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Separation and detection of acidic and neutral impurities in illicit heroin via capillary electrophoresis

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Abstract

The separation and detection of acidic and neutral impurities in illicit heroin using capillary electrophoresis (CE) is described. Separations were achieved using charged cyclodextrin modified micellar electrokinetic capillary chromatography. The use of the anionic β-cyclodextrin sulfobutyl ether 1V in combination with sodium dodecyl sulfate significantly increased resolution. Improved selectivity and/or sensitivity in detection was obtained using photodiode array ultraviolet and laser-induced fluorescence detection. The phenanthrene-like heroin impurities exhibit high native fluorescence under krypton–fluoride laser excitation (248 nm). The limit of detection by laser-induced fluorescence detection for one of these solutes (acetylthebaol) is 1.8 ng/ml, 500 times more sensitive than UV. This methodology is applicable to analysis of both crude and refined heroin.

1. Introduction

The analysis of acidic and neutral impurities in illicitly produced heroin is important for intelligence purposes [1]. These impurities (e.g., see Table 1), which arise from acetylation of various opiate alkaloids present in crude morphine during illicit heroin production, are generally found at levels below 0.5%. Capillary GC with flame ionization, nitrogen–phosphorus, electron-capture or electron-impact mass spectrometric detection have all been previously reported for the analysis of these solutes [2–4]. In addition, high-performance liquid chromatography (HPLC) with ultraviolet (UV), photodiode array-UV (PDA-UV), fluorescence, electrochemical, electron-impact mass spectrometric and thermospray mass spectrometric detection have also been employed [5–11]. Recently, MECC with UV and conventional fluorescence detection were shown to afford significantly greater resolution vs HPLC for the analysis of these neutral impurities [11]. However, the identities of the various components were not addressed in that study. In general, GC affords higher resolution than MECC for these compounds (due to its higher peak capacity); however, derivatizations are commonly required for the former technique.

This study describes an improved separation for acidic and neutral impurities in illicit heroin using charged cyclodextrin (CD) modified

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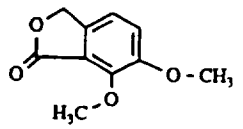
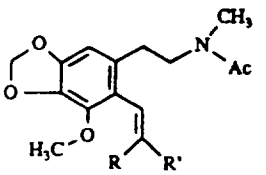
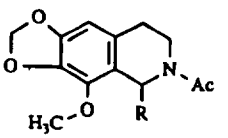
Table 1
Some acidic and neutral acetylated rearrangement products of opium alkaloids



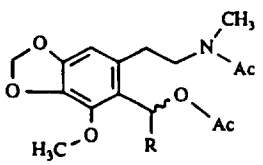
1.



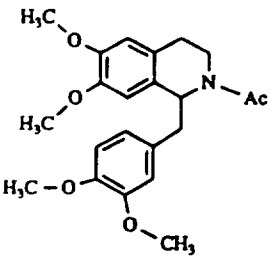
2. R=H, R'=COOH
6. "E", R-R'=1.
7. "Z", R-R=1.



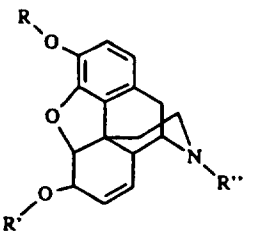
3. R=1.



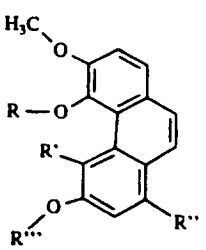
4 erythro, R=1.
5 threo, R=1.



8.



9. R,R',R''=Ac
10. R=CH3 R',R''=Ac



11. R=Ac, R'=CH2CH2NAcCH3, R''=H, R'''=CH3
12. R=Ac, R'=H, R''=CH2CH2NAcCH3, R'''=CH3
13. R=Ac, R' R''=H, R'''=CH3

Compound	Name
1	Meconin
2	3-(2-[N-Methylacetamido-ethyl])-4,5-methylenedioxy-6-methoxyphenylacrylic acid
3	N-Acetylnornarcotine
4	*erythro*-1-Acetyloxy-N-acetylanhydro-1,9-dihydronornarceine
5	*threo*-1-Acetyloxy-N-acetylanhydro-1,9-dihydronornarceine
6	*cis*-N-Acetylandronornarceine
7	*trans*-N-Acetylandronornarceine
8	N-Acetylnorlaudanosine
9	N,3,6-Triacetylnormorphine
10	N,6-Diacetylnorcodeine
11	3,6-Dimethoxy-4-acetyloxy-5-[2-(N-methylacetamido)]ethylphenanthrene
12	3,6-Dimethoxy-4-acetyloxy-8-[2-(N-methylacetamido)]ethylphenanthrene
13	Acetylthebaol

MECC. The use of PDA-UV and laser-induced fluorescence (LIF) detection to increase selectivity and/or sensitivity, as well as aid in the identification of the peaks, is also discussed. The developed methodology is applicable to both crude and refined heroin.

2. Experimental

2.1. Equipment

An Applied Biosystems Model 270A-HT capillary electrophoresis unit (San Jose, CA, USA) interfaced with a PE Nelson Turbochrom 3 chromatographic data system (Cupertino, CA, USA) was used for preliminary method development. A Beckman Pace 5500 capillary electrophoresis system (Fullerton, CA, USA) was used for experiments involving PDA-UV detection. The CE-LIF system was home-built and was similar to one previously described [12]. Fluorescence excitation was provided by a Potomac Model GX-500 Pulsed Laser (Lanham, MD, USA) operating at 248 nm. The laser beam was first spectroscopically filtered with an ARC Interference Filter (Acton, MA, USA) and then focused onto the capillary with a UV-grade biconvex lens (focal length 50 mm). Emission was collected at a 90-degree angle to the incident laser beam with a Carl Zeiss 10X, UV grade microscope (numerical aperture 0.5) (Thornwood, NY, USA). After passing through a Melles Griot UG1 or bandpass filter (400 ± 40 nm) (Irvin, CA, USA), the collected emission was detected by a photomultiplier tube. The current output of the photomultiplier was fed into a EG&G Boxcar Averager (Princeton, NJ, USA) and its voltage output was displayed on a PC via a Beckman A/D Interfacing Module. In a typical experiment, a laser pulse rate of 1 kHz with an averaged excitation power of approximately 0.5 mW was used. Absorption detection on the home-built CE instrument was carried out using a Spectra-Physics Spectra Focus Fast Scanning UV–Vis Detector (Fremont, CA, USA).

The fused-silica capillaries (Polymicro Technologies, Scottsdale, AZ, USA) used in this study were conditioned by successively washing for 10 min each with 1 *M* sodium hydroxide, water, and finally the run buffer. When changing run buffers or for the initial daily use, the capillary was washed with run buffer for 30 min. After daily use, the capillary was washed with distilled/deionized water for 10 min. Injections were either by vacuum (1.0 s) when using the 270A-HT unit, by pressure (2.5 s) with the Pace CE system, or gravity with the home-built system (20 s). Larger injections for improved sensitivity (10.0 s) were used when analyzing refined heroin samples on the Pace CE instrument.

2.2. Materials

Sodium dodecyl sulfate (SDS) (Mallinckrodt, Paris, KY, USA), β-cyclodextrin (β-CD) (Sigma, St. Louis, MO, USA) and β-cyclodextrin sulfobutyl ether 1V (β-CD SBE 1V) (CyDex L.C., Overland Park, KS, USA) were used as received. Sulfuric acid, sodium borate and sodium phosphate (monobasic) were reagent grade. Deionized water from a Millipore Milli-Q System (Bedford, MA, USA) was used to prepare all buffers. Acetonitrile, ethyl ether and methylene chloride were all HPLC grade (Baxter Scientific, Muskegon, MI, USA). A stock solution containing 10 m*M* borate and 10 m*M* phosphate (pH 9.0) was used for the preparation of all run buffers. All standards used in this study were synthesized at the Special Testing and Research Laboratory.

2.3. Sample preparation

For the analysis of crude heroin, an amount of sample equivalent to 50 mg of heroin was placed into a 15-ml conical, glass-stoppered centrifuge tube with 4 ml of 0.5 *M* sulfuric acid. After thorough mixing, the solution was extracted once with 5.0 ml of ethyl ether–methylene chloride (60:40, v/v). The organic layer was transferred to a 5.0-ml silanized reaction vial, where it was evaporated to dryness under a stream of nitrogen. The extract was reconstituted in 750 μl of acetonitrile–water (38:62) with the aid of a vortex. The solution was filtered through a Whatman (Clifton, NJ, USA) 0.45-μm UniPrep PVDF syringeless filter prior to injection onto the Beckman CE instrument.

For refined samples, the same basic procedure as for crude heroin was used except that a sample equivalent to 100 mg of heroin was utilized (to increase the relative concentration of impurities); additionally, the final extract was

reconstituted in 50 μl of injection solvent consisting of run buffer diluted 10 to 1 with acetonitrile–water (10:90, v/v) .

3. Results and discussion

3.1. Separation of acidic and neutral heroin impurities

Choice of organic modifier and temperature

The run buffer in our previous work contained 15% acetonitrile and 85 m*M* SDS–8.5 m*M* phosphate–8.5 m*M* borate [11]. In that study, acetonitrile was found to be the organic modifier of choice over methanol and tetrahydrofuran because it gave a more uniform peak distribution pattern and best overall resolution. For these reasons, and also for better solute detectability at lower wavelengths, acetonitrile was utilized in this study as well. In contrast to our previous work, however, separations were carried out at 30°C (instead of 50°C) in order to minimize degradative reactions (especially hydrolysis).

Effect of SDS concentration

As shown in Fig. 1, there are significant changes in migration times and resolution with varying SDS concentration. As expected, migration times increase with higher SDS concentrations because of the increased phase ratios (i.e., volumes of micellar phase divided by volumes of aqueous phase). Of the three concentrations used, 42.5 m*M* SDS was the best in terms of resolution and speed of analysis; the increased resolution vs 21.2 m*M* is a result of operating at more favorable k' values and over an increased elution range [13].

Effect of acetonitrile concentration

Varying the concentration of the organic modifier (Fig. 2) indicates that the best resolution is obtained with 15% acetonitrile. Higher concentrations of acetonitrile decrease the osmotic flow, resulting in an increased elution range. In addition, k' values tend to decrease with an increase in organic modifier, due to more favorable partitioning of solutes between the micelle and the run buffer. The resulting electropherograms represent a compromise between both factors. The actual elution range, i.e., t_{mc}/t_o (time of micelle divided by time of neutral marker) is difficult to measure because of the solubility of micelle markers in organic modifiers. As shown in Fig. 2, 10% acetonitrile results in excessive k' values, while 20% results in decreased capacity factors.

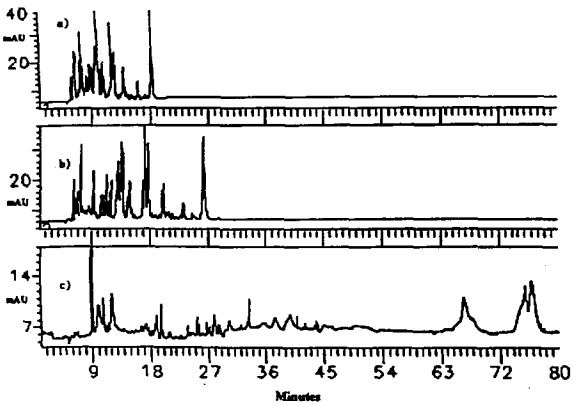


Fig. 1. Effect of SDS concentration on the separation of an acid extract of a Southwest Asian crude heroin base sample: (a) 21.2 m*M* SDS, (b) 42.5 m*M* SDS, (c) 85 m*M* SDS. A 78 cm (56 cm length to detector) × 50 μm capillary at 30°C was used with a voltage of 30 kV. In addition to SDS, the run buffer contained 8.5 m*M* phosphate and 8.5 m*M* borate at pH 9.0 plus 15% acetonitrile. UV detection at 210 nm.

Effect of addition of CDs

The effects of the addition of CDs are shown in Fig. 3. When β-CD was added to the buffer (Fig. 3b), a slight decrease in migration times for most solutes, some selectivity changes and increased resolution were all observed, especially for those solutes eluting after 20 min. This is consistent with the findings of Nishi et al. [14], who have shown that the addition of CDs to a run buffer containing SDS results in reduced k' values and selectivity changes. This arises because the solutes are distributed between the aqueous phase, the micelle and the CD. Since the neutral CD migrates with osmotic flow, any inclusion complexes formed would result not only in possible selectivity changes but also in reduced k' values.

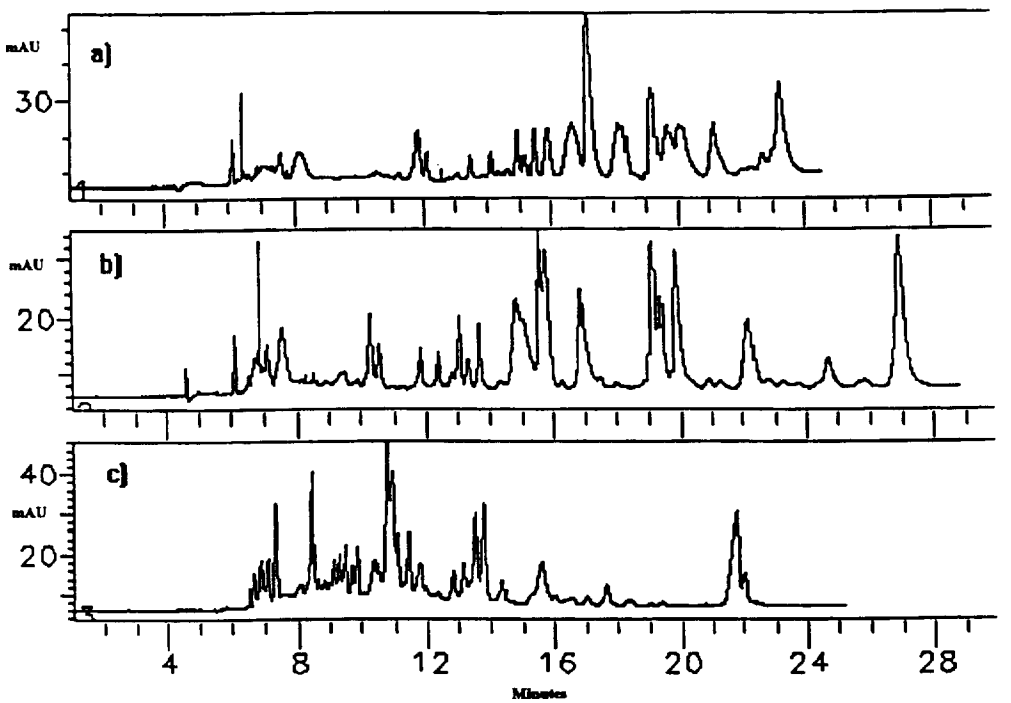


Fig. 2. Same as Fig. 1, except: (a) 9.0 m*M* phosphate, 9.0 m*M* borate, 45.0 m*M* SDS, and 10% acetonitrile, (c) 8.0 m*M* phosphate, 8.0 m*M* borate, 40.0 m*M* SDS, and 20% acetonitrile.

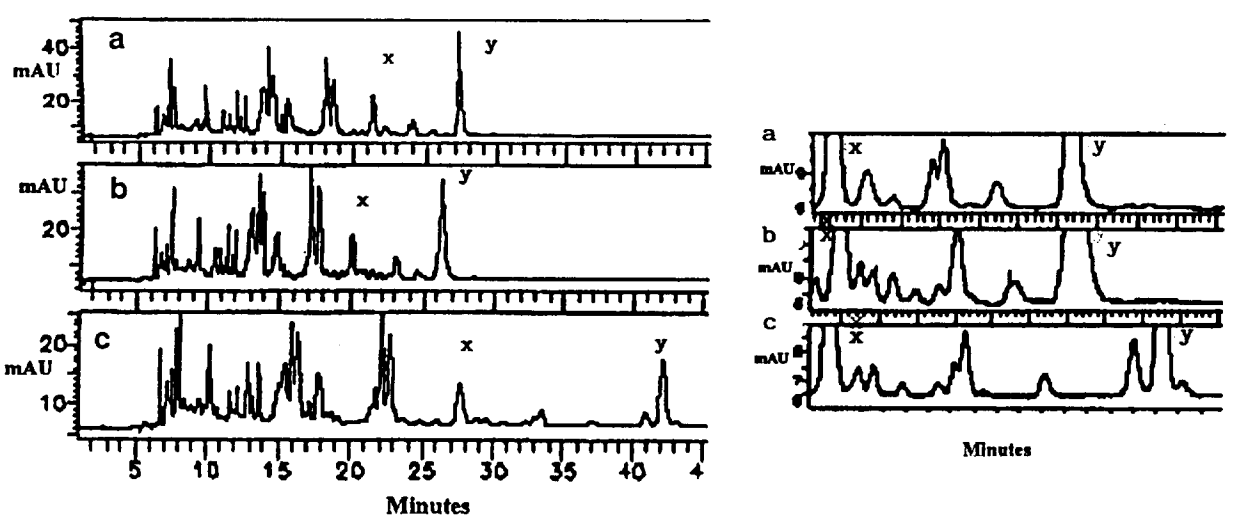


Fig. 3. Same as Fig. 1, except: (a) 42.5 m*M* SDS, (b) 42.5 m*M* SDS + 6.5 m*M* β-CD, (c) 42.5 m*M* SDS + 6.5 m*M* β-CD SBE 1V.

With β-CD SBE 1V (Fig. 3c), the migration time of every solute increases, with greater changes occurring for the more hydrophobic, late migrating compounds. Similar selectivity changes occur with either CD; however, there is a significant increase in resolution for β-CD SBE 1V vs β-CD, especially for the more hydrophobic solutes. Migration times and resolution both increase with the concentration of β-CD SBE 1V; the selected concentration (7.5 m*M*) is a compromise between speed of analysis and resolution.

The basis of the effect of the β-CD SBE 1V can be explained by examining a resolution equation for MECC. Extending the work of Ghowsi et al. [15] for the effect of the anionic CD, and assuming the independence of the micelle and CD [14], the following expression can be derived:

$$R_s = \frac{\Delta\mu_{ep^*}}{\mu_{mc^*} + \mu_{cd^*} + \mu_{eo}} \frac{\sqrt{N}}{4} \quad (1)$$

where R_s = resolution, $\Delta\mu_{ep^*}$ = mobility difference between the solutes, μ_{mc^*} and μ_{cd^*} = average mobilities of the two solutes, μ_{eo} = electroosmotic mobility, and N = the number of theoretical plates. The effective mobilities μ_{mc^*} and μ_{cd^*} are defined by Eqs. 2 and 3, respectively:

$$\mu_{mc^*} = \left(\frac{\eta_{mc}}{\eta_{aq} + \eta_{mc} + \eta_{cd}}\right)\mu_{mc} \quad (2)$$

$$\mu_{cd^*} = \left(\frac{\eta_{cd}}{\eta_{aq} + \eta_{mc} + \eta_{cd}}\right)\mu_{cd} \quad (3)$$

where η_{aq}, η_{mc} and η_{cd} are the total moles of the solutes in the aqueous phase, micelle and CD, respectively, and μ_{mc} and μ_{cd} are the mobilities of the micelle and CD complex, respectively. Eq. 1 indicates that resolution will improve when mobility is opposed to osmotic flow (which is the situation that exists with the anionic CD). Furthermore, as shown in Eqs. 1 and 3, μ_{cd^*} and R_s both increase with an increase in η_{cd}. The moles of solute in the CD phase will increase with both the concentration of the CD and value of the partition coefficient for the CD complex. Therefore, another anionic CD that forms a stronger inclusion complex may improve resolution, especially for the more hydrophilic compounds. Unfortunately, the availability of such reagents is very limited at the present time.[1]

As shown in Fig. 4, a further increase in resolution is obtained for the system containing β-CD SBE 1V by lowering the concentration of acetonitrile in the run buffer. This arises since (for many solutes) decreasing the organic modifier increases the k' values to a range where the anionic CD has a greater effect, i.e., effectively increasing μ_{mc^*} (compare Figs. 2a and 3c). Since the separation shown in Fig. 4 was run on a different instrument (with a different length from the detector window to the end of the capillary), the voltage was changed in order to obtain approximately the same field strength and similar run times.

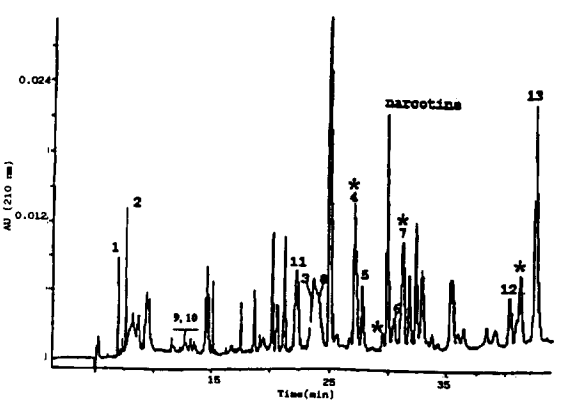


Fig. 4. A 57 cm (50 cm length to detector) × 50 μm capillary at 30°C was used with a voltage of 21 kV. The run buffer contained 9.0 m*M* phosphate, 9.0 m*M* borate, 45.0 m*M* SDS, 6.9 m*M* β-CD SBE 1V, and 10% acetonitrile. The identities of the numbered compounds are shown in Table 1. An asterisk refers to peaks containing unknown compounds with phenanthrene moieties.

[1] Ghowsi et al. [15] also showed that N depends on various parameters including μ_{eo}. However, this expression is neglected in Eq. 1 because it is not relevant to this situation.

3.2. Detection of acidic and neutral heroin impurities

PDA-UV detection

In order to facilitate the identification of the various components elucidated in Fig. 4, PDA-UV detection was used. Compounds 1–13 (Table 1) were identified to be present in the sample by comparing migration times[2] and UV spectra with standard compounds, an approach which greatly increases specificity of identification. The UV spectra of the compounds listed in Table 1 are shown in Fig 5. PDA-UV detection can aid in identifying unknown compounds present in the heroin sample. For example, there are several peaks in Fig. 4 that exhibit spectra which indicate that they have a phenanthrene backbone, suggesting that they originate from thebaine [16]. For the entire electropherogram, an examination of the UV spectra at various points of the peaks indicates that extensive co-migration of solutes exists. A partial solution, as shown in Fig. 6, is offered by the use of multi-wavelength detection. The simultaneous PDA-UV analysis at 210, 258, 304 and 350 nm affords increased selectivity and/or sensitivity of detection over the use of a single wavelength. An examination of UV spectra and/or isoabsorbance plots can be used to predict appropriate wavelengths of detection. For example, the choice of 258 nm was based on the presence of phenanthrene compounds, 304 nm for solutes with extended UV conjugation, and 350 nm for compounds 6 and 7; 210 nm was utilized for good general sensitivity.

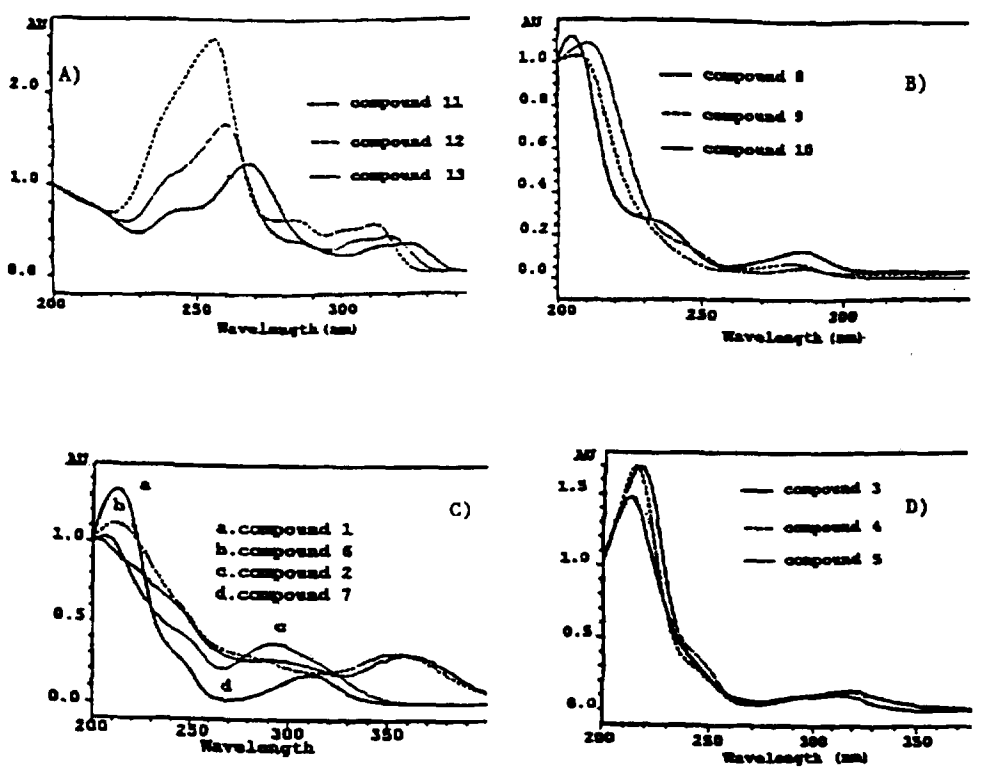


Fig. 5. Normalized UV spectra of standards shown in Table 1.

[2] Migration time matches obtained by co-injecting standard and sample.

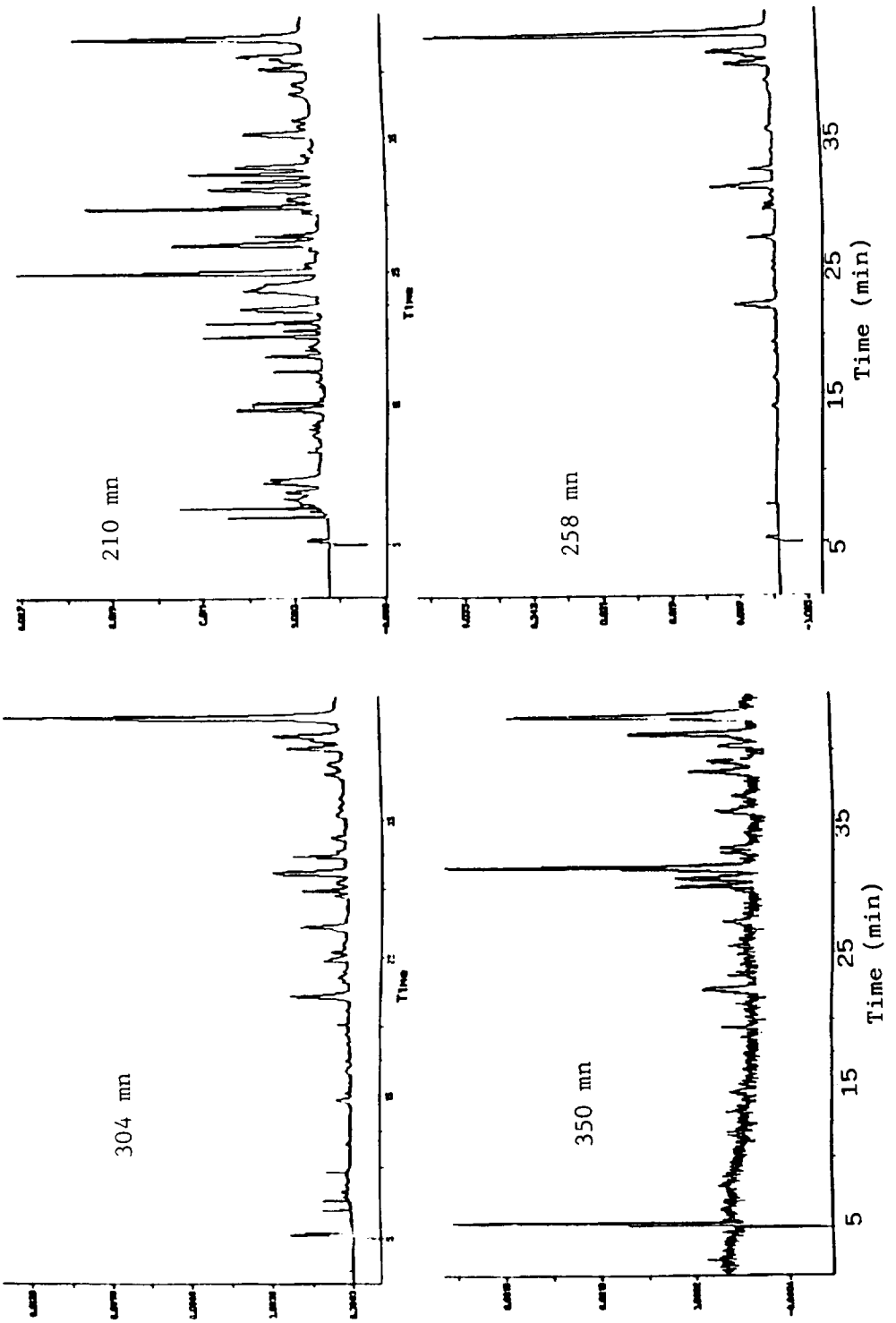


Fig. 6. Multi-wavelength detection of sample shown in Fig. 4.

Refined heroin, which invariably contains much lower levels of the acidic and neutral impurities, can also be analyzed (e.g., see Fig. 7). However, the identities of most of the solutes, except the heroin and acetylcodeine carried through during extraction, is presently not known.

3.3. LIF detection

For phenanthrene compounds, it was previously shown that conventional fluorescence detection with excitation at 257 nm improved S/N ratios by approximately one order of magnitude [11]. In the present study, LIF detection with excitation at 248 nm significantly increased both selectivity and sensitivity. For acetylthebaol, LIF detection was found to be approximately 500 times more sensitive than UV detection at 260 nm. The limit of detection ($S/N = 2$) was 1.8 ng/ml for LIF vs 1.0 μg/ml for UV. A comparison of UV vs LIF detection for the analysis of a refined heroin sample is shown in Fig. 8.

3.4. Reproducibility

Short- and long-term variability for migration times, relative migration times, areas and relative areas for selected compounds present in the crude heroin sample are shown in Table 2. For migration times, short-term precision was <0.60% with a considerably higher long-term precision of <3.0%. Long-term precision improved significantly by using relative migration time values (<1.5%). Similarly, the short-term precision for peak areas (between 4 and 8%) improved to <5.10% by using relative peak areas. The long-term precision for area and relative area is, for the most part, similar to that obtained for short-term precision. The results show that CE is acceptable for derivation of intelligence via analysis of illicit heroin. By

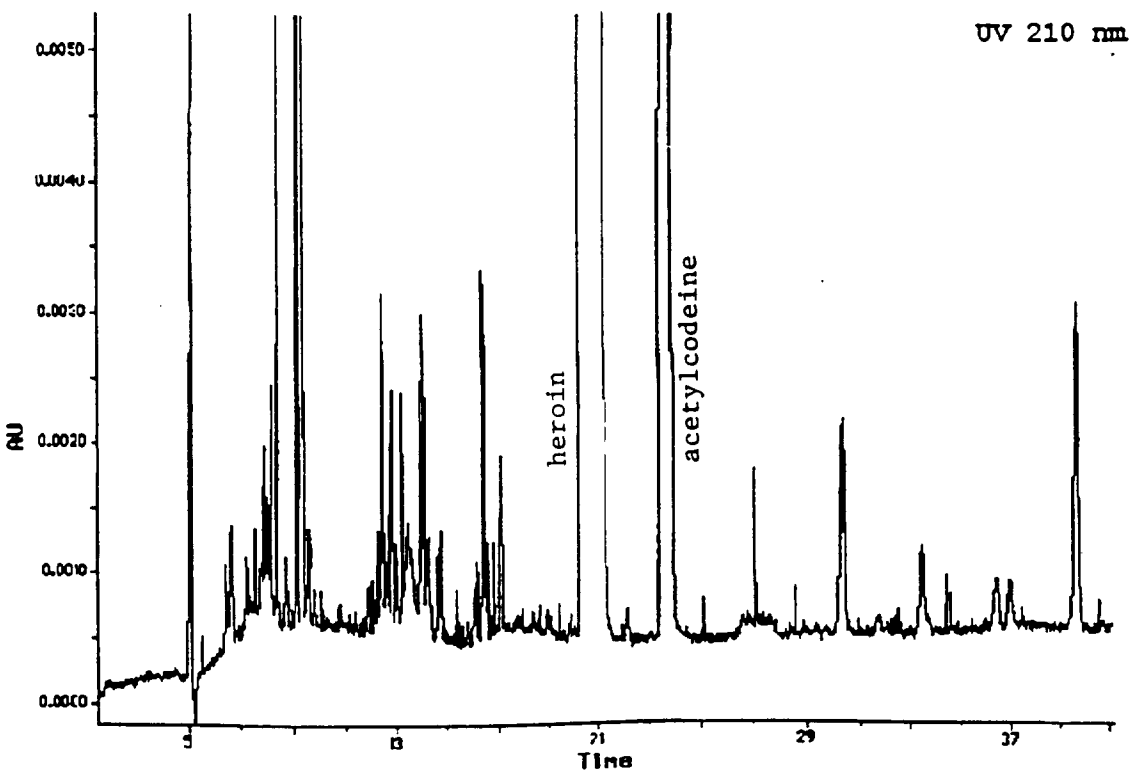


Fig. 7. Electropherogram of a Southeast Asian heroin hydrochloride exhibit. Conditions as in Fig. 4.

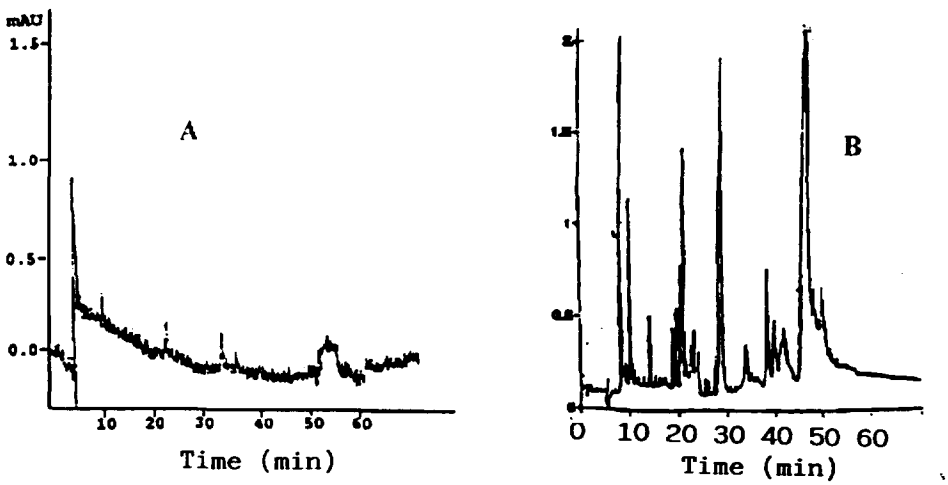


Fig. 8. Comparison of (A) UV detection at 260 nm and (B) LIF detection with krypton–fluoride laser for the CE analysis of a refined Southwest Asian heroin hydrochloride exhibit. Conditions as in Fig. 4 except for a 60 cm (55 cm length to detector) × 50 μm capillary and a voltage of 15 kV.

virtue of the improvement obtained by using relative values, employing internal standard(s) is clearly desirable. In addition, structurally related internal standards at various points in the electropherogram, as have been previously used for GC analysis [4], would both improve the MECC precision and allow for extraction variability.

4. Conclusions

The separation via MECC of acidic and neutral impurities in heroin was significantly improved by adding a counter migrating charged cyclodextrin to the run buffer. The highly complex samples are particularly amenable to a multiple detection scheme such as PDA-UV and LIF detection. Improved selectivity and/or sensitivity is obtained with both detection techniques. For solutes containing a phenanthrene moiety, LIF detection using a UV laser can offer greater than 2 orders of magnitude lower limits of detection versus UV detection.

Acknowledgements

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Table 2
Short- and long-term relative standard deviations (%) of migration times (mt), relative migration times (rmt), areas and relative areas (rarea) for heroin impurities

Parameter	1	11	Narcotine	13
mt	0.20 (0.65)	0.50 (1.70)	0.56 (2.21)	0.50 (3.00)
rmt[a]	0.50 (1.46)	–	0.00 (0.71)	0.46 (1.38)
area	5.00 (0.49)	4.3 (2.4)	3.92 (3.04)	7.81 (10.30
rarea[a]	3.10 (2.21)	–	4.03 (4.22)	5.02 (7.60)

[a] Relative to compound 11.
Within-day variations ($n = 5$); day-to-day variations over 7 day period ($n = 4$). Same sample extract used for run-to-run and day-to-day studies. Sample stored in freezer and thawed prior to analysis. Compound numbers as in Fig. 1 and separation conditions as in Fig. 5; values in parentheses represent day-to-day variability.

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