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Analysis of Heroin Drug Seizures by Micellar Electrokinetic Capillary Chromatography (MECC)

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ABSTRACT: A rapid procedure using Micellar Electrokinetic Capillary Chromatography (MECC) is presented for the quantitation of illicit heroin samples. This analytical system resolves heroin from accompanying impurities and adulterants enabling accurate quantitation via the use of an internal standard. An aqueous run buffer consisting of 40 mmol sodium dodecyl sulfate, 8.5 mmol sodium phosphate, 8.5 mmol sodium borate and 15% acetonitrile is used with a 27 cm × 50 μm fused silica capillary column. Linearity, accuracy and reproducibility studies of heroin using this method are established. Comparisons to a commonly used gas chromatographic method show excellent correlation. Due to its high resolution and speed, this MECC system also serves as a screening procedure to detect impurities and adulterants present in heroin samples. Relative migration times of various opiates and adulterants are reported. With minor exceptions, complete separation of numerous compounds is achieved within five minutes, including compounds that are difficult to analyze by gas chromatography such as morphine, O^6-acetylmorphine, aspirin and salicylic acid.

KEYWORDS: forensic science, MECC, capillary electrophoresis, CE, quantitation, heroin, screening

Gas chromatography (GC) and high performance liquid chromatography (HPLC) [*1,2*] are common chromatographic techniques employed for the analysis of illicit heroin exhibits. Both of these methods have their own specific advantages and disadvantages. Capillary GC allows for fast run times and high resolution, however, derivatization is required for the analysis of certain impurities such as morphine and O^6-acetylmorphine and adulterants such as aspirin and salicylic acid. HPLC does not require derivatization, but compared to GC, lacks resolution and speed. In addition, HPLC requires copious amounts of solvent leading to high acquisition and disposal costs. MECC, a mode of capillary electrophoresis, does not require derivatization and produces low flow rates. Several MECC applications have been published in the biological [*3–7*], environmental [*8–10*], and forensic [*11,12*] sectors. Also, three comprehensive reviews [*13–15*] and a book [*16*] have been devoted entirely to MECC.

MECC incorporates a micelle in the run buffer allowing for the separation of both charged and neutral compounds in a single run. Compounds are separated by a combination of electrophoresis and chromatography. In this electroosmotically driven system, solutes separate by electrophoresis and by partitioning differently within the micellar pseudophase. In a separation not optimized for speed, Weinberger and Lurie [*17*] reported taking less than fourteen minutes to resolve nine compounds present in a heroin exhibit using sodium dodecyl sulfate (SDS). However, no quantitative studies were performed. As reported by Trenerry et al. [*18*], most of these compounds could be separated within a similar run time using the positive micelle cetyltrimethylammonium bromide (CTAB), which offers a different selectivity and order of elution than obtained using SDS. The method was shown to be applicable for the quantitation of heroin and certain impurities.

The following method uses MECC and SDS for both the qualitative and quantitative analyses of heroin exhibits. Impurities and adulterants found in heroin samples can be determined in less than 5 minutes.

Materials and Methods

Chemicals and Reagents

The heroin standards (99% + purity) were obtained from the Drug Enforcement Administration (DEA, Special Testing and Research Laboratory, McLean, VA). N-propyl-p-hydroxybenzoate (NPPB) from K&K Laboratories was the internal standard. The run buffer contained sodium borate, sodium phosphate, and sodium dodecyl sulfate (SDS) from Sigma Chemical and Aldrich Chemical. HPLC-Grade water and acetonitrile were used. The impurities and adulterants found in Table 1 were obtained from the following sources: DEA Special Testing and Research Laboratory, Mallinckrodt, Aldrich, Sigma, P&B, K&K, and Supro.

Procedure

MECC was performed with a Beckman P/ACE System 2100 capillary electropherometer equipped with a Deuterium lamp and a detector operated at 214 nm. An uncoated fused silica capillary column, 27 cm × 50 μm, with the detector window 7.0 cm from the outlet end was used. The system was run at 30°C with an applied voltage of 20 kilovolts (740 v/cm). All data were processed by Beckman System Gold software, Version 7.1.

The run buffer consisted of 85:15 (water:acetonitrile, v/v) con-

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TABLE 1—*Migration times relative to heroin for various adulterants (a) and impurities (i) found in illicit heroin samples.*

Impurities and Adulterants	Relative Migration Time	Impurities and Adulterants	Relative Migration Time
Isonicotinamide (a)	0.50	Aspirin (a)	0.88
Nicotinamide (a)	0.50	NPPB (Int Std)	0.94
Phenacetin (a)	0.51	Procaine (a)	0.97
Acetaminophen (a)	0.53	Heroin	1.00
Caffeine (a)	0.60	Acetylcodeine (i)	1.09
Morphine (i)	0.63	Lidocaine (a)	1.12
Hydromorphone (a)	0.66	Salicylic acid (a)	1.13
O^6-Acetylmorphine (i)	0.74	Papaverine (i)	1.15
Phenylpropanolamine (a)	0.75	Thebaine (i)	1.18
Codeine (i)	0.79	Cocaine (a)	1.40
Methaqualone (a)	0.82	Noscapine (i)	1.47
Phenobarbital (a)	0.83	Quinine (a)	1.62
Strychnine (a)	0.87	Diphenhydramine (a)	2.31

taining final concentrations of 40 mmol SDS, 8.5 mmol sodium borate, and 8.5 mmol sodium phosphate, pH 8.5.

Samples and standards were diluted with run buffer containing 0.4 mg/mL NPPB. Each was sonicated and filtered prior to injection. Unless noted otherwise, MECC runs consisted of a single one second high-pressure injection, a five minute run time and a two minute high-pressure rinse with run buffer. The run buffer was changed after every five injections.

Results and Discussion

Migration times in MECC depend on the capacity of the system, which is determined by the micelle partition coefficient and the stationary to mobile phase ratio. By reducing the SDS concentration, the volume of stationary phase decreases, creating faster migration times. Coupling this effect with a shorter capillary produces a system that performs the heroin separation in under five minutes as opposed to the fourteen minute runs reported by Weinberger and Lurie's [*17*]. Illicit heroin samples routinely contain a variety of adulterants and impurities. Migration times relative to heroin for common impurities and common adulterants are shown in Table 1. The compounds were diluted to 0.5 mg/mL and were analyzed for six minutes followed by a two minute rinse. Figure 1 is an electropherogram that illustrates the separation obtained for a heroin sample containing at least thirteen components.

Different run times and flush schemes were examined over fifteen injections for an illicit heroin sample diluted to 0.20 mg/mL. Three and five minute runs having no rinses were followed by three minute runs having 30 second and 2 minute rinses. The resulting data in Table 2 shows that the analyses with rinses offered better reproducibility with the best results obtained with 2 minute rinses. The rinses serve to wash off retained components which could alter the capillary surface. In addition, rinsing prevents ion depletion in the capillary itself. This phenomenon, which results from electrolysis at the electrodes, could yield pH changes in the run buffer and thus effect the electroosmotic flow, the main driving force in MECC. The small difference between 3 and 5 minute runs indicates that continuing the run for two minutes after all the peaks elute has little effect on the reproducibility. One of the major advantages of MECC is the ability to stop a run after the peak of interest elutes and to start the next run within two minutes. By rinsing, late eluting peaks are eliminated and initial run conditions are reestablished. For all the experiments, fresh run buffer was

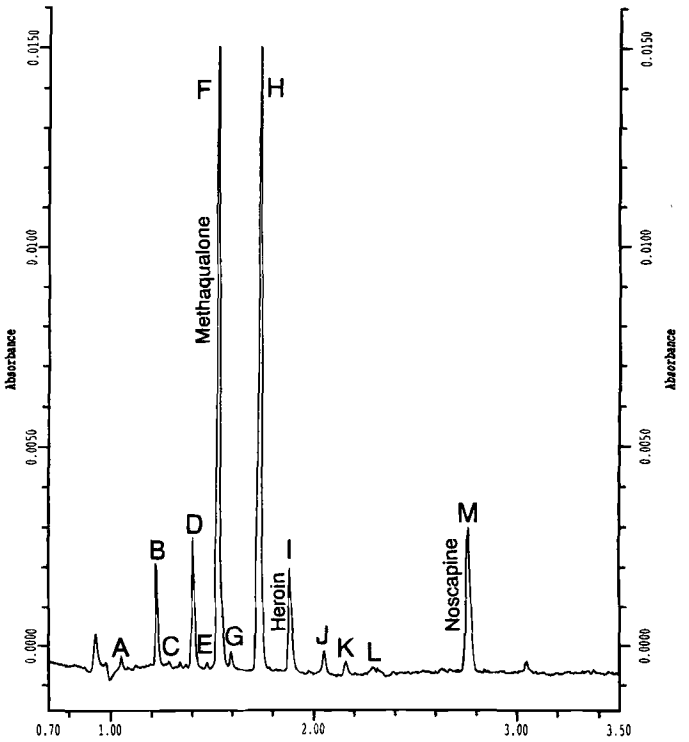

FIG. 1—*An electropherogram of an illicit heroin sample in which the following components have been qualitatively identified. A: Phenacetin, B: Caffeine, C: Morphine, D: O^6-Acetylmorphine, E: Codeine, F: Methaqualone, G: Phenobarbital, H: NPPB (internal standard), I: Heroin, J: Acetylcodeine, K: Papaverine, L: Thebaine, M: Noscapine.*

TABLE 2—*Reproducibility of heroin peak area to internal standard peak area in an illicit heroin sample with varying run conditions.*

Run Time (minutes)	Rinse Time (minute)	RSD[a] of Area Ratio (%)
3	0	3.1
5	0	2.7
3	0.5	2.0
3	2	1.2

[a]RSD = Relative Standard Deviation.

used after every 5 injections. This is recommended to prevent ion depletion in the run buffer reservoirs [*19*]. Occasionally, a dozen injections were made from the same run buffer without observing any noticeable effects to the chromatography. However, to ensure accurate quantitative results, the run buffer was changed after every five injections.

Linearity studies were conducted for heroin hydrochloride, heroin base, heroin citrate, heroin tartrate, acetylcodeine and O^6-acetylmorphine at concentrations ranging from 0.05–2.00 mg/mL. Analyzing the ratio of these compounds' peak areas to NPPB peak areas all produced correlation coefficients of 0.999 or greater. Linearity beyond 2.00 mg/mL was not examined.

Reproducibility of standard heroin hydrochloride migration times and peak area ratios was examined at 0.05, 0.10, 0.30, 0.40 and 0.50 mg/mL, correlating to normal working concentrations. Fifteen injections of each were made utilizing three minute run

TABLE 3—*Reproducibility of migration times and peak areas for heroin hydrochloride standards*

Standard Concentration (mg/mL)	RSD of Absolute Migration Time (%)	Standard Deviation of Area Ratio[a]	RSD of Area Ratio[a] (%)
0.05	0.69	0.00214	2.4
0.10	0.72	0.00432	2.2
0.30	0.60	0.00767	1.5
0.40	0.54	0.00697	0.99
0.50	0.58	0.00722	0.82

[a]Peak area ratio determined relative to the internal standard

TABLE 4—*Reproducibility of migration times and peak areas for methagualone, heroin, and noscapine in an illicit heroin sample.*

Peak ID	RSD of Absolute Migration Time	RSD of Migration Time Ratio[a]	RSD of Absolute Peak Area	RSD of Peak Area Ratio[a]
Methaqualone	0.53%	0.26%	2.7%	1.1%
Heroin	0.52%	0.23%	3.2%	1.2%
Noscapine	0.50%	0.27%	2.7%	2.4%

[a]Migration time ratios and peak area ratios were determined relative to the internal standard.

TABLE 5—*Comparison of MECC to GC. GC conditions: HP5880 GC, FID, 12m × 25 μm HP-1 capillary column, oven temp-250°C, injector temp-270°C, detector temp-280°C. Internal standard was tetracosane (0.4 mg/mL). Difference is determined from MECC to GC.*

Sample ID	GC Results (%)	MECC Results (%)	Difference (%)
Known #1	89.1	92.3	3.5
Known #2	62.1	61.2	1.4
Known #3	51.9	50.3	3.1
Known #4	12.3	12.3	0.0
Known #5	10.4	10.1	2.9
Known #6	25.7	24.7	3.9
Known #7	20.4	20.1	1.5
Known #8	84.3	81.9	2.8
Known #9	39.2	38.4	2.0
Known #10	40.2	39.2	2.5
Known #11	18.5	18.6	0.5
Known #12	28.5	28.1	1.4
Known #13	24.1	23.8	1.2
Known #14	14.5	14.3	1.4
Illicit #1	25.6	24.9	2.7
Illicit #2	25.9	26.7	3.0
Illicit #3	26.4	26.1	1.1
Illicit #4	26.7	25.8	3.4
Illicit #5	34.2	34.8	1.7
Illicit #6	23.6	23.0	2.5

times and three minute rinses. Table 3 contains the resulting data. As shown earlier, two minute rinses would have been sufficient.

An illicit sample of heroin (0.2 mg/mL) containing methaqualone and noscapine (see Fig. 1) was used to further validate the reproducibility of the methodology. Table 4 shows reproducibility data of these compounds' migration times and peak areas. For both cases, using an internal standard improves the reproducibility of the data.

Fourteen heroin mixtures were prepared at various concentrations and with different adulterants to mimic illicit samples. Each known sample was ground via mortar and pestle to insure homogeneity. Approximately 50 milligrams of each was diluted to 0.30 mg/mL. Six street samples were also prepared at similar concentrations. These known and unknown samples were quantitated by both MECC and GC. As can be seen in Table 5, the values obtained from MECC and GC are similar. The run times for the two methods were similar, however, resolution for all adulterants was better with MECC.

Conclusion

Micellar electrokinetic capillary chromatography is an analytical technique which eliminates some of the disadvantages encountered with GC and HPLC. MECC quantitates heroin while enabling screening of adulterants and impurities in less than five minutes. Coupling the above system with a diode array or rapid scanning UV detector would further enhance specificity of detection.

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