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Profiling of impurities in heroin by capillary electrochromatography and laser-induced fluorescence detection

Ira S. Lurie[a,*], Deon S. Anex[b,1], Yolanda Fintschenko[b], Wen-Yee Choi[b]

[a]*US Drug Enforcement Administration, Special Testing and Research Laboratory, 3650 Concorde Parkway, Suite 200, Chantilly, VA 20151, USA*

[b]*Chemical and Radiation Detection Laboratory, Sandia National Laboratories, P.O. Box 969, Mail Stop 9671, Livermore, CA 94551, USA*

Abstract

Capillary electrochromatography (CEC) with laser-induced fluorescence (LIF) detection was investigated for the analysis of acidic and neutral impurities in heroin. The phenanthrene-like heroin impurities exhibit high native fluorescence when excited with a doubled argon ion laser (operating at 257 nm). The limit of detection for acetylthebaol is 66 pg ml^{-1}. CEC–LIF analysis of heroin samples of different geographical origin gave distinguishable peak-enriched chromatograms. A sulfonic acid C_{12} polymer monolith column provided similar resolving power to a 1.5 mm non-porous ODS column for the isocratic analysis of a refined heroin sample. Analysis of a crude heroin sample via a multi-step gradient CEC resolved a significantly higher number of peaks than gradient high-performance liquid chromatography or micellar electrokinetic capillary chromatography. Published by Elsevier Science B.V.

Keywords: Electrochromatography; Heroin; Acetylthebaol

1. Introduction

Impurity profiling of illicit drugs such as heroin is important for deriving strategic or tactical intelligence [1]. Strategic intelligence involves the determination of geographical origin and/or synthetic route. Tactical intelligence involves the determination of whether two or more exhibits came from an identical source, i.e., same batch from the same laboratory. Both organic and inorganic impurities are present in virtually all illicitly produced drugs. The analysis of these impurities, which are complex mixtures of solutes often present at trace levels, require methodologies which offer high degrees of resolution, specificity and sensitivity.

Acidic and neutral organic impurities in heroin, which arise from the acetylation of opiate alkaloids present in the crude morphine used in illicit heroin production, are usually found at trace levels. These solutes are attractive for impurity profiling since they can be easily isolated from the bulk heroin matrix. Capillary GC with various detection schemes such as flame ionization [2,3], nitrogen–phosphorous [2], electron-capture [4], or electron impact mass spectrometric [3] have been used for the analysis of acidic and neutral heroin impurities. HPLC in combination with conventional UV [5–8], photodiode array (DAD) UV [9,10], fluorescence (FL) [6,7,11], electrochemical [7], electron impact [8], or thermospray mass spectrometric detection [8] have also been investigated. In addition, micellar electrokinetic

*Corresponding author.

[1]Present address: Eksigent Technologies, 2021 Las Posita Court, Suite 161, Livermore CA 94550, USA.

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capillary chromatography (MECC) with single-wavelength UV [10], DAD UV [12], conventional FL [10] and laser-induced fluorescence (LIF) detection [12] have been investigated for the analysis of acid and neutral heroin extracts. Although GC affords the highest peak capacity, the analysis of compounds that are polar, thermolabile and non-volatile can be problematic; therefore, derivatization is commonly employed for heroin impurity profiling by GC. HPLC and MECC are amenable to solutes that are difficult to analyze by GC. The relatively small peak efficiency and peak capacity afforded by HPLC relative to GC is partially compensated for by using highly sensitive and relatively selective fluorescence detection. Although MECC offers high peak efficiency, its peak capacity is limited by the finite migration window t_{mc}/t_o. Capillary electrochromatography (CEC), which is also viable for compounds that are problematic by GC, offers high peak efficiency; however, due to the presence of a stationary phase (an infinite migration window), CEC has a potentially higher peak capacity than MECC. In addition, limitations in sensitivity exist for CEC (as well as MECC) with conventional UV detection, due to the small pathlength of the detection cell. This limitation can be compensated for with a high sensitivity UV cell (Z cell configuration) and FL detection. CEC with a high sensitivity UV cell has been used for profiling of cannabis impurities [13]. LIF detection with CEC has been reported for the fingerprinting of methamphetamine impurities [14].

In this study, CEC with LIF detection is investigated for impurity profiling of heroin.

2. Experimental

2.1. Reagents and analytes

Tris(hydroxymethyl)aminomethane (Tris) was obtained from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI, USA) and Aldrich (Milwaukee, WI, USA.) Phosphoric acid and sodium hydroxide were reagent grade. Deionized water was obtained using a Millipore Milli-Q purification system (Bedford, MA, USA) and a Labconco Water Pro purification system (Kansans City, MO, USA). Acetylthebaol (AT) was synthesized at the Special Testing and Research laboratory. All other chemicals were reagent grade.

The CEC mobile phase was prepared by combining appropriate ratios of 5.0 m*M* Tris buffer (pH 9.0), and acetonitrile. The HPLC mobile phases were internally mixed from solvent reservoirs containing acetonitrile and phosphate buffer (pH 2.1). The phosphate buffer was a mixture of 3480 ml water, 120 ml 2 *M* sodium hydroxide, and 40.0 ml of phosphoric acid.

2.2. Analytical solutions

For CEC and HPLC, acidic extracts derived from approximately 1.5 mg and .063 mg equivalents, respectively, of a crude heroin sample, were dissolved in 100 μl of acetonitrile–Tris buffer pH 9.0 (30:70) and 1000 μl of acetonitrile–phosphate buffer pH 2.1 (30:70). For refined heroin samples, 50 mg equivalents were prepared as above for CEC analysis. The acidic extracts were prepared by dissolving an appropriate quantity of sample in 4.0 ml of 1.0 *M* sulfuric acid, and extracting it with 5.0 ml of diethyl ether–methylene chloride (60:40). The extracts were then evaporated to dryness with nitrogen at 80 °C, and reconstituted with 1.0 ml of methylene chloride for CEC (5.0 ml was used for HPLC); aliquots of this reconstituted solution were used for all HPLC and CEC analyses.

2.3. Instrumentation

The CEC–LIF experiments were performed using a previously described laboratory-built system [14]. CEC columns containing 1.5 μm non-porous ODS II particles were prepared in the laboratory as previously reported [15]. The columns were 75 μm I.D. 365 μm O.D. and had a total length of 32 cm (of which 23.5 cm was packed), with the detection windows (~2 mm long) approximately 2 mm downstream of the outlet frit. Columns were conditioned as previously reported [14]. CEC columns containing porous sulfonic acid C_{12} polymer monoliths in UV-transparent PTFE-coated capillaries were also prepared in the laboratory using a previously reported methodology [16,17]. The columns were 100 μm I.D. 365 μm O.D. and had a total packed length of 39 cm. The

polymer in the detection volume was removed by the 257 nm laser through a depolymerization process. Where the laser passed though the column (about 10 cm from the outlet of the column), the polymer was removed.

A Hewlett–Packard Model 1100 HPLC system (Waldbronn, Germany) equipped with a quaternary pumping system, a 1100 series DAD UV detector, and a Model 1046A FL detector, was used for HPLC analyses. A 11.0 cm×4.7 mm I.D. HPLC cartridge system (5 μm Partisil ODS 3) operated at ambient temperature was used (Whatman, Clifton, NJ, USA.)

Table 1
Acetylthebaol limits of detection

Technique	LOD
CEC–LIF	66 pg ml^{-1} [a]
MECC–LIF	2 ng ml^{-1} [b]
MECC–UV	1 μg ml^{-1} [b]
HPLC–FL	1 ng ml^{-1} [c]
HPLC–UV	500 ng ml^{-1} [c]

[a] Conditions: 5.0 m*M* tris pH 9.0–acetonitrile (20:80). A sulfonic acid C_{12} porous monolith [39 cm (effective length 29 cm)×100 μm] was used at ambient temperature at a voltage of 20 kV.
[b] Ref. [12].
[c] Ref. [7].

3. Results and discussion

The presence of numerous, highly fluorescent acidic and neutral phenanthrene compounds in heroin is well established. These solutes are easily detected by LIF detection using a doubled argon laser operating at 257 nm. As shown in Fig. 1, the most abundant phenanthrene-type impurity (AT) has a UV excitation maximum at 258 nm. Using CEC with LIF detection the limit of detection (LOD) for this solute was 66 pg ml^{-1} (S/N=3). This compares quite favorably with LODs for AT using MECC with LIF, conventional UV detection, or HPLC with conventional fluorescence or UV detection (see Table 1). The LOD for CEC using a doubled argon laser was approximately 30 times lower than MECC with a krypton–fluoride laser operating at 248 nm, and approximately 15 times lower than HPLC with conventional fluorescence with excitation at 256 nm. Several factors contribute to the differences in the various techniques, including column efficiency, injection size and detector type. In previous studies, fluorescence detection was found to be 500 times more sensitive than UV detection (see Table 1).

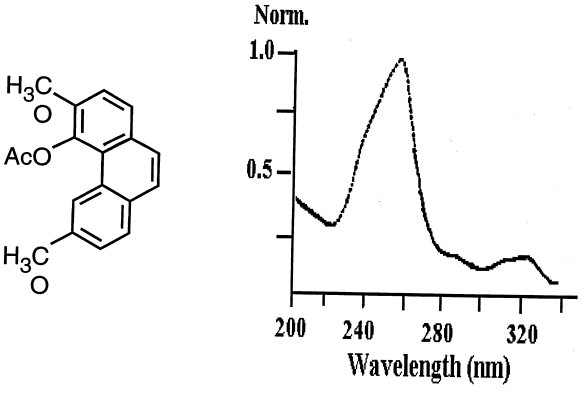

Fig. 1. DAD UV spectra of acetylthebaol. Conditions: phosphate pH 2.1–acetonitrile (linear gradient 30–50% acetonitrile). Flow-rate 1.5 ml min^{-1}. A Whatman 5 μm ODS 3 (11.0 cm×4.7 mm I.D.) column was used at ambient temperature.

A previous study using a 1.5 μm non-porous ODS column provided a high-resolution separation of methamphetamine neutral impurities [14]. However, packed columns can be tedious to prepare, and are subject to bubble formation during CEC separations (especially with systems which do not provide for pressurizing the inlet and outlet vials). In contrast, monolithic columns do not contain the in situ frits which may cause difficulties of this sort. A comparison of the CEC–LIF separation of a refined Southeast Asian heroin extract using a 1.5 μm non-porous ODS column versus a sulfonic acid C_{12} porous monolith column is shown in Fig. 2. Approximately the same number of peaks were resolved (resolution of 1) with either column. For the late eluting acetylthebaol, the plate count was approximately 63 000 m^{-1} for both stationary phases. For the packed column, peaks eluting between 7 and 10 min gave plate counts of approximately 135 000 m. In contrast, for the monolith column, peaks eluting at 5.4 and 7.4 min gave plate counts of approximately 58 000 m^{-1}. Although there was a similar retention time range for both columns, vastly different chromatographic profiles were obtained. A higher organic content in the mobile phase is required for the monolithic versus the packed column in order to

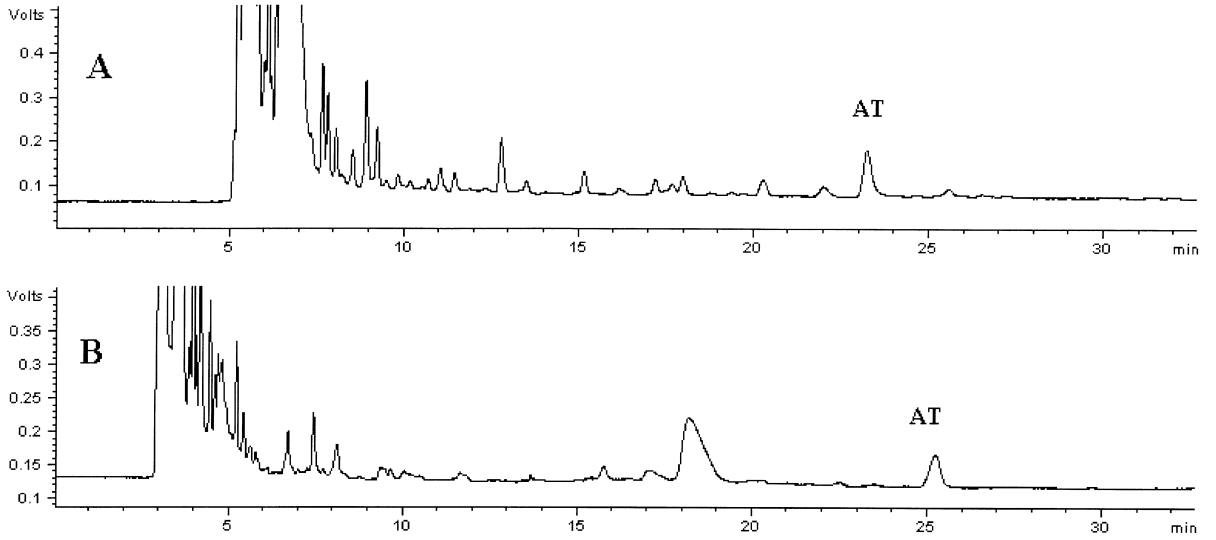

Fig. 2. CEC with LIF detection of acidic–neutral extract of refined Southeast Asian heroin. (A) Conditions: 5.0 m*M* Tris pH 9.0–acetonitrile (70:30). A 1.5 μm non-porous ODS II column was used at ambient temperature at a voltage of 15 kV. (B) Conditions: 5.0 m*M* Tris pH 9.0–acetonitrile (50:50). A sulfonic acid C_{12} porous monolith column was used at ambient temperature at a voltage of 20 kV.

obtain comparable retention times. The reason for these findings is not clear; however, it appears that the polymer backbone contributes considerably to the column hydrophobicity. Monoliths can be viewed as a single, large porous particle without a secondary pore structure [18]. Thus, in a sense they resemble packed beds of non-porous particles. Differences exist in the hydrophicity and surface area between the octadecyl chains of the packed column and the dodecyl moieties in the monolith. The silica backbone of the former column, and the methacrylate backbone as well as sulfonic acid moieties on the

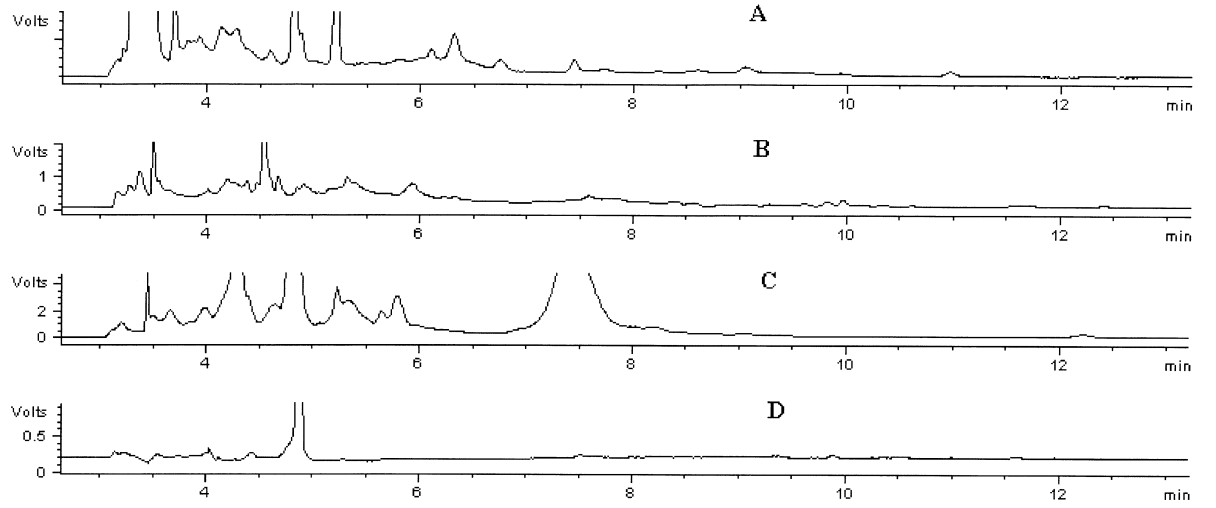

Fig. 3. CEC with LIF detection of acidic–neutral extracts of (A) refined Southeast Asian heroin, (B) refined South American heroin, (C) crude Southwest Asian heroin and (D) procedural blank. Conditions: 5.0 m*M* Tris pH 9.0–acetonitrile, (60:40). A sulfonic acid C_{12} porous monolith column was used at ambient temperature at a voltage of 20 kV.

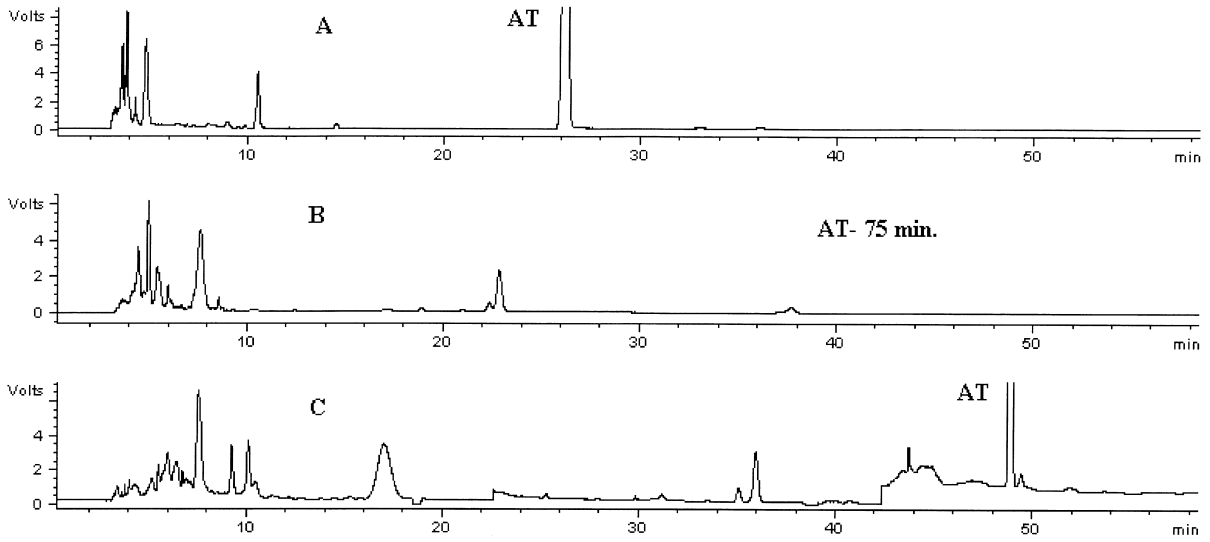

Fig. 4. CEC with LIF detection of acidic–neutral extract of crude Southwest Asian heroin. (A) Conditions: 5.0 m*M* Tris pH 9.0–acetonitrile (50:50). A sulfonic acid C_{12} porous monolith column was used at ambient temperature at a voltage of 20 kV. (B) Conditions: 5.0 m*M* Tris pH 9.0–acetonitrile (60:40). A sulfonic acid C_{12} porous monolith column was used at ambient temperature at a voltage of 20 kV. (C) Conditions: 5.0 m*M* Tris pH 9.0–acetonitrile (initial 70:30 for 18.6 min, intermediate 60:40 for 20 min and final 50:50). A sulfonic acid C_{12} porous monolith column was used at ambient temperature at a voltage of 20 kV.

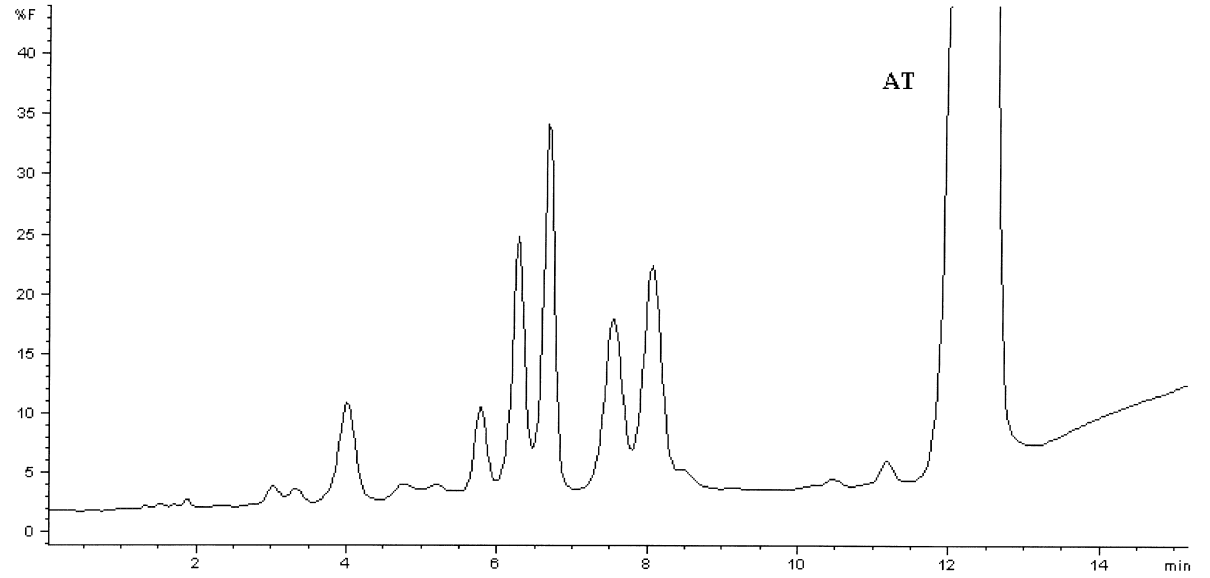

Fig. 5. HPLC gradient with conventional fluorescence detection (excitation 257 nm and emission 390 nm) of acidic–neutral extract of crude Southwest Asian heroin. Conditions: phosphate buffer pH 2.1–acetonitrile (15-min linear gradient 30– 50% acetonitrile). A Whatman 5-ODS 3 column was used at ambient temperature at a flow-rate of 1.5 ml min^{-1}.

latter column, can contribute to osmotic flow and retention.

In view of the comparable resolving power of the monolith versus the non-porous particle column, and the problems with bubble formation using the latter column, additional studies were conducted using the monolith stationary phase. A comparison of the chromatographic profiles for extracts from refined Southeast Asian, refined South American, crude Southwest Asian heroin, and a procedural blank, is shown in Fig. 3. The three heroin types exhibit vastly different chromatographic patterns, with most peaks arising from the sample and not the procedural blank. As expected, the refined heroin samples (for which more extensive cleanup procedures were used) exhibit lower levels of impurites compared to crude heroin exhibits. A lower amount of acetonitrile (40% versus 50%) was used for these chromatographic runs in order to increase the resolution of the earlier eluting peaks (cf. Fig. 2B with 3A). In spite of the change in the solvent strength of the mobile phase, however, significant overlap of the early eluting peaks was observed (see Fig. 3). In addition, the lower mobile phase solvent strength resulted in excessive retention of the late eluting compounds such as AT (75 versus 25 min; cf. Figs. 4B and 4A).Therefore, a multi-step gradient was used to improve separation. As shown in Fig. 4C, a vastly improved separation was obtained for the separation of a crude Southwest Asian heroin using a three step-gradient. Starting the run with a lower amount of acetonitrile (30% versus 40%) greatly improved the separation of the earlier eluting compounds (cf. Figs. 4B and C), while switching to 40% acetonitrile maintained or improved the separation of the mid-range eluting solutes (cf. Figs. 4B and C). The final step of 50% acetonitrile eluted AT in under 50 min (Fig. 4C).These results suggest that a gradient elution with an even weaker starting mobile and stronger final mobile phase would be superior to the multi-step gradient. A continuous gradient using more extreme elution conditions should result in further improved separations and faster analyses. In addition, the observed abrupt baseline changes (see Fig. 4) and the possibility of artifactual patterns in the electropherograms would be avoided using a continuous gradient.

It is of interest to compare the multi-step gradient CEC separation with other liquid phase techniques such as gradient HPLC and MECC (see Figs. 4–6). CEC resolved (resolution of 1) approximately 2.5 times and approximately 30% more peaks than HPLC and MECC, respectively. Differences in peak capacity, selectivity and sensitivity of detection are contributing factors. Step gradient CEC is expected to have a greater peak capacity than gradient HPLC [19]. In contrast, due to the finite migration time of the micelle, MECC (as normally practiced) is expected to have a limited peak capacity [20]. HPLC, MECC and CEC, where the major separation mecha-

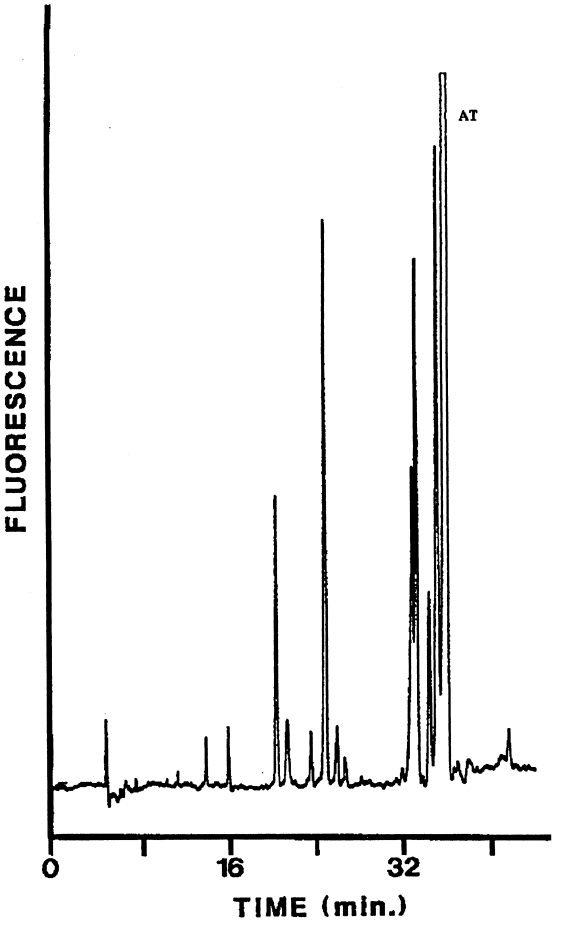

Fig. 6. MECC with conventional fluorescence detection (excitation 257 nm and emission 400 nm) of acidic–neutral extract of crude Southwest Asian heroin. Conditions: 100 m*M* sodium dodecylsulfate, 10 m*M* phosphate, 10 m*M* borate (pH 9.0)–acetonitrile (85:15). A 72 cm (50 cm length to detector)×50 μm capillary was used at 60°C at a voltage of 30 kV.

nism depends on hydrophobic interactions, would be expected to have similar selectivity. However, a comparison of chromatographic profiles indicate major selectivity differences between the techniques, especially for CEC (cf. Figs. 4–6). However, due to the high excitation power of the laser used for the LIF detection, the CEC chromatogram could also represent non-phenanthrene compounds.

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