J Forensic Sci, January 2006, Vol. 51, No. 1
doi:10.1111/j.1556-4029.2005.00017.x
Available online at: www.blackwell-synergy.com

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Analysis of Carbohydrates in Seized Heroin Using Capillary Electrophoresis

ABSTRACT: Illicitly produced heroin is commonly cut with carbohydrates to increase bulk. The analysis of these solutes is important for legal and intelligence purposes. A capillary electrophoresis (CE) method was developed for the qualitative analysis of dextrose, lactose, sucrose, inositol, and mannitol in heroin exhibits. For this method, a 64 cm (55.5 cm to detector window) by 50 μm capillary was used with the Agilent Basic Anion Buffer modified to pH 12.1. This separation was performed at 25°C with a voltage of 20 kV and indirect detection with 2,6-pyridinedicarboxylic acid as the visualization reagent. The methodology is also applicable for the screening of inorganic and organic anions using indirect detection, and acidic adulterants using direct detection. For a run time of 13 min, the relative standard deviation ($n = 6$) of the methodology was better than 0.36% for migration times and less than 2.6% for corrected peak areas. For the analysis of carbohydrates and acidic adulterants in seized heroin, excellent agreement was obtained between CE and nuclear magnetic resonance spectroscopy.

KEYWORDS: forensic science, seized heroin, inorganic anions, organic acids, acidic adulterants, carbohydrates, capillary electrophoresis, nuclear magnetic resonance spectroscopy

The determination of diluents such as carbohydrates in heroin samples is important for legal and intelligence purposes (1). Microscopic techniques such as optical crystallography are highly dependent on operator skill (2), and become difficult to interpret with low levels of carbohydrates. Gas chromatography (GC) (3–6) and high-performance liquid chromatography (HPLC) (2,7,8) have been used for the analysis of sugars and polyhydric alcohols in seized heroin exhibits. GC, unlike HPLC, requires derivatization and allows for the separation of anomers of reducing sugars, which can increase the complexity of analysis. HPLC inherently lacks resolution and requires prior sample cleanup.

Carbohydrates can be analyzed without derivatization or prior sample cleanup using capillary electrophoresis (CE). Owing to the weak acidities and poor UV chromophores of these solutes, most CE methods employ high pH run buffers with indirect UV detection (9–15). Ishi et al. (16) directly analyzed carbohydrates in cocaine using micellar electrokinetic chromatography (MEKC) with indirect detection using a highly alkaline pH buffer with phthalate as the visualization reagent. The methodology is also applicable to the analysis of cocaine, basic cocaine impurities, and basic adulterants, and would be applicable for seized heroin. However, more selective and precise methodology already exists for the determination of heroin, basic impurities, and basic adulterants using CE in the capillary zone electrophoresis (CZE) mode (17).

In this study, sugars and polyhydric alcohols in seized heroin are directly screened for using CZE with a pH adjusted Agilent Basic Anion buffer (11–15), indirect detection, and 2,6-pyridinedicarboxylic acid (PDC) as the visualization reagent. The methodology is also applicable to the screening of inorganic and organic anions (e.g., sulfate and tartrate) as well as acidic adulterants (e.g., phenobarbital) present in heroin samples.

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Received 28 Feb. 2005; and in revised form 13 June and 4 Aug. 2005; accepted 20 Aug. 2005; published 26 Dec. 2005.

Experimental

Reagents

All standards used were obtained from the reference collection of the Special Testing and Research Laboratory (Dulles, VA). Basic Anion Buffer was obtained from Agilent Technologies (Waldbronn, Germany), while 1 N sodium hydroxide was obtained from MicroSolv Corporation (Eatontown, NJ). Deionized and high-purity water (HPLC grade water) was obtained from a Millipore Synergy 185 water system (Bedford, MA). Maleic acid was acquired from Fluka (Buchs, Switzerland). Deuterium oxide (D_2O) containing 0.05 weight percent 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt was obtained from Aldrich (St. Louis, MO).

CE Analysis

Run Buffer—The run buffer was prepared by adding 250 μL of 1 N sodium hydroxide to a 50 mL bottle of Basic Anion Buffer.

Standards and Samples—The standards were diluted in reference compound solution (fructose 1.3 mg/mL HPLC grade water), and 500 μL of filtered standard solution (SRI 0.5 μm nylon filter) was added into a polypropylene CE injection vial. The samples were prepared by weighing approximately 10 mg of heroin sample in a small test tube, pipetting in 1.0 mL of reference compound solution (fructose 1.3 mg/mL HPLC grade water) and vortexing for approximately 30 sec. Filtered sample solution (500 μL, SRI 0.5 μm nylon filter) was added into a polypropylene CE injection vial.

Apparatus and Operating Conditions—An Agilent Model HP^{3D}CE Capillary Electrophoresis System equipped with a diode array detector was used for the CE experiments. All experiments were carried out with fused silica 64 cm (55.5 cm to detector window) by 50 μm i.d. capillaries obtained from Polymicro Technologies (Phoenix, AZ). For conditioning a new capillary, the same prerinses were used as for regular analysis. The prerinse consisted of a 2 min flush of outlet home vial to waste, followed by a 2 min flush of inlet home vial to waste. The home vials contained the run

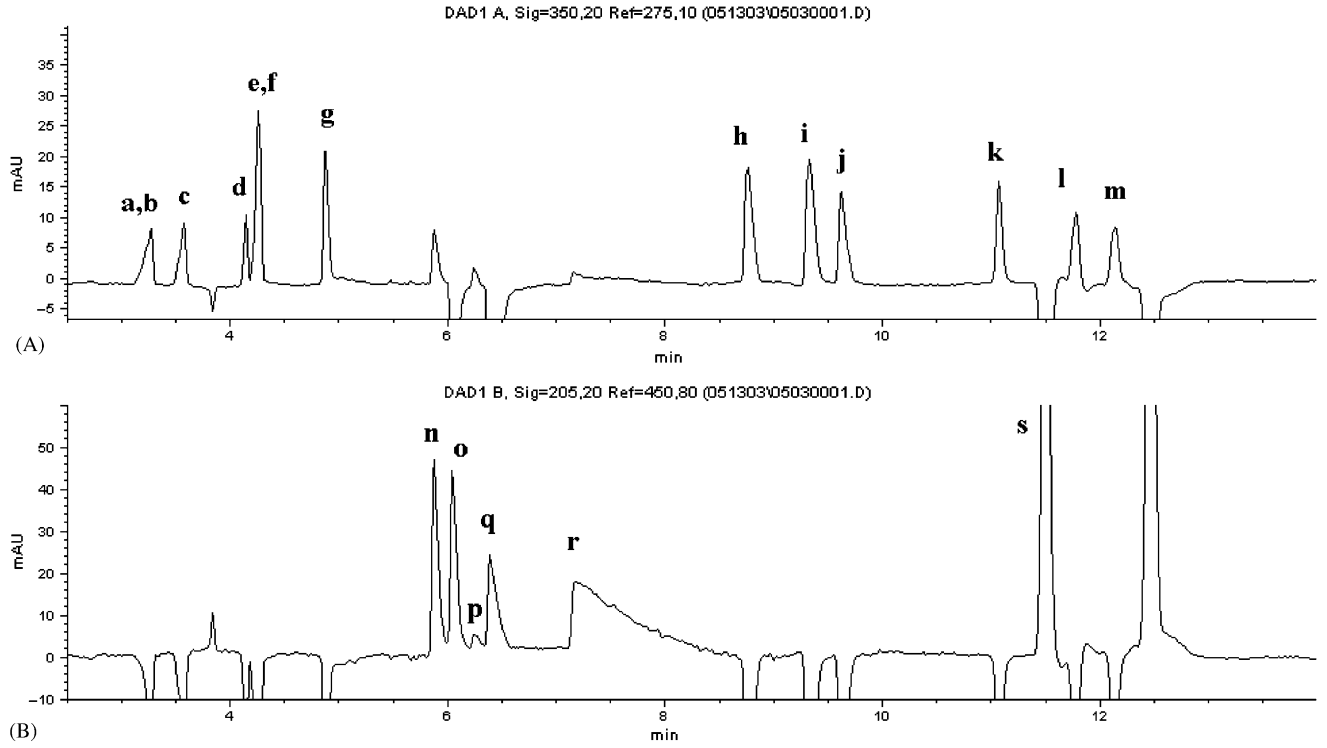

FIG. 1—*Capillary zone electrophoresis separation of standard mixture of inorganic and organic anions, carbohydrates, and acidic adulterants using: (A) indirect detection and (B) direct detection. Experimental conditions are described in capillary electrophoresis analysis section. Peaks: (a) bromide (0.33 mg/mL potassium bromide), (b) chloride (0.32 mg/mL sodium chloride), (c) sulfate (0.40 mg/mL magnesium sulfate), (d) citrate (0.36 mg/mL sodium citrate), (e) tartrate (0.34 mg/mL tartaric acid), (f) phosphate (0.34 mg/mL potassium phosphate monobasic (g) acetate (0.31 mg/mL sodium acetate), (h) fructose (0.95 mg/mL), (i) dextrose (1.0 mg/mL), (j) lactose (1.0 mg/mL), (k) sucrose (0.97 mg/mL), (l) mannitol (1.0 mg/mL), (m) inositol (0.98 mg/mL), (n) phenobarbital (0.94 mg/mL), (o) theophylline (0.82 mg/mL), (p) aspirin (1.0 mg/mL), (q) acetaminophen (0.84 mg/mL), (r) salicylic acid (0.50 mg/mL), (s) caffeine (0.82 mg/mL).*

buffer used for electrophoresis. Polypropylene vials (2.0 mL) were used for all CE experiments. The separations were performed at 25°C at a voltage of 20 kV. CE runs were carried out with both indirect UV detection at 275 nm with a bandwidth of 10 nm and direct UV detection at 205 nm with a bandwidth of 20 nm. Pressure injections of 150 mbar sec were employed. For overnight storage, the capillary was first washed with water for 20 min and then stored in water.

TABLE 1—*Precision and sensitivity.*

Anion	% RSD ($n = 6$) Migration Time	% RSD ($n = 6$) Relative Migration Time	% RSD ($n = 6$) Corrected Peak Area	% RSD ($n = 6$) Relative Corrected Area	Detection limit* (μg/mL)
(a) Bromide	0.17	0.43	2.0	2.8	9
(b) Chloride	0.17	0.43	2.0	2.8	4
(c) Sulfate	0.18	0.43	3.5	4.4	2
(d) Citrate	0.30	0.52	3.8	2.7	9
(e) Tartrate	0.28	0.48	1.6	2.1	12
(f) Phosphate	0.28	0.48	1.6	2.1	7
(g) Acetate	0.23	0.44	2.4	1.5	6
(h) Fructose (reference)	0.35	1.00	2.1	1.0	12
(i) Dextrose	0.35	0.01	0.8	1.4	13
(j) Lactose	0.26	0.12	1.0	2.2	17
(k) Sucrose	0.19	0.28	1.8	0.5	19
(l) Mannitol	0.24	0.44	1.3	1.5	30
(m) Inositol	0.28	0.51	2.6	3.0	37
(n) Phenobarbital	0.33	0.52	2.8	5.0	10
(o) Theophylline	0.27	0.33	3.0	5.1	10
(p) Aspirin	0.29	0.33	6.4	9.4	533 (144)†
(q) Acetaminophen	0.48	1.00	6.2	1.0	10
(r) Salicylic acid	0.47	0.60	1.4	5.7	296
(s) Caffeine	0.36	0.38	2.4	5.2	3

*Detection limit 2 × signal to noise.
†Indirect detection.

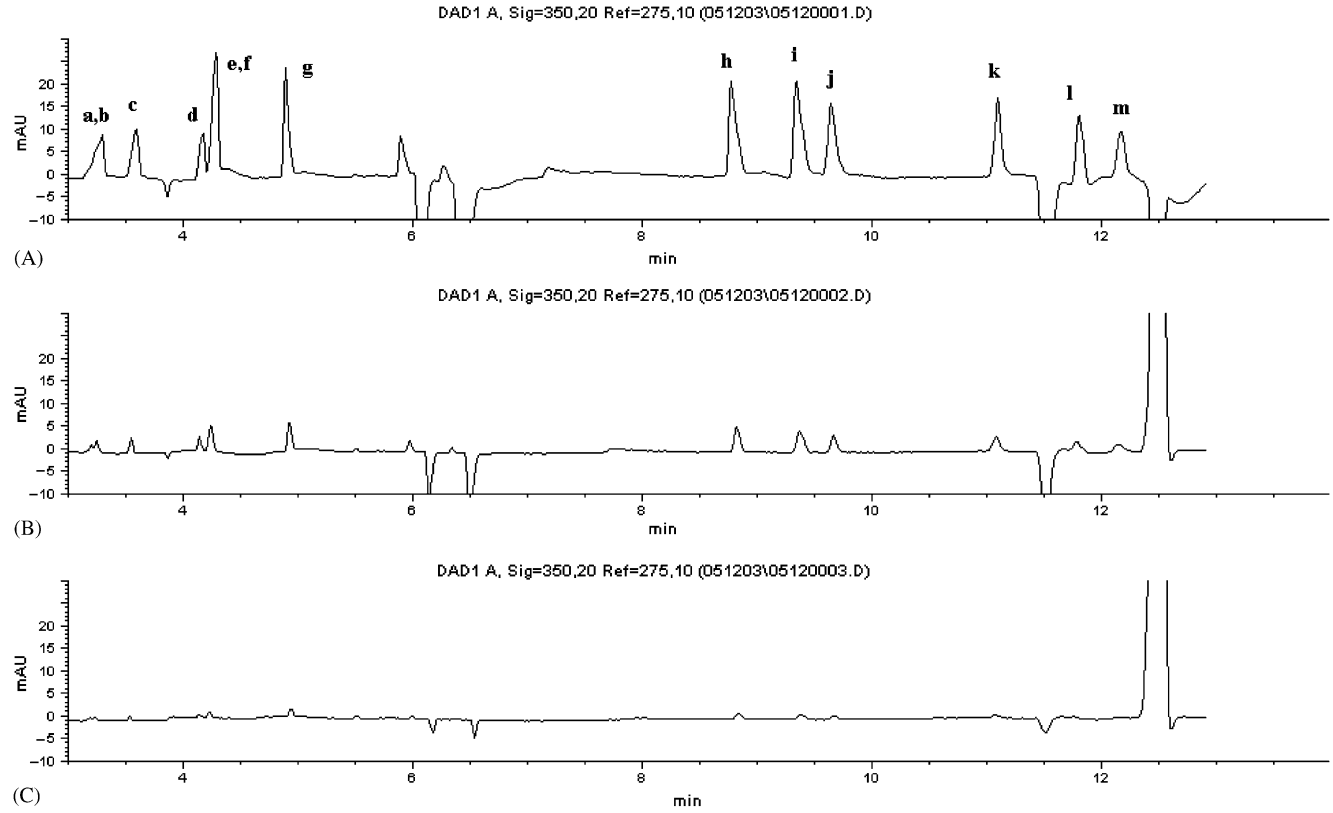

FIG. 2—*Capillary zone electrophoresis separation of standard mixture of inorganic and organic anions, and carbohydrates at (A) original dilution, (B) diluted 1/5 with water, and (C) diluted 1/25 with water. Experimental conditions are described in capillary electrophoresis analysis section. See Fig. 1 for compound identity and concentration of original dilution.*

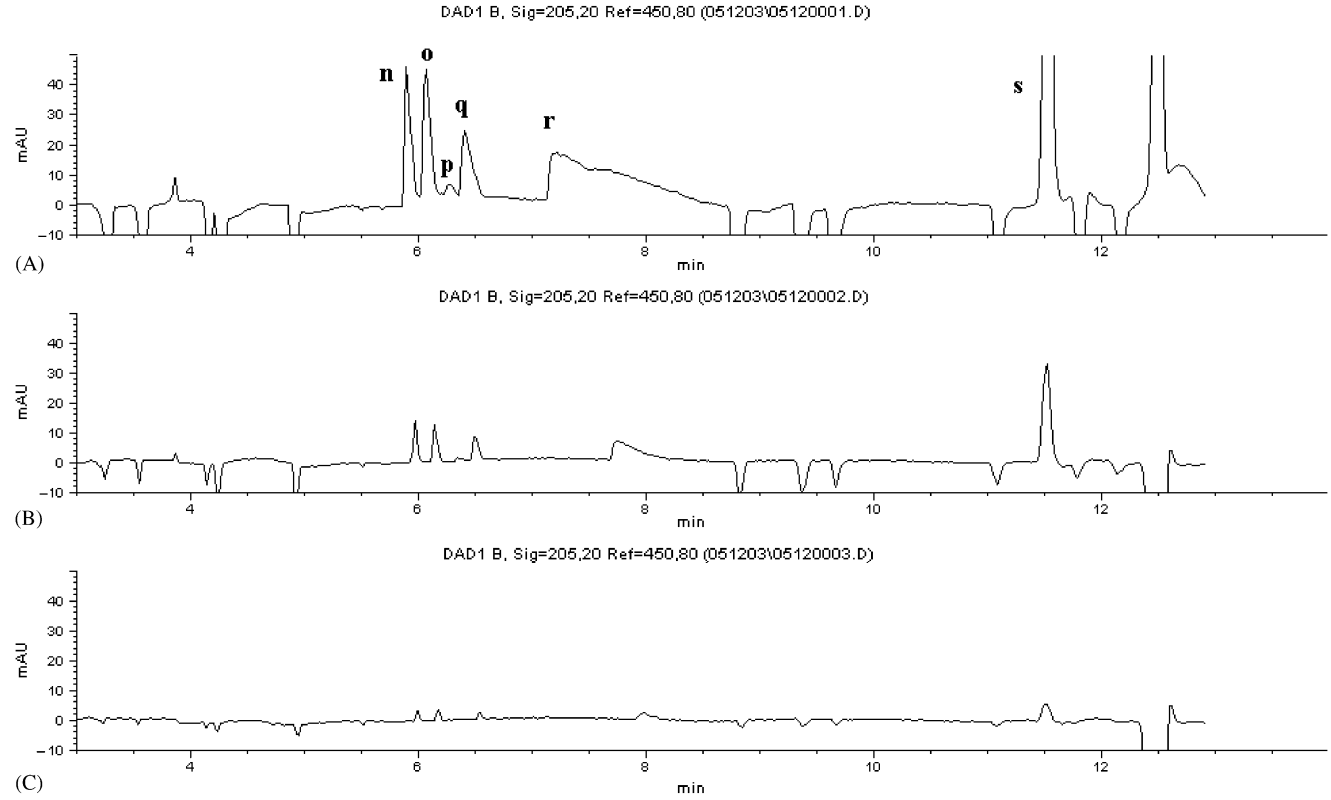

FIG. 3—*Capillary zone electrophoresis separation of standard mixture of acidic adulterants at (A) original dilution, (B) diluted 1/5 with water, and (C) diluted 1/25 with water. Experimental conditions are described in capillary electrophoresis analysis section. See Fig. 1 for compound identity and concentration of original dilution.*

TABLE 2—*Comparison of CE and NMR for screening heroin samples.*

Sample Type	CE and NMR
Tan powder	Lactose and mannitol
Tan chunky powder	Lactose and mannitol
Brown chunky powder	Lactose and mannitol
Beige powder	Lactose and mannitol
Tar	Dextrose*
Brown powder	Lactose
Tar	Lactose
Dark brown powder	Lactose
Brown powder	Lactose and acetaminophen
Light brown powder	Lactose and caffeine
Off-white powder	Acetaminophen
Light brown powder	Lactose and acetaminophen
Light brown powder	Caffeine
Brown plastic material	Caffeine
Off-white powder	Mannitol, inositol, and lactose
Brown powder	Inositol and acetaminophen
Brown powder	Lactose and caffeine
Brown powder	Inositol and acetaminophen
Light brown powder	Caffeine
Brown powder	Inositol and acetaminophen

*Acetylated sugar present in NMR spectrum obstructed confirmation. Some dextrose peaks present but spectrum too busy.

CE, capillary electrophoresis; NMR, nuclear magnetic resonance.

Nuclear Magnetic Resonance (NMR) Analysis

Standards and Sample—Sugar and acidic adulterant identifications were performed using the NMR data obtained for the quantitation of heroin and adulterants. Samples were dissolved in 1.0 mL of internal standard solution (5 mg/mL maleic acid in D_2O, containing 0.05 weight percent 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt [TSP] as chemical shift reference). If insolubles were present after mixing, 1 mL of D_2O without internal standard or chemical shift reference was added, and sonication was performed for 15 min, followed by mixing and filtration. The sample concentrations were between 10 and 30 mg/mL.

Apparatus and Operating Conditions—A Varian Mercury 400 MHz NMR (Varian NMR Systems, Palo Alto, CA) was used for the NMR experiments. A Nalorac 5 mm indirect detection probe with PulseTune (Varian NMR Systems) was used with the following settings: 2 min delay for thermal equilibration followed by automatic proton probe tuning, solvent lock, deuterium gradient shimming. Eight scans were acquired using a pulse width of 5 μsec (90° pulse), spectral width of 6410 Hz (− 3 to 13 ppm), oversampling of 4 with inline digital signal processing, 45 sec between scans, 64,000 data points (acquisition time of 5 sec), unweighted Fourier transform with two times zero filling, phase, drift, and baseline correction. The regions from 3.2 to 5.5 and 0 to 10 ppm were examined for sugar peaks and acidic adulterants, respectively.

Results and Discussion

In order to analyze carbohydrates in heroin samples, the utility of the Agilent Basic Anion Buffer was investigated. Although an excellent separation was obtained for the five carbohydrates of interest (dextrose, lactose, sucrose, mannitol, and inositol), the mannitol and inositol peak heights significantly decreased during multiple injections. Replacing the glass vials recommended by the manufacturer (18) with plastic vials for the run buffer resulted in reproducible separations for the carbohydrates. The reason for this phenomenon is not clear.

As the Basic Anion Buffer is capable of also separating inorganic and organic anions present in heroin samples, the separation of these solutes in relation to the carbohydrates was investigated. The pH was found to be a critical parameter effecting the separation. Using the Agilent Basic Anion Buffer as received (pH 11.9), results in bromide and chloride, tartrate and phosphate, and

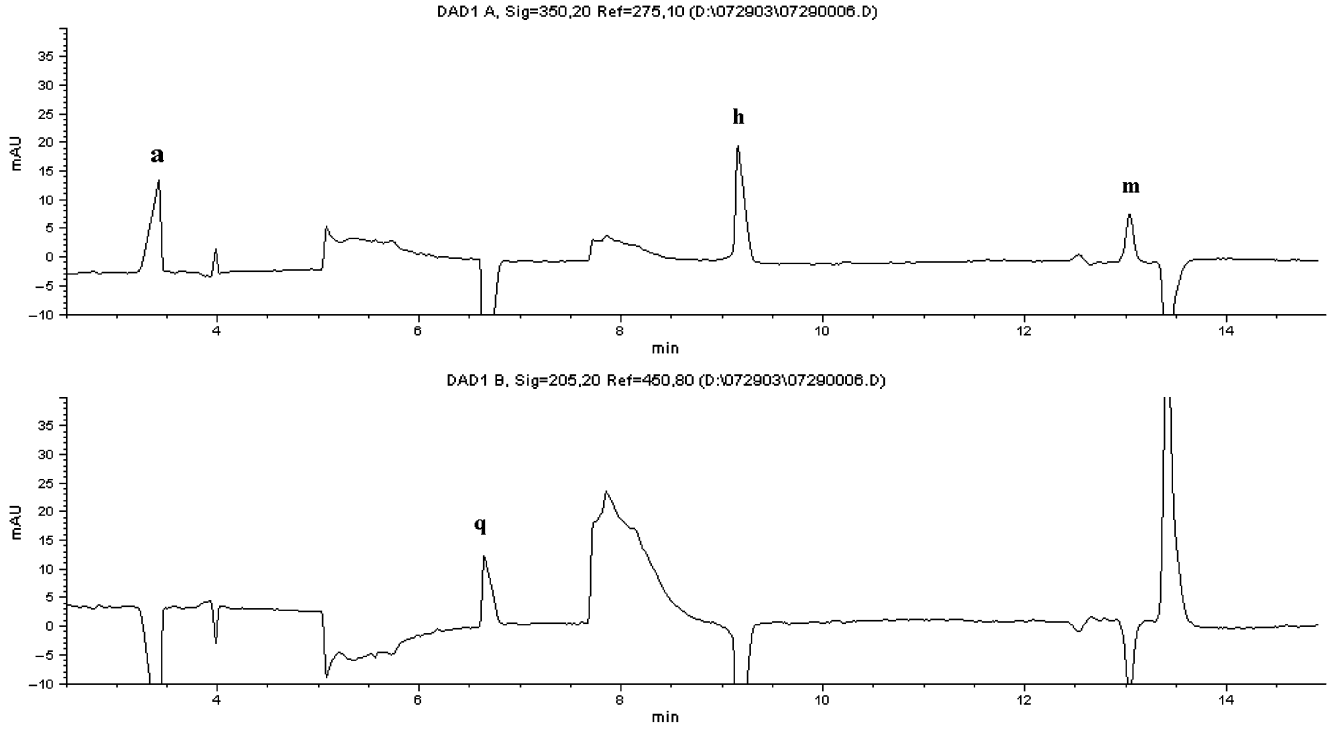

FIG. 4—*Capillary zone electrophoresis of a seized heroin sample. Experimental conditions are described in capillary electrophoresis analysis section. See Fig. 1 for compound identity.*

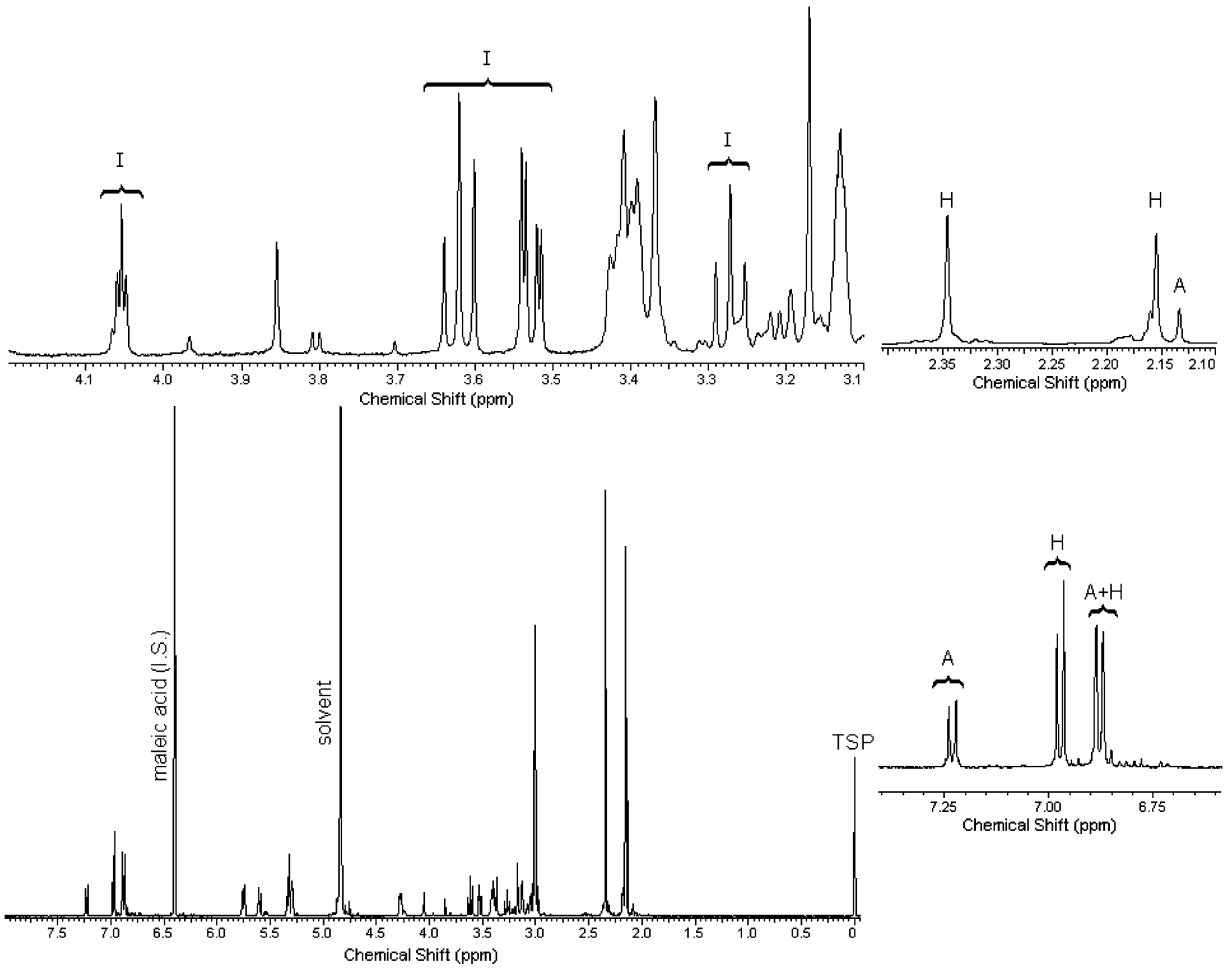

FIG. 5—*Nuclear magnetic resonance proton spectrum of a seized heroin sample with insets showing the major peaks of heroin (H), acetaminophen (A), and inositol (I). D_2O was used as solvent, 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt as 0 ppm reference, and maleic acid as internal standard. Other peaks are due to heroin and other alkaloids.*

phenobarbital and theophylline co-migrating, while sucrose is not adequately resolved from caffeine. At a pH of 12.1, (50 mL reagent + 250 μL 1 N sodium hydroxide) bromide and chloride as well as tartrate and phosphate co-migrate. Finally at pH 12.2, (50 mL reagent+500 μL 1 N sodium hydroxide) bromide and chloride, tartrate and phosphate, as well as caffeine and mannitol, co-migrate. The best overall separation of carbohydrates in the presence of inorganic and organic anions and acidic adulterants was obtained at pH 12.1 and is shown in Fig. 1. The inorganic and organic anions, as well as carbohydrates, are detected by indirect detection using 2,6-pyridinedicarboxylic acid as the chromophore, while the acidic adulterants are detected by direct UV detection. The repeatability and sensitivity of the methodology is presented in Table 1. Excellent migration time precision, and for the most part, good peak area precision is obtained for the carbohydrates as well as the inorganic and organic anions and acidic adulterants. The lower peak area precision for aspirin and acetaminophen is still acceptable for qualitative analysis. With fructose as a reference compound, relative migration times gave only improved precision for nearby migrating solutes such as lactose and sucrose. Also, with fructose as a reference compound, the relative corrected area gave an overall improvement in precision over the corrected area.

The use of the reference compound is also beneficial if excessive electroosmotic flow (EOF) changes occur due to capillary fouling or ambient temperature changes. Most solutes, except for aspirin and salicylic acid, have detection limits <100 μg/mL, which allow for the screening at levels down to 1% w/w. The use of direct detection over indirect detection is preferred for the acidic adulterants due to the greater specificity of detection of the former mode. Owing to the relatively poor detection limits of aspirin using direct detection, indirect detection is preferred for this solute. Although the methodology is used for qualitative purposes, linearity of response is required, especially at the lower limits of detection, for reliable screening. For successive 1/5 dilutions of the standard mixture, linear responses are obtained for both indirect and direct detection as shown in Figs. 2 and 3.

Comparable reproducibility to the analysis of standards as was obtained for samples. For three sets of five consecutive injections of samples, the average %RSD (relative standard deviation) for migration time and corrected area for the fructose internal standard was 0.46 and 2.0, respectively. CE was compared to NMR for the analysis of carbohydrates and acidic adulterants in seized heroin samples. For 19 heroin samples, excellent agreement for the identification of the above solutes was obtained using CE and NMR (see Table 2). The CE analysis and NMR analysis of one of these samples are shown in Figs. 4 and 5, respectively. For one of the samples, the presence of acetylated sugar obstructed NMR confirmation. The CE migration times and relative migration times were used for the determination of the presence of carbo-

hydrates, while migration times and UV spectra were used for the determination of the presence of acidic adulterants.

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