulterants the shorter capillary is sufficient. Figures of merit for heroin are shown in Table 4. Excellent linearity, excellent run-to-run area precision and quantitative accuracy are obtained for this solute. Excellent run-to-run migration time precision is also obtained for heroin (RSD 0.07%). Relative migration time data for heroin, moderately basic impurities and mostly moderately basic adulterants (nicotinamide and aminopyrene are weak bases) are shown in Table 9. Screening for adulterants is facilitated by the use of automated library searches.

Table 9. Relative migration times for heroin and related compounds using a dynamically coated capillary system

Solute	Relative migration time
Thiamine	0.547
Nicotinamide	0.645
Quinine	0.655
Chloroquine (d or l)	0.711
Chloroquine (d or l)	0.716
Quinine impurity	0.805
Heroin	0.924
Dipyrone	0.947
Lidocaine	0.960
Aminopyrene	0.960
O6-Monoacetylmorphine	0.962
O3-Monoacetylmorphine	0.975
Acetylcodeine	1.00 (7.6 min)
Morphine	1.01
Papaverine	1.03
Strychnine	1.04

Table 9. Continued

Solute	Relative migration time
Codeine	1.06
l-Ephedrine	1.08
l-Pseudoephedrine	1.08
d-Ephedrine	1.09
Thebaine	1.11
d-Pseudoephedrine	1.12
Noscapine	1.12
Procaine	1.15
d-Chlorpheniramine	1.17
d-Brompheniramine	1.18
Tetracaine	1.21
Cocaine	1.22
trans-Doxepin	1.22
cis-Doxepin	1.23
Diphenhydramine	1.23
Yohimbine	1.27
Benactyzine	1.27
Chlorpromazine	1.31
Bromodiphenhydraminine	1.36

3.6 Identification of major alkaloids in hallucinogenic mushrooms

Psilocin ("Psilocybe mushroom species") and bufotenine (Bufo toad species) comigrate (Fig. 4A). This is not surprising since the fully protonated positional isomers psilocin and bufotenine (differing only in position of a phenol functional group) would be expected to have similar mobilities. In addition, psilocybin (Psilocybe mushroom species) migrates near t_o with significant tailing using CElixir Reagent B (pH 2.5) (Fig. 4A). This is due to the amphoteric nature of psilocybin at pH 2.5 (fully ionized tertiary amine group and mostly ionized phosphate group). Lowering the pH to 1.8 partially protonates the psilocybin phosphate group (*i.e.*, imparts a greater $\Delta+$ charge) and therefore increases the effective mobility of this solute. As a result, psilocybin migrates further from t_o with an improved peak shape (Fig. 4B). As expected, the relative mobilities of psilocybin and bufotenine, which are also fully protonated at pH 1.8, are similar to their relative mobilities at pH 2.4. However, due to secondary equilibrium, psilocin and bufotenine are fully resolved after the addition of 50 mM HP-β-CD to the CElixir Reagent B (pH 1.8) buffer with good peak shape for psilocybin (Fig. 4C). Since psilocin and bufotenine have easily distinguishable diode array UVs (Figure *5*) and are not found together, the pH 1.8 run buffer which gives shorter migration times for psilocybin is recommended. Excellent run-to-run precision is obtained for these solutes (RSD $\leq$ 0.39%).

3.7 Identification of neutral, acidic, and weakly basic adulterants

Methodology has been previously reported for the identification of weakly basic, acidic, and neutral adulterants in heroin using dynamically coated capillaries [49]. This same methodology is applicable to the identification of these adulterants in phenethylamine and cocaine samples. Relative migration time data for neutral, acidic and

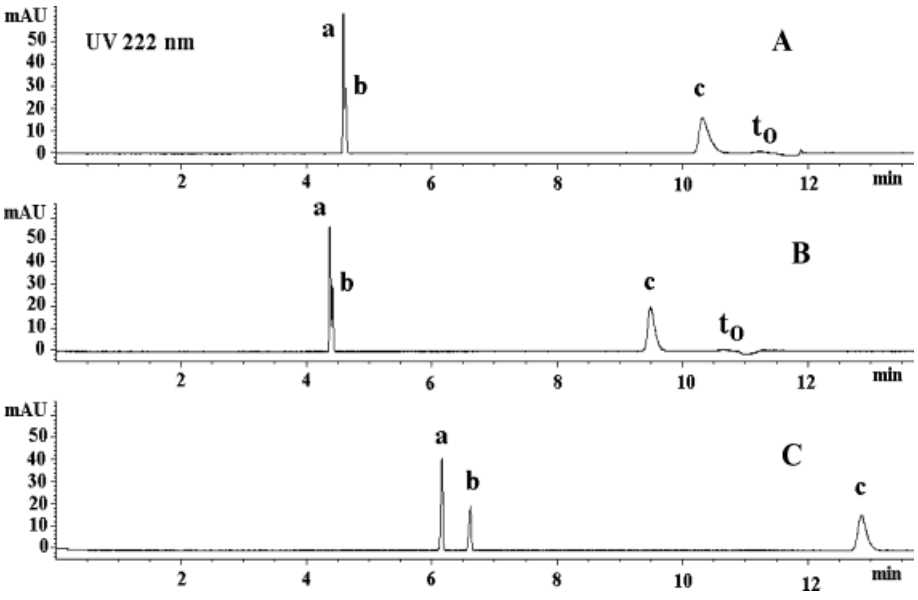

Figure 4. Comparison of CE separations of standard mixture of (a) psilocin (0.05 mg/mL), (b) bufotenine (0.04 mg/mL), and (c) psilocybin (0.07 mg/mL) using various run buffers. A 32 cm (23.5 cm to the detector window) × 50 μm ID fused-silica capillary was used at 15°C with a voltage of 10 kV with 100 mbar·s injections. (A) Anionic coating reagent and run buffer consisting of CElixir Reagent B (pH 2.5). (B) Anionic coating reagent and run buffer consisting of CElixir Reagent B (pH 1.8). (C) Anionic coating reagent and run buffer consisting of CElixir Reagent B (pH 1.8) + 50 mM HP-β-CD.

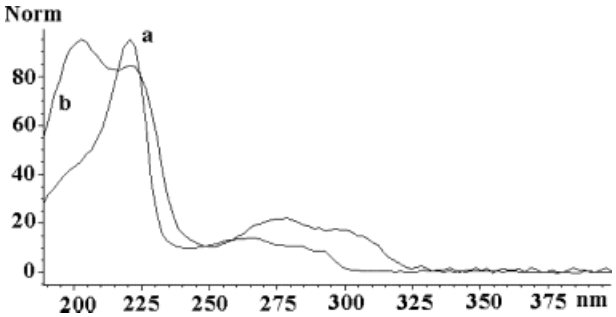

Figure 5. Diode array UV spectra of (a) psilocin, and (b) bufotenine obtained using experimental conditions described in Fig. 4B.

weakly basic adulterants are shown in Table 10. Screening for these adulterants is facilitated by the use of automated library searches.

3.8 Quantitative analysis of GHB and GBL

The separation of GHB and GBL using dynamically coated capillaries is shown in Figure 6. A pH 6.5 run buffer (50 mM phosphate + 3% SDS) is chosen to minimize the chemical interconversion of GHB and GBL [55]. As demonstrated in Figs. 6A and B, no interconversion is obtained for either solute under these conditions. Furthermore, no interconversion occurs even for solutions sitting overnight in the autosampler. This run buffer is used instead of 50 mM phosphate-borate (adulterant analysis) because the latter reagent gives a peak, which can interfere with GBL. While GBL, a neutral solute, is retained by the micelle, the negatively charged SDS aggregate should repel the anionic GHB. The longer migration time of the latter solute is probably due to the high mobility of this relatively small solute in the anodic direction. The triangular GHB peak is caused by electromigration dispersion of the anionic solute present at a relatively high concentration (weak extinction coefficient). According to Weinberger [56], quantitative results are still maintained as long

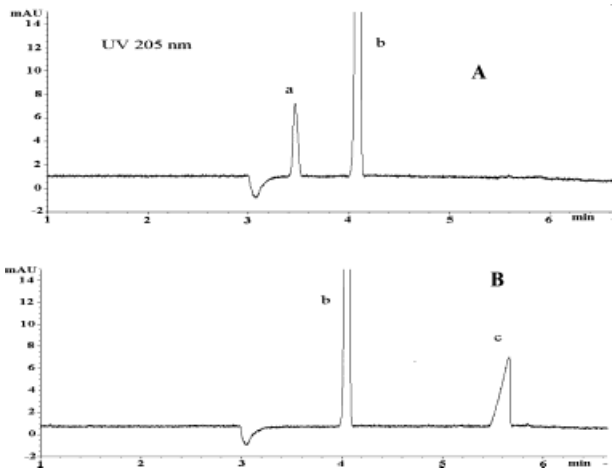

Figure 6. Electropherograms of (A) standard mixture of (a) GBL (7.0 mg/mL), and (b) resorcinol (0.1 mg/mL) (internal standard) and (B) standard mixture of (b) resorcinol (internal standard) and (c) GHB (3.5 mg/mL). A 32 cm (23.5 cm to the detector window) × 50 μm ID fused-silica capillary was used with CE conditions as described in Section 2.

Table 10. Relative migration times for weakly basic, acidic, and neutral solutes using a dynamically coated capillary system

Solute	Relative migration time
t_o (DMSO)	0.504
Nicotinamide	0.591
Acetaminophen	0.660
Theophylline	0.673
Dipyrone (1)	0.738
Caffeine	0.764
Aspirin	0.809
Salicylic acid	0.860
Anitipyrene	0.948
Phenobarbital	1.000 (6.8 min)
Ibuprofen	1.110
Aminopyrene	1.110
Phenacetin	1.130
Dipyrone (2)	1.130
Benzocaine	1.250
Thiamine	1.320
Morphine	1.350
Codeine	1.440
O3-Monoacetylmorphine	1.440
Procaine	1.450
Pseudoephedrine	1.450
Ephedrine	1.450
Lidocaine	1.460
Heroin	1.470
O6-Monoacetylmorphine	1.470
Acetylcodeine	1.470
Noscapine	1.470
Quinine	1.480
Chloroquine	1.480
Yohimbine	1.480
Strychnine	1.480
Thebaine	1.480
Xylazine	1.490
Cocaine	1.490
Tetracaine	1.490
cis- and *trans*-Doxepin	1.500
Brompheniramine	1.500
Methorphan	1.500
Papaverine	1.500
Chlorpheniramine	1.510
Diphenhydramine	1.510

as peak areas are used and sufficient resolution is designed into the separation. This is the case as indicated by the figures of merit (see Table 4). Excellent linearity, excellent run-to-run area precision, and good quantitative accuracy are obtained for these solutes. Excellent run-to-run precision is also obtained for these solutes (RSD $\leq$ 0.12%). Due to a very weak extinction coefficient, 1,4-butanediol was not detected as high as 10.0 mg/mL concentration. Unlike GHB, this solute is easily analyzed by GC [55]. Although GBL is also easily analyzed by GC, this compound can be encountered in combination with GHB.

3.9 Reproducibility

Certain test solutes were analyzed by multiple CE systems (consecutively, on the same day) over a two-week period. d,l-Methamphetamine, d,l-MDMA and d,l-*n*-butylamphetamine were separated using CElixir Reagent B (pH 2.5) and CElixir Reagent B (pH 2.5) with 50 mM HP-β-CD. Theophylline and caffeine were resolved by using 50 mM phosphate-borate (pH 6.5) with 3% SDS. Although no significant changes in the phenethylamine separations occurred, the EOF for the SDS system decreased from 4.5×10^{-4} to 3.6×10^{-4}. However, for the SDS system, reproducible separations were obtained (RSDs of effective mobilities $< 1.0\%$).

Since different lots of the same CD can vary in both the degree of substitution* and the position of substituents, each time a CD from a new batch is received, test mixtures are analyzed. For multiple lots of both CDs there were no significant changes for most separations tested. For a most recent lot of HP-β-CD, d-methamphetamine comigrated with d-pseudoephedrine. Changing the CD concentration from 50 mM to 45 mM resolved these solutes and gave the expected separation for the other compounds in the test mixtures.

3.10 Applications of the methodology to seized drugs exhibits

Examples of the above methodology for the analysis of seized drug samples are shown in Figs. 7–9. These examples include the analysis of an illicit methamphetamine

* Using electrospray-MS, the manufacturer of DM-β-CD has shown that different lots have different degrees of substitution.

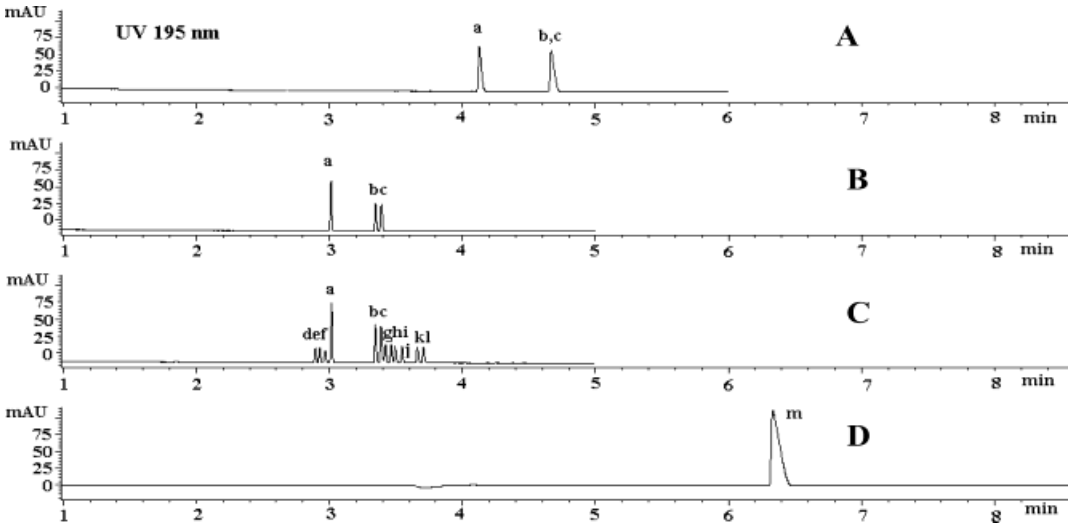

Figure 7. CE analysis of an illicit methamphetamine tablet (same sample vial) using multiple run buffers with a 32 cm (23.5 cm to the detector window) $\times$ 50 μm ID fused-silica capillary operating at 15°C. (A) Electropherogram with anionic coating reagent and run buffer consisting of CElixir Reagent B (pH 2.5) with a voltage of 10 kV and 100 mbar·s injection. Identity of peaks: (a) d-methamphetamine, (b) l- or d-*n*-butylamphetamine (internal standard), and (c) l- or d-*n*-butylamphetamine. (B) Electropherogram with anionic coating reagent and run buffer consisting of CElixir Reagent B (pH 2.5) + 50 mM HP-β-CD with a voltage of 20 kV and 100 mbar·s injection. Identities of peaks identical to (A). (C) Electropherogram of co-injection of 100 mbar·s of sample and 35 mbar·s of standard with CE conditions as in (B). Identity of peaks are same as (A) except for (d) l-amphetamine, (e) d-amphetamine, (f) l-methamphetamine, (g) l or d-MDA, (h) l or d-MDA, (i) l or d-MDMA, (j) l or d-MDMA, (k) l or d-MDEA, and (l) l or d-MDEA. (D) Electropherogram with anionic coating reagent and run buffer consisting of 50 mM phosphate-borate (pH 6.5) + 3% SDS with a voltage of 8.5 kV and 100 mbar·s injection. Identity of peak is (m) caffeine.

tablet (a "Thai Tab"), a seized heroin HCl sample, an LSD exhibit, and a hallucinogenic mushroom. The illicit methamphetamine tablet is analyzed (same sample injection vial) by three of the above CE systems on the same capillary. The methamphetamine content (24.2%) is first measured (Fig. 7A), followed by a chiral determination for which methamphetamine enantiomers are present (Fig. 7B), proceeded by a confirmation of the d-methamphetamine isomer by co-injection (Fig. 7C), and finally an acidic, neutral, and weakly basic adulterant screen (Fig. 7D) which indicates the presence of caffeine (migration time and UV library search). A chiral determination without co-injection is necessary in case the sample also contains a small amount of the other enantiomer, which could be difficult to confirm by co-injection.

A brown powder containing heroin HCl is analyzed by two of the above CE systems on the same capillary. The heroin HCl content (27.8%) is first measured and also library searched (Fig. 8A). The presence of quinine is indicated by both UV spectra and migration time. The identification of basic impurities by both library search and migration time helps to eliminate these commonly occurring peaks as possible adulterants. An acidic, neutral and weakly basic adulterant screen (Fig. 8B) indicates the presence of benzocaine (migration time and UV library search). The amount of LSD in a blotter sample (40 μg per blotter) (Fig. 9A), and the identification of psilocin and psilocybin in a mushroom exhibit (Fig. 9B) are determined by consecutively using two of the above systems, again using the same capillary.

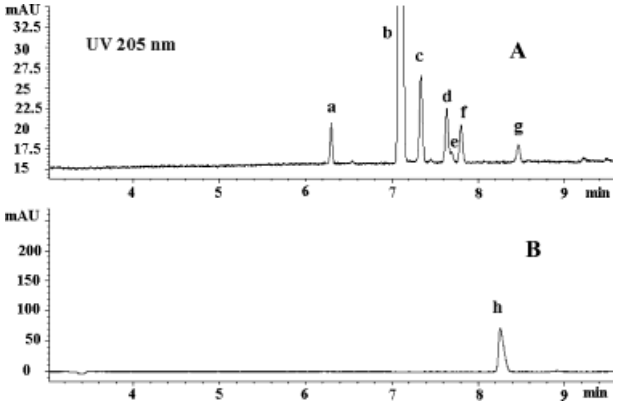

Figure 8. CE analysis of a seized heroin HCl sample (same sample vial) using multiple run buffers with a 32 cm (23.5 cm to the detector window) × 50 μm ID fused-silica capillary operating at 15°C. (A) Electropherogram with anionic coating reagent and run buffer consisting of CElixir Reagent B (pH 2.5) + 100 mM DM-β-CD with a voltage of 13 kV and 250 mbar·s injection. Identities of peaks: (a) quinine, (b) heroin, (c) O6-monoacetylmorphine, (d) acetylcodeine, (e) morphine, (f) papaverine, and (g) noscapine. (B) Electropherogram with anionic coating reagent and run buffer consisting of 50 mM phosphate-borate (pH 6.5) + 3% SDS with a voltage of 8.5 kV and 100 mbar·s injection. Identity of peak is (h) benzocaine.

4 Concluding remarks

These data demonstrate that a wide variety of seized drugs can be analyzed on a single capillary using dynamic coatings generated from the same polymeric cationic coating

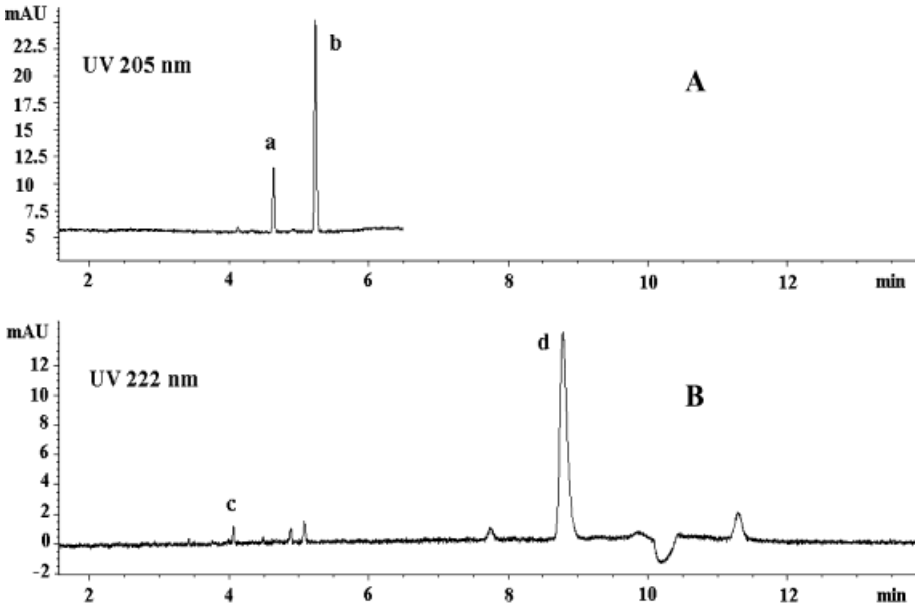

Figure 9. CE analysis of seized LSD and psilocybin exhibits using a 32 cm (23.5 cm to the detector window) × 50 μm ID fused-silica capillary at 15°C. (A) Electropherogram obtained under conditions as described in experimental section for LSD. Identities of peaks are (a) LSD, and (b) tetracaine (internal standard). (B) Electropherogram obtained under conditions as described in experimental section for hallucinogenic mushrooms. Identities of peaks are (c) psilocin, and (d) psilocybin.

reagent and a suite of anionic coating buffers. Validated methods presented are applicable to routine forensic drug analysis. Dynamic coatings with short capillaries give rapid, precise and reproducible separations.

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