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# Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis

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**Abstract**

Rapid, precise, accurate, and reproducible methodology using capillary electrophoresis (CE) with dynamically coated capillaries for the analysis of heroin and its basic impurities and adulterants is presented. Highly selective determination of the above solutes is obtained by analyzing the same sample preparation by two CE methods. For the determination of heroin, its basic impurities and basic adulterants, dynamic coating of the capillary surface is accomplished using a commercially available reagent kit with an added cyclodextrin ((CD) polycation coating followed by polyanion coating with dimethyl-β-cyclodextrin or hydroxypropyl-β-cyclodextrin). The addition of a cyclodextrin to the run buffer significantly improves the separation of these solutes. Neutral, acidic, and weakly basic adulterants which migrate near or after $t_0$ do not interfere with the more mobile basic solutes. The determination of neutral, acidic, and weakly basic adulterants in heroin is accomplished using a modification of the above commercially available reagent kit. After first coating with a polycation, a negative coating is obtained using a surfactant sodium dodecyl sulfate. Micellar electrokinetic chromatography (MEKC) with dynamically coated capillaries gives an excellent separation of the neutral, acidic, and weakly basic solutes, with considerably shorter run times compared to conventional MEKC. In addition for this system, most basic solutes in heroin have longer migration times than the uncharged and acidic compounds.


*Keywords:* Dynamic coating; Coated capillaries; Forensic analysis; Heroin; Cyclodextrins

## 1. Introduction

Analytical information derived from the analysis of heroin (diacetylmorphine), a highly abused drug, is important for legal and intelligence purposes. For judicial purposes, the determination of the amount and/or identity of heroin (and other controlled substances) is frequently required. For strategic and tactical intelligence, a more in-depth analysis of the heroin sample is usually necessary which includes the determination of impurities, adulterants and diluents. Strategic intelligence for diacetylmorphine typically involves the determination of a geographical origin and/or manufacturing process [1]. Tactical intelligence ascertains whether two or more exhibits came from an identical source, i.e. same batch from the same laboratory [1]. Organic and inorganic impurities arise from the heroin manufacturing process. Heroin exhibits can also be cut with adulterants and diluents. Adulterants are pharmaceuticals (e.g. quinine and phenobarbital), which are added to mimic heroin. Diluents are substances (e.g. starch and sugars) that are added merely to dilute the drug.

One of the analytical techniques used for obtaining strategic intelligence (of primary importance to the Drug Enforcement Administration) involves the determination of the ratios of basic impurities such as morphine, codeine, O3-monoacetylmorphine, O6-monoacetylmorphine, acetylcodeine, noscapine, and papaverine to diacetylmorphine. These ratios are indicative of the originally manufactured heroin since the absolute values for impurities can change if adulterants or diluents are subsequently added. For tactical intelligence, the determination of ratios of adulterants to heroin are useful. The analysis of adulterants and/or basic impurities in heroin is accomplished using gas chromatography (GC) [2,3], high-performance liquid chromatography (HPLC) [4–6], and capillary electrophoresis (CE) [7–11].

HPLC and CE are advantageous over GC (packed and capillary) for the analysis of the above solutes since morphine, O6-monoacetylmorphine, noscapine, aspirin, salicylic acid,

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acetaminophen, and phenobarbital can exhibit poor gas chromatographic performance. Although reversed phase gradient HPLC allows for the simultaneous analysis of heroin, basic impurities, and basic, acidic and neutral adulterants, the technique suffers from limited peak capacity and, therefore, limited resolving power. In addition, since solute hydrophobicity plays a dominant role in the separation mechanism, basic, acidic and neutral compounds separate in the same chromatographic region.

Compared to HPLC, CE offers high resolving power, selective detection, shorter run times and economy of use. Micellar electrokinetic chromatography (MEKC) [7–10,12] and capillary zone electrophoresis (CZE) with a neutral cyclodextrin (CD) [11] have been used for the analysis of adulterants and/or basic impurities in heroin. Due to either incomplete separations, marginal run-to-run precision, or limitation to certain class of compounds, none of the reported methodology was satisfactory for our purposes.

Dynamically coated capillaries with neutral CD added to the run buffer appear well suited for the analysis of basic solutes in heroin. For the CZE separation of basic solutes, faster and more precise migration times and higher plate counts are obtained using coated versus uncoated capillaries [13–17] (run buffers without added CDs). The addition of CD to the run buffer with a dynamically coated system not only provides more rapid separations (versus uncoated capillaries), and highly precise separations, but also affords the additional selectivity required for the analysis of enantiomers of epinephrine [18] and the major alkaloids in opium [19]. For the above separations, a coating procedure developed by Chevigne and Janssens [20] is used. The methodology for 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 is the latter coating reagent. The p$K_a$ of the polyanion is lower than that of a silanol, and therefore, the coating retains a consistent electroosmotic flow (EOF) at all workable pH values. This is more robust than an uncoated capillary. This process gives rise to a highly reproducible EOF at a low pH, and to a capillary surface with more favorable kinetics. This approach gives excellent separation of basic solutes, free of interference from neutral and acidic compounds. However, neutral compounds migrate poorly and are separated either slightly before or at $t_0$, while acidic compounds migrate after $t_0$.

The use of dynamic coated capillaries in the normal polarity MEKC mode allows for the analysis of acidic and neutral solutes, even at low pH values. Katayama et al. [21] and Bendahl et al. [22] analyzed solutes using a bilayered coating with an anionic polymer and sodium dodecylsulfate (SDS) in the run buffer (pH 2–7). Rodriguez-Delgado et. al [23] employed polyethylenimine as the cationic polymer followed by a run buffer (pH 1.5) containing SDS as the anionic coating reagent. These same authors [23] demonstrated the significant advantages of their approach versus the use of anionic polymer coated capillaries and reversed polarity MEKC with low pH run buffers. For polymer coated capillaries, the coating procedure is laborious and time-consuming. For reversed polarity MEKC separations, neutral solutes migrating close to EOF marker cannot be detected, long analysis times are usually required and poor reproducibility is obtained.

In this report (for a single sample preparation), the analyses of heroin, its basic impurities and adulterants using dynamically coated capillaries, both in the CZE mode with added neutral CD, and the MEKC mode with SDS as the anionic coating, are described.

## 2. Experimental

### 2.1. Reagents

All drug standards were obtained from the reference collection of the Drug Enforcement Adminsitration Special Testing and Research Laboratory (Dulles, VA, USA). CElixir Reagent A, CElixir Reagent B (pH 2.5), 50 mM phosphate–borate (pH 6.5) and 50 mM phosphate (pH 6.5) were obtained from MicroSolv Technology (Eatontown, NJ, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD), dimethyl-β-cyclodextrin (DM-β-CD) and SDS were acquired from Sigma (St. Louis, MO, USA). Sodium phosphate (monobasic), phosphoric acid, and sodium hydroxide were reagent grade. HPLC-grade methanol was obtained from Burdick and Jackson (Muskegon, MI, USA). High-purity, deionized water was obtained from a Millipore Milli-Q-Gradient A10 water system (Bedford, MA, USA).

### 2.2. Run buffer

The run buffers for CE analysis consisted of (1) 100 mM DM-β-CD in CElixir Reagent B (pH 2.5), (2) 100 mM HP-β-CD in CElixir Reagent B (pH 2.5), and (3) 103.2 mM SDS in 50 mM phosphate–borate (pH 6.5). Reagent 1 was prepared by weighing 1330 mg of DM-β-CD into a 25 ml Erlenmeyer flask and pipetting 10.0 ml of CElixir Reagent B (pH 2.5), followed by vigorous shaking. Reagent 2 was prepared by weighing 1576 mg of HP-β-CD into a 25 ml Erlenmeyer flask and pipetting 10.0 ml of CElixir Reagent B (pH 2.5), followed by vigorous shaking. Finally, reagent 3 was prepared by weighing 300 mg of SDS into a 25 ml Erlenmeyer flask and pipetting 10.0 ml of 50 mM phosphate–borate (pH 6.5), followed by vigorous shaking. These solutions, as well as other reagents used for CE analyses, were filtered through an SRI 0.5 μm nylon filter (Eatontown, NJ, USA).

### 2.3. Injection solvent

The solvent consisted of a 2:8 mixture of methanol and 3.75 mM phosphate buffer (pH 3.2). The buffer was prepared by weighing 1034 mg of sodium phosphate monobasic into

a 100 ml volumetric flask and then diluting to volume with water. The pH was adjusted to 2.6 by a dropwise addition of phosphoric acid. The resulting solution was transferred to a 2000 ml volumetric flask and diluted to volume with water.

### 2.4. Apparatus

An Agilent Model HP$^{3D}$CE capillary electrophoresis system equipped with a diode array detector (Waldbronn, Germany) was used for CE experiments. Experiments using run buffers 1 and 2 were carried out with fused silica 64 cm (55.5 cm to detector window) × 50 μm i.d. capillaries, obtained from Polymicro Technologies (Phoenix, AZ, USA). Experiments using run buffer 3 were carried out with fused silica 32 cm (23.5 cm to detector window) × 50 μm i.d. capillaries, also obtained from Polymicro Technologies.

For conditioning new dynamically coated capillaries for use with run buffers 1 and 2, the same conditioning steps as for regular analysis were used. The capillaries were first flushed with 0.1 M sodium hydroxide for 2 min, then water for 2 min, followed by CElixir Reagent A for 2 min, and finally run buffer 1 or 2 for 4 min. For overnight or prolonged storage, the capillary was flushed with water for 20 min and stored with the inlet and outlet dipped in water.

For conditioning new dynamically coated capillaries for use with run buffer 3, the columns were first flushed with 0.1 M sodium hydroxide for 1 min, then water for 1 min, followed by CElixir Reagent A for 1 min, then 50 mM phosphate–borate (pH 6.5) for 1 min and finally run buffer 3 for 6 min. For subsequent injections, only 2-min flushes with run buffer 3 were required. For overnight or prolonged storage the capillary was stored with run buffer (the inlet and outlet dipped in water).

For the CE methodology, 2.0 ml CE glass vials are used with waste vials filled to 500 μl of water, and all others (including flush vials and run buffers) filled to 1000 μl.

### 2.5. Standards and samples for CE

#### 2.5.1. Preparation of a standard mixture of heroin, moderately basic impurities, and moderately basic adulterants (excluding O6-monoacetylmorphine·HCl)

(1) Weigh (using weighing paper or weighing boat) an appropriate amount of standard heroin·HCl (DAM), O3-sulfamate (O3), morphine·HCl (M), acetylcodeine·HCl (AC), papaverine·HCl (P), codeine·HCl (C), noscapine (N), thiamine·HCl (TH), quinine·HCl (Q), procaine·HCl (PrO), and diphenhydramine·HCl (DP) into a 100 ml volumetric flask, in order to obtain a final concentration of approximately 0.40 mg/ml (DAM), 0.005 mg/ml (O3), 0.005 mg/ml (M), 0.01 mg/ml (AC), 0.005 mg/ml (P), 0.005 mg/ml (C), 0.005 mg/ml (N), 0.02 mg/ml (TH), 0.02 mg/ml (Q), 0.02 mg/ml (PrO) and 0.02 mg/ml (DP), respectively. Dilute to volume with injection solvent, sonicate for 15 min, then vortex and transfer approximately 1000 μl of filtered standard solution (SRI 0.5 μm nylon filter) into a 2 ml glass CE injection vial. Make sure there are no air bubbles on the bottom of glass vial.

#### 2.5.2. Preparation of a standard mixture of O6-monoaetylmorphine

Weigh (using weighing paper or weighing boat) an appropriate amount of standard O6-monoacetylmorphine·HCl (O6) into a 100 ml volumetric flask in order to obtain a final concentration of approximately 0.015 mg/ml (O6). Dilute to volume with injection solvent, sonicate for 15 min, then vortex and transfer approximately 1000 μl of filtered standard solution (SRI 0.5 μm nylon filter) into a 2 ml glass CE injection vial. Make sure there are no air bubbles on the bottom of glass vial.

#### 2.5.3. Preparation of a standard mixture of acidic, weakly basic, and neutral adulterants

Weigh (using weighing paper or weighing boat) an appropriate amount of standard acetaminophen, theophylline, caffeine, aspirin, salicylic acid, antipyrene, phenobarbital, and phenacetin into a 100 ml volumetric flask in order to obtain a final concentration of each component of approximately 0.10 mg/ml. Dilute to volume with injection solvent, sonicate for 15 min, then vortex and transfer approximately 1000 μl of filtered standard solution (SRI 0.5 μm nylon filter) into a 2 ml glass CE injection vial. Make sure there are no air bubbles on bottom of glass vial.

#### 2.5.4. Preparation of a heroin·HCl sample

Weigh 20 mg equivalent of heroin·HCl into a 50 ml volumetric flask (using weighing paper or weighing boat). Dilute to volume with injection solvent, sonicate for 15 min, then vortex and transfer approximately 1000 μL of filtered standard solution (SRI 0.5 μm nylon filter) into a 2 ml glass CE injection vial. Make sure there are no air bubbles on the bottom of glass vial.

#### 2.5.5. Preparation of a heroin base sample (50% base or greater)

Weigh 10 mg equivalent of heroin·HCl into a 50 ml volumetric flask (using weighing paper or weighing boat). Dilute to volume with injection solvent, sonicate for 15 min, then vortex and transfer approximately 1000 μl of filtered standard solution (SRI 0.5 μm nylon filter) into a 2 ml glass CE injection vial. Make sure there are no air bubbles on the bottom of glass vial.

## 3. Results and discussion

The dynamically coated capillary approach used for the analysis of phenethylamines [14], based on a predominantly free zone mechanism, gave poor resolution of heroin and basic impurities (morphine, codeine, O6-monoacetylmorphine, O3-monoacetylmorphine, acetylcodeine, noscapine and papaverine). As in previous studies using dynamically coated

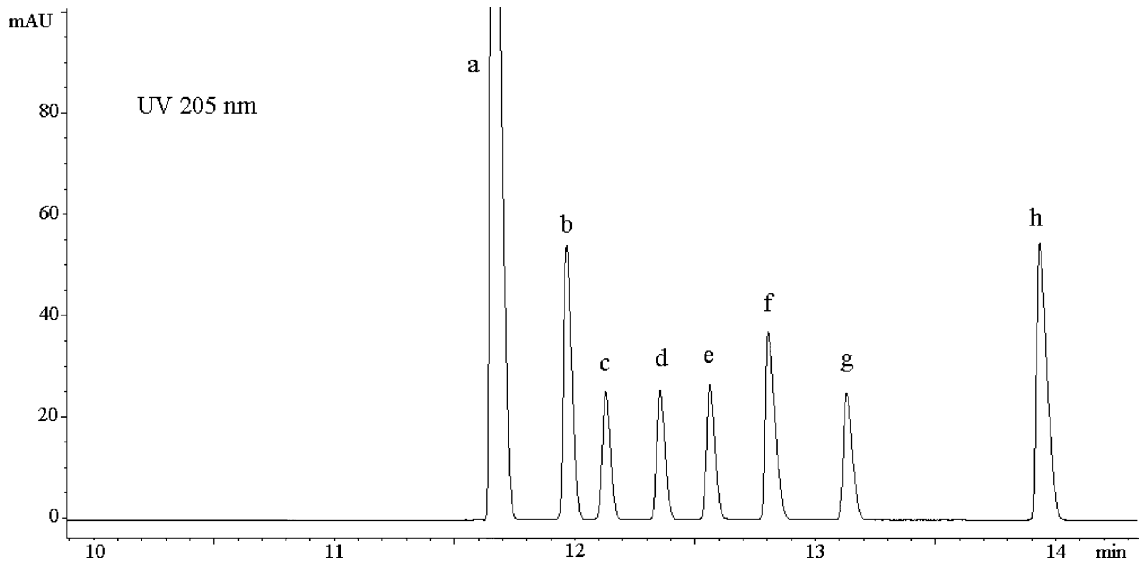


Fig. 1. Electropherogram of a standard mixture of (a) heroin (DAM) (0.40 mg/ml), (b) O6-monoacetylmorphine (O6) (0.09 mg/ml), (c) O3-monoacetylmorphine (O3) (0.04 mg/ml), (d) morphine (MOR) (0.04 mg/ml), (e) acetylcodeine (ACO) (0.04 mg/ml), (f) papaverine (PAP) (0.04 mg/ml), (g) codeine (COD) (0.04 mg/ml), and (h) noscapine (NOS) (0.06 mg/ml). A 64 cm (55.5 cm to detector window) × 50 μm i.d. fused silica capillary operating at 25 °C and 30 kV with UV detection at 205 nm was used. Pressure injections of 500 mbar s were used with a run buffer consisting of Celixir Reagent B (pH 2.5) + 100 mM DM-β-CD.

capillaries, the addition of CD(s) to the run buffer significantly affects selectivity through secondary equilibria [18,19]. For a 32 cm (23.5 cm to detector window) × 50 μm i.d. capillary operated with a voltage of 20 kV at 15 °C, various combinations of DM-β-CD and HP-β-CD were investigated for the separation of the solutes of interest. Although the best separation was obtained using 100 mM DM-β-CD, acetylcodeine and morphine were only partially resolved. This separation was further optimized by doubling the length of the capillary to 64 cm (55.5 cm to detector window) and increasing the temperature to 25 °C. As shown in Fig. 1, heroin, morphine, codeine, O6-monoacetylmorphine, O3-monoacetylmorphine, acetylcodeine, noscapine, and papaverine are all baseline resolved using the improved conditions.

The relative migration times (RMT) of heroin, basic[1] impurities, basic adulterants (relative to acetylcodeine) under these same conditions are shown in Table 1. The above system was highly selective for the separation of the basic solutes in the presence of acidic and neutral adulterants. Under these conditions, neutral solutes migrated poorly separated at or near $t_0$ (22.9 min), while acidic adulterants migrated after $t_0$. As illustrated, the basic impurities were relatively free of interferences from basic adulterants (Table 1). Lidocaine and aminopyrene co-migrated with O3-monoacetylmorphine, and dipyrone co-migrated with O6-monoacetylmorphine. As shown in Fig. 2, O3-monoacetylmorphine and O6-monoacetylmorphine were resolved from these interfering adulterants using run buffer 2. Since aminopyrene and dipyrone have much greater extinction coefficients than certain heroin impurities at 260 nm, these adulterants could be determined (depending on relative concentrations) at this higher wavelength using run buffer 2 (Fig. 2). Although certain adulterants were not resolved, they are typically not encountered together.

Linearity was examined for heroin and basic impurities using an external standard procedure. As shown in Table 2, excellent linearity was obtained ($0.99993 \geq R^2 \geq 0.99996$), with plots of peak area versus concentration passing through the origin. Also, as shown in Table 3, outstanding run-to-run migration time precision was obtained for heroin and the basic impurities (R.S.D.s ≤0.071%). Good run-to-run corrected area (area/migration time) precision was obtained for heroin, and for the basic impurities (R.S.D.s ≤2.00%, see Table 3). Although the EOF decreased with continued usage of the capillary over a 2-week period (EOF decreased from $1.03 \times 10^{-4}$ to $0.803 \times 10^{-4}$ $cm^2\,V^{-1}\,s^{-1}$), reproducible separations were obtained (R.S.D.s of effective mobilities <0.9%).

For the analysis of acidic, weakly basic,[2] and neutral adulterants in heroin a similar approach to Rodriguez-Delgado et al. [23] was used. In the present methodology, a proprietary reagent (CElixir Reagent A) was used instead of polyethylenimine as the source of the cationic polymer. In addition, background electrolyte (run buffer without SDS) was used instead of water as the intermediate flush reagent between cationic and anionic coating steps. Reagent A and SDS are immiscible but soluble in the background electrolyte.

[1] Basic impurities and basic adulterants shown in Table 1 have p$K_a$ values >3.5 and are mostly ionized at the run buffer pH of 2.5.

[2] p$K_a$'s ≤3.5.

Table 1
Relative migration times of heroin, basic impurities and basic adulterants

| Solute | RMT (min) |
|---|---|
| Thiamine | 0.562 |
| Nicotinamide | 0.666 |
| D- or L-chloroquine | 0.713 |
| D- or L-chloroquine | 0.717 |
| Quinine | 0.815 |
| Quinine impurity | 0.856 |
| Heroin | 0.927 |
| O6-monoacetylmorphine | 0.953 |
| Dipyrone | 0.957 |
| O3-monoacetylmorphine | 0.965 |
| Aminopyrene | 0.968 |
| Lidocaine | 0.968 |
| Morphine | 0.983 |
| Acetylcodeine | 1.00 (12.4) |
| Papaverine | 1.02 |
| Strychnine | 1.03 |
| Codeine | 1.05 |
| L-Ephedrine | 1.07 |
| L-Pseudoephedrine | 1.07 |
| D-Ephedrine | 1.08 |
| Xylazine | 1.10 |
| D-Pseudoephedrine | 1.10 |
| Noscapine | 1.11 |
| Thebaine | 1.12 |
| Procaine | 1.14 |
| Chlorpheniramine | 1.15 |
| Brompheniramine | 1.16 |
| Cocaine | 1.22 |
| *trans*-Doxepin | 1.23 |
| Diphenhydramine | 1.23 |
| Tetracaine | 1.23 |
| *cis*-Doxepin | 1.24 |
| $t_0$ | 1.85 |

See Fig. 1 for CE conditions.

For the MEKC dynamic coating separation of acidic, weakly basic, and neutral adulterants in heroin (acetaminophen, theophylline, caffeine, aspirin, salicylic acid, antipyrene, phenobarbital, and phenacetin), the effect of SDS concentration, temperature, and run buffer pH was investigated. A 32 cm (23.5 cm to detector window) × 50 μm i.d. capillary was used with a voltage of 8.5 kV. Since peak shapes and peak efficiency improved with increased SDS concentration (background electrolyte 50 mM phosphate, pH 2.5), a 3% SDS coating reagent was found to be a good compromise between electrophoretic performance and current. A separation temperature of 15 °C was preferred (run buffer 50 mM phosphate + 3% SDS, pH 2.6), since higher temperatures degraded the separation between acetaminophen and theophylline. For the separation of acidic, weakly basic and neutral adulterants in heroin, a pH would be desirable that allowed for the highly selective determination of the acidic, weakly basic, and neutral solutes in the presence of highly basic compounds. At a pH range of 2.5–6.5, most of the basic solutes in heroin under these conditions are fully ionized and will ion pair with SDS (either on stationary phase or run buffer) and therefore migrate after the acidic and neutral solutes. For this pH range, there was a significant change in separation selectivity, with all solutes only resolved at pH 6.5. This selectivity change is not surprising since solutes such as theophylline ($pK_b$ 3.5 [24]), aspirin ($pK_a$ 3.5 [24]), salicylic acid ($pK_a$ 3.0 [24]) and antipyrene ($pK_b$ 1.4 [24]) change their ionization state over this pH range. For dynamic coating with SDS, factors such as partitioning into micelle, mobility in free zone solution, repulsion of negatively charged solutes from the micelle, ion pairing of the micelle with positively charged solutes and hydrophobic and hydrophilic interactions with the SDS on the wall surface all contribute to selectivity. An electropherogram depicting the separation of all of the above solutes is shown in Fig. 3.

Table 2
Results for linearity study

| Solute | Linearity range (mg/ml) | Correlation coefficient ($R^2$) |
|---|---|---|
| Heroin | 0.0125–0.802 | 0.99994 |
| O6 | 0.000706–0.181 | 0.99995 |
| O3 | 0.000663–0.0848 | 0.99993 |
| Morphine | 0.000600–0.0768 | 0.99996 |
| Acetylcodeine | 0.000625–0.0800 | 0.99994 |
| Papaverine[a] | 0.000325–0.0832 | 0.99996 |
| Codeine | 0.000625–0.0800 | 0.99995 |
| Noscapine | 0.000563–0.1440 | 0.99996 |

See Fig. 1 for CE conditions.
[a] UV 252 nm.

Table 3
Run-to-run precision (R.S.D. (%); $n = 5$)

| Solute | MT | Ca |
|---|---|---|
| Heroin | 0.058 | 2.00 |
| O6 | 0.059 | 1.98 |
| O3 | 0.061 | 1.65 |
| Morphine | 0.063 | 1.75 |
| Acetylcodeine | 0.061 | 1.89 |
| Papaverine[a] | 0.060 | 1.90 |
| Codeine | 0.066 | 1.73 |
| Noscapine | 0.071 | 1.88 |

See Fig. 1 for CE conditions.
[a] UV 252 nm.

Fifty millimolar phosphate–borate buffer (pH 6.5) was used instead of 50 mM phosphate buffer (pH 6.5) since the migration time of salicylic acid versus other solutes using phosphate buffer significantly changed over time. It is interesting to compare the separation of the acid and neutral solutes with coated capillaries versus uncoated capillaries using a 3% SDS 50 mM phosphate–borate buffer, pH 6.5 run buffer. For both systems, an identical migration order is obtained for the solutes detected with considerably longer migration times for uncoated versus coated capillaries (e.g. Phenobarbital at 33.3 min uncoated versus 6.8 min coated). This result is due in part to the lower EOF of the uncoated capillary ($t_0$ = 5.1 min) versus the coated capillary ($t_0$ = 3.5 min) where even after 80 min the last solute (phenacetin) is not detected. Lack of interaction of solute with SDS on

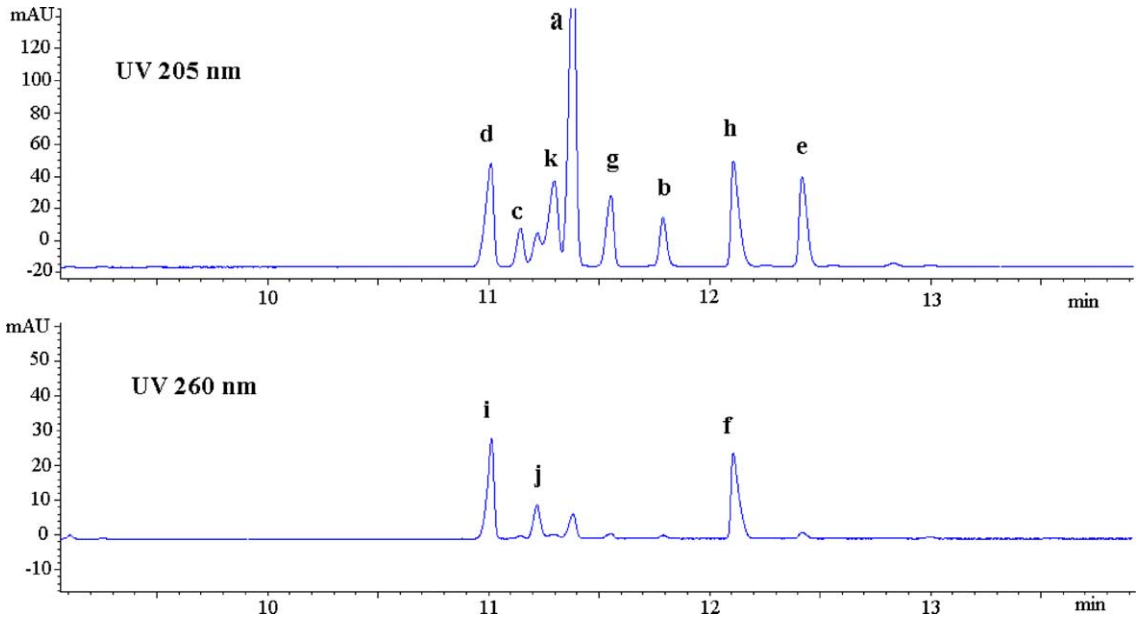


Fig. 2. Electropherogram of a standard mixture of basic impurities and selected adulterants. Peak identities identical to Fig. 1 except for (i) aminopyrene, (j) dipyrone, and (k) lidocaine. All solute concentrations at 0.05 mg/ml except for heroin (0.4 mg/ml). A 64 cm (55.5 cm to detector window) × 50 μm i.d. fused silica capillary operating at 25 °C and 30 kV with UV detection at 205 nm was used. Pressure injections of 500 mbar s were used with a run buffer consisting of CElixir Reagent B (pH 2.5) + 100 mM HP-β-CD.

the wall in the uncoated system could also be playing a role in the differences in migration times. For the coated system, relative migration time data for acidic, weakly basic, and neutral adulterants as well as moderately basic adulterants and impurities (relative to phenobarbital) is shown in Table 4. The above system is highly selective for the separation of the acidic, weakly basic and neutral solutes in the presence of the moderately basic compounds. The moderately basic impurities and adulterants which have p$K_a$ values ≥6.2 are significantly ionized at the run buffer pH of 6.5, and therefore ion pair with the SDS headgroups. Hydrophobic interactions with the SDS micelles also play a role in their migration.

Linearity was examined for the above acidic and neutral adulterants using an external standard procedure. As shown in Table 5, excellent linearity was obtained (0.99985 ≥

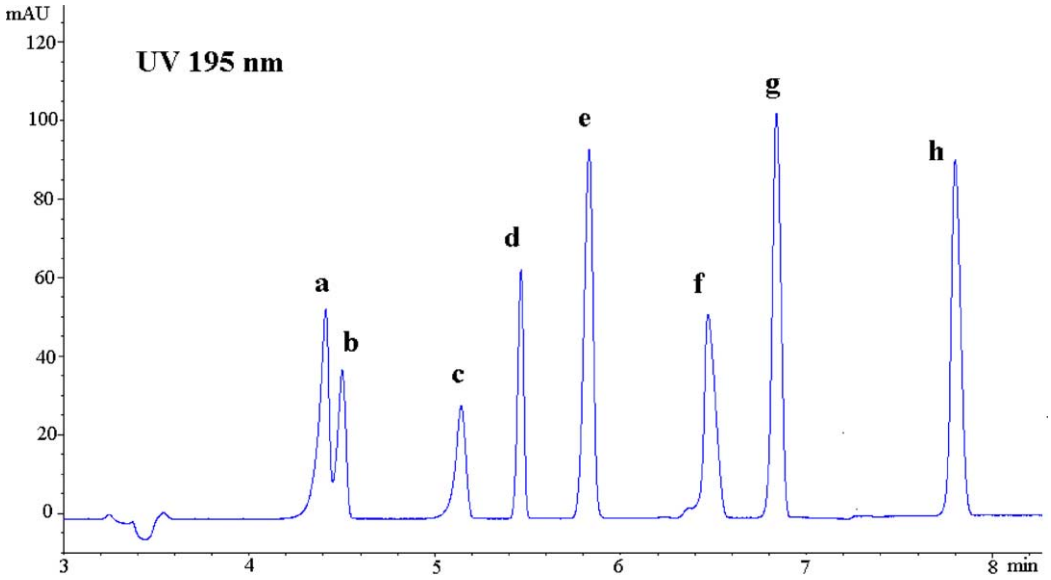


Fig. 3. Electropherogram of a standard mixture concentrations of (a) acetaminophen (0.08 mg/ml), (b) theophylline (0.09 mg/ml), (c) caffeine (0.09 mg/ml), (d) aspirin (0.09 mg/ml), (e) salicylic acid (0.09 mg/ml), (f) antipyrene (0.10 mg/ml), (g) phenobarbital (0.10 mg/ml), (h) phenacetin (0.10 mg/ml). A 32 cm (23.5 cm to detector window) × 50 μm i.d. fused silica capillary operating at 15 °C and 8.5 kV with UV detection at 195 nm was used. Pressure injections of 100 mbar s were used with a run buffer consisting of 50 mM phosphate–borate (pH 6.5) + 103.2 mM (3%, w/w) SDS.

Table 4
Relative migration times of acidic and neutral adulterants, basic impurities and basic adulterants

| Solute | RMT (min) |
|---|---|
| $t_0$ | 0.504 |
| Nicotinamide | 0.591 |
| Acetominophen | 0.660 |
| Theophylline | 0.673 |
| Dipyrone (1) | 0.738 |
| Caffeine | 0.764 |
| Aspirin | 0.809 |
| Salicylic acid | 0.860 |
| Anitipyrene | 0.948 |
| Phenobarbital | 1.00 (6.8) |
| Ibuprofen | 1.11 |
| Aminopyrene | 1.11 |
| Phenacetin | 1.13 |
| Dipyrone (2) | 1.13 |
| Benzocaine | 1.25 |
| Thiamine | 1.32 |
| Morphine | 1.35 |
| Codeine | 1.44 |
| O3-monoacetylmorphine | 1.44 |
| Procaine | 1.45 |
| Pseudoephedrine | 1.45 |
| Ephedrine | 1.45 |
| Lidocaine | 1.46 |
| Heroin | 1.47 |
| O6-monoacetylmorphine | 1.47 |
| Acetylcodeine | 147 |
| Noscapine | 1.47 |
| Quinine | 1.48 |
| Chloroquine | 1.48 |
| Yohimbine | 1.48 |
| Strychnine | 1.48 |
| Thebaine | 1.48 |
| Xylazine | 1.49 |
| Cocaine | 1.49 |
| Tetracaine | 1.49 |
| *cis*- and *trans*-Doxepin | 1.50 |
| Brompheniramine | 1.50 |
| Methorphan | 1.50 |
| Papaverine | 1.50 |
| Chlorpheniramine | 1.51 |
| Diphenhydramine | 1.51 |

See Fig. 3 for CE conditions.

Table 5
Results for linearity study

| Solute | Linearity range (mg/ml) | Correlation coefficient ($R^2$) |
|---|---|---|
| Acetaminophen | 0.00698–0.893 | 0.99999 |
| Theophylline | 0.00647–0.207 | 0.99998 |
| Caffeine | 0.00737–0.943 | 0.99999 |
| Aspirin | 0.00366–0.468 | 0.99999 |
| Salicylic acid | 0.00352–0.450 | 0.99985 |
| Antipyrene | 0.00658–0.421 | 0.99999 |
| Phenobarbital | 0.00354–0.454 | 0.99992 |
| Phenacetin | 0.00304–0.0486 | 0.99999 |

See Fig. 3 for CE conditions.

Table 6
Run-to-run precision (R.S.D. (%) $n = 5$)

| Solute | Coating procedure[a] | MT | Ca |
|---|---|---|---|
| Acetaminophen | Full | 0.72 | 1.58 |
| | Partial | 0.13 | 0.73 |
| Theofylline | Full | 0.74 | 1.16 |
| | Partial | 0.13 | 0.59 |
| Caffeine | Full | 0.86 | 1.55 |
| | Partial | 0.13 | 0.85 |
| Aspirin | Full | 0.95 | 2.06 |
| | Partial | 0.16 | 0.88 |
| Salicylic acid | Full | 0.99 | 1.73 |
| | Partial | 0.15 | 0.72 |
| Antipyrene | Full | 1.08 | 1.50 |
| | Partial | 0.13 | 0.86 |
| Phenobarbital | Full | 1.15 | 1.50 |
| | Partial | 0.17 | 0.73 |
| Phenacetin | Full | 1.48 | 1.53 |
| | Partial | 0.45 | 0.64 |

See Fig. 3 for CE conditions.
[a] Full: 1 min 0.1 M sodium hydroxide, 1 min water, 1 min reagent A, 1 min 50 mM phosphateborate (pH 6.5), 6 min 103.2 mM (3%, w/w) SDS in 50 mM phosphate–borate (pH 6.5); partial: 2 min 103.2 mM (3%, w/w) SDS in 50 mM phosphate–borate (pH 6.5).

$R^2 \geq 0.99999$), with plots of area standard versus concentration passing through the origin. Although overall good run-to-run corrected area precision (area/migration time) (1.2% $\geq$ R.S.D.s $\geq$ 2.1%) was obtained, the run-to-run migration time precision was fair (0.7% $\geq$ R.S.D.s $\geq$ 1.5%) (see Table 6). Considerable improvement in migration time precision was obtained by only partially recoating between injections (0.1% $\geq$ R.S.D.s $\geq$ 0.5%) (see Table 6). This improvement was accomplished by using 2-min flushes of run buffer between injections, versus 10-min flushes including washing with base and water and recoating with reagent A and SDS. Using the revised conditions excellent overall corrected area was obtained (0.6% $\geq$ R.S.D.s $\geq$ 0.9%). Rodriguez-Delgado et al. [23] using dynamically coated MECC with full recoating at pH 1.5 for polyphenolic solutes obtained run-to-run migration time precision of 0.2% $\geq$ R.S.D.s $\geq$ 1.8%. Bendahl et al. [22] employing dynamically coated MEKC, with only a partial recoat between injections at pH 6 and 7 for neutral and acidic solutes, reported run-to-run migration time precision of 0.6% $\geq$ R.S.D.s $\geq$ 1.2%. Although in the present study the EOF decreased with continued usage of the capillary over a 2-week period (EOF decreased from $4.3 \times 10^{-4}$ to $3 \times 10^{-4}$ $cm^2\,V^{-1}\,s^{-1}$), reproducible separations were obtained (R.S.D.s of effective mobilities <1.0%).

For the above methodologies, quantitation by external standard is used. External standard methods require precise injections that are obtainable with proper routine maintenance procedures. Worn out seals on electrodes, dirty electrodes, and dirty pre-punchers, can all contribute to imprecise injections. Finding proper internal standard(s) for

Table 7
Comparison of quantiation of heroin samples (calculated as the base) using CE and HPLC

| Sample | DAM | | O6/DAM | | O3/DAM | | MOR/DAM | | ACO/DAM | | PAP/DAM | | COD/DAM | | NOS/DAM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LC | CE | LC | CE | LC | CE | LC | CE | LC | CE | LC | CE | LC | CE | LC | CE |
| 1 | 39.2 | 41.8 | 0.085 | 0.077 | 0.045 | 0.043 | 0.005 | 0.007 | 0.043 | 0.042 | 0.026 | 0.028 | 0 | 0 | 0.002 | 0.002 |
| 2[a] | 48.6 | 52.8 | 0.043 | 0.038 | 0.004 | 0.004[b] | 0.003 | 0.003 | 0.026 | 0.026 | 0.013 | 0.014 | 0 | 0 | 0.007 | 0.008 |
| 3[c] | 69.3 | 68.6 | 0.028 | 0.025 | 0.002 | 0.003 | 0.005 | 0.005 | 0.027 | 0.027 | 0.005[d] | 0.005 | 0 | 0 | 0 | 0 |
| 4[e] | 65.2 | 65.3 | 0.028 | 0.022 | 0.002 | 0.003 | 0.005 | 0.004 | 0.027 | 0.027 | 0.004[d] | 0.004 | 0 | 0 | 0 | 0 |
| 5[f] | 22.8 | 22.5 | 1.11 | 1.04 | 0.011 | 0.013 | 0.241 | 0.261 | 0.065 | 0.067 | 0.089 | 0.096 | 0.011 | 0.011 | 0.035 | 0.037 |
| 6[g] | 62.2 | 60.5 | 0.135 | 0.132 | 0.013 | 0.012 | 0.016 | 0.017 | 0.044 | 0.045 | 0.014 | 0.014 | 0.002 | 0.001 | 0 | 0 |
| 7 | 75.8 | 75.4 | 0.014 | 0.013 | 0 | 0 | 0 | 0 | 0.129 | 0.125 | 0 | 0 | 0 | 0 | 0 | 0 |

HPLC same as published procedure except for 3.0 mm column i.d. and flow rate of 0.75 ml/min.
[a] Lidocaine/DAM (0.023 LC, 0.025 CE); caffeine/DAM (0.023 LC, 0.021 CE).
[b] O3 determined using run buffer 2.
[c] Diphenhydramine/DAM (papaverine interfered with diphenhydramine LC (0.015 CE); caffeine/DAM (0.009 LC, 0.007 CE).
[d] Higher wavelength detection in LC allowed the determination of papaverine in the presence of diphenhydramine.
[e] Diphenhydramine/DAM (papaverine interfered with diphenhydramine LC (0.018 CE).
[f] Procaine/DAM (0.732 LC, 0.640 CE; caffeine/DAM (0.079 LC, 0.076 CE).
[g] Thiamine/DAM (0.074 LC, 0.068 CE).

the above methods can be difficult. An internal standard should be resolved from over 40 target solutes, and not be present in heroin.

Overall good agreement is shown for the analysis of heroin samples by CE and HPLC (see Table 7). Electropherograms of a "clean" heroin·HCl exhibit (sample 7) as well as a "dirty" heroin·HCl sample (sample 1) are shown in Figs. 4 and 5, respectively. Multi-wavelength detection is used to improve overall signal-to-noise of the various solutes and to increase quantitative accuracy. While most solutes have highest sensitivity at lower wavelengths (e.g. 205 nm), papaverine has improved signal-to-noise at a higher wavelength (252 nm). Since peak area at a given wavelength is dependent on a solute's extinction coefficient, analysis at multiple wavelengths can indicate the presence of an interfering solute (for a single component the quantitative values should agree). Also as mentioned earlier, at higher wavelengths certain solutes which co-migrate, can be determined by selective detection. Although peak purity analysis is routinely performed (comparison of multiple spectrum across peak), this algorithm as well as other available peak purity techniques are not viable at low solute concentrations (lack of spectral data). Library searches are also routinely performed, not only to screen for adulterants but also to help insure that the right solute is being quantitated.

The CE methodology for strategic intelligence (heroin profiling) has proven to be rugged and reliable. Since

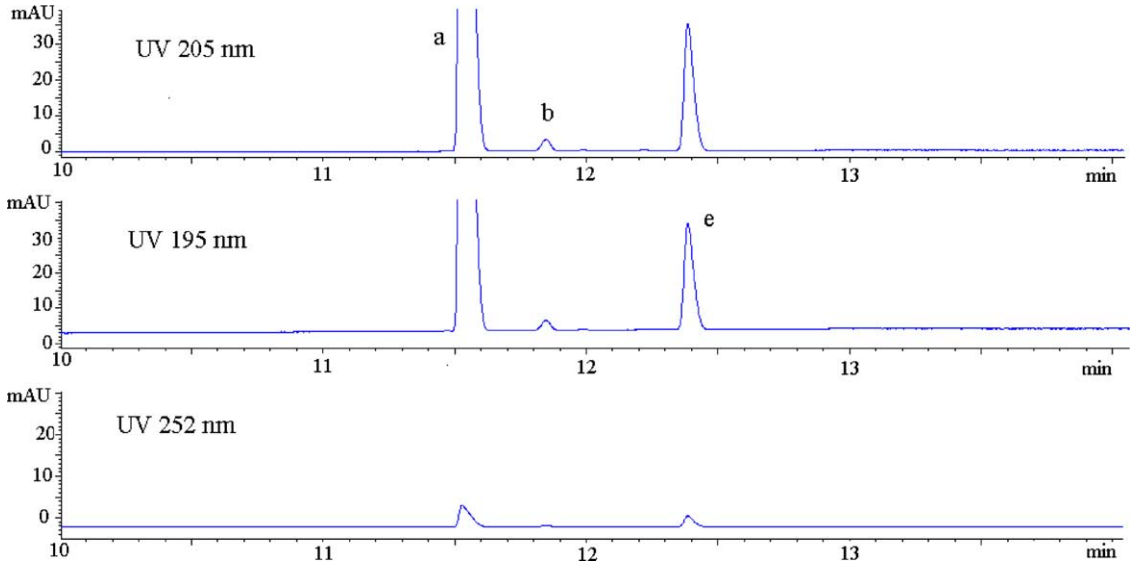


Fig. 4. Electropherogram of a "clean" heroin·HCl sample. Peak identities and CE conditions identical to Fig. 1.

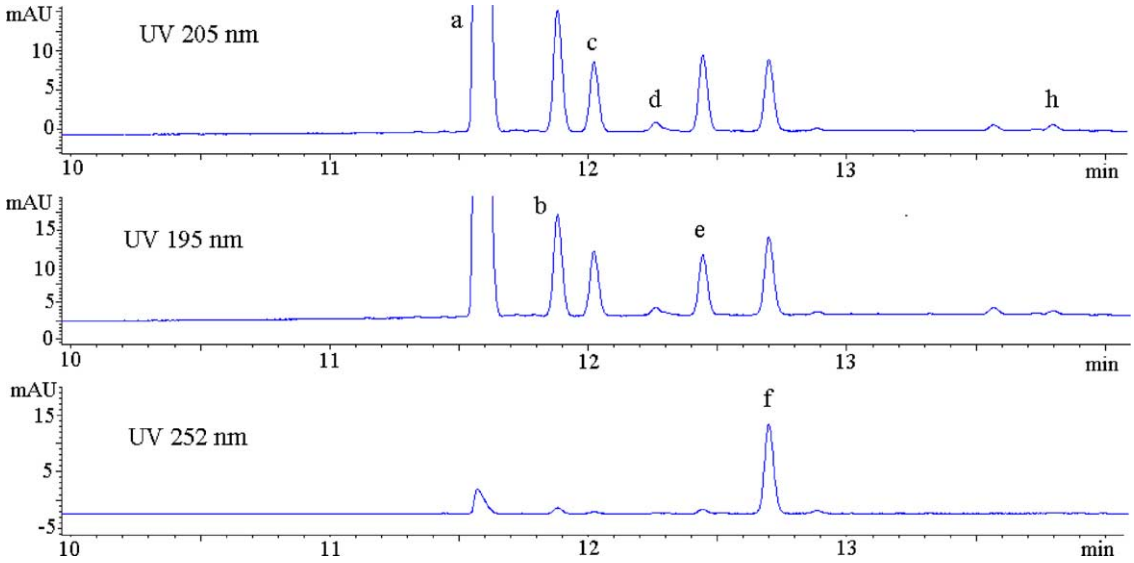


Fig. 5. Electropherogram of a "dirty" heroin·HCl sample. Peak identities and CE conditions identical to Fig. 1.

implementation for routine use, hundreds of samples have been successfully analyzed.

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