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Review

Application of micellar electrokinetic capillary chromatography to the analysis of illicit drug seizures

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Abstract

The application of micellar electrokinetic capillary chromatography (MECC) to the analysis of illicit drug seizures is presented. Areas investigated include general screening and qualitative and, in some instances, quantitative analysis of various drugs, including heroin, opium, cocaine, amphetamines, LSD and anabolic steroids. Due to its high efficiency, high selectivity and general applicability, MECC is well suited for forensic drug analyses. © 1997 Elsevier Science B.V.

Keywords: Reviews; Opium; Forensic analysis; Heroin; Cocaine; Amphetamines; Steroids; Alkaloids; Lysergic acid diethylamide

Contents

1. Introduction

The analysis of illicit drugs seized or purchased undercover by law enforcement authorities is important for legal and intelligence gathering purposes [1]. Comprehensive analysis of drug exhibits involves qualitative and, in some instances, quantitative analyses of both the controlled substance(s) and also any adulterants, diluents, by-products and/or impurities present in the sample. Capillary gas chromatography (cGC) [2] and high-performance liquid chromatography (HPLC) [3] are the most widely used separation techniques for these analyses. However, although GC affords high resolving power,

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good precision and accuracy, problems can arise for drug substances that are thermally degradable, polar or non-volatile. For example, lysergic acid diethylamide (LSD), psilocybin and certain anabolic steroids are thermally labile. In addition, phenethylamines, such as a amphetamine and methamphetamine, are polar solutes that exhibit poor chromatographic performance when analyzed in their salt forms; in these cases, prior conversion to the respective free bases is required. Other non-volatile adulterants and diluents (e.g., sugars) can be analyzed by GC only after prior derivatization.

HPLC, although applicable to solutes that are thermally degradable, polar or non-volatile, is a relatively low resolution technique compared with GC. This mandates slower analysis times for HPLC versus GC for many solutes. In addition, HPLC is less amenable to drug profiling, where high resolution is required. Finally, HPLC involves degassing and filtering of solvents and priming of pumps and, in general, it has more cumbersome set-up techniques than GC.

In contrast to these techniques, capillary electrophoresis (CE) is both amenable to the analysis of thermally degradable, polar or non-volatile solutes and it is also a high resolution technique. In addition, it has relatively easy set-up procedures and greater economy of operation compared to GC and HPLC. For these reasons, CE has generated much interest in the area of the analysis of illicit drugs [4].

Many exhibits require the analysis of neutral solutes or solutes with similar charge-to-size ratios. Such exhibits require more specialized CE techniques employing pseudo-stationary phases, such as micellar electrokinetic chromatography (MECC). Since all solutes must elute at or before the time of the micelle (t_{mc}), MECC rivals temperature programming GC and gradient elution HPLC in its versatility. In this vein, the utility of MECC for the analysis of various controlled substances is described. MECC applications presented in this article (including experimental parameters) are given in Table 1.

2. Applicability to general forensic analysis

MECC has been applied to a wide range of controlled substances, including heroin, cocaine, opium alkaloids, amphetamines, hallucinogens, barbiturates, benzodiazepines and cannabinoids [5]. For example, an electropherogram of a complex mixture of eighteen different drugs is shown in Fig. 1 (migration times relative to heroin are presented in Table 2). Notably, each solute exhibited excellent peak shape, with no apparent breakdown in the run buffer.

It is clear from these data that MECC represents an excellent technique for drug screening. As noted above, this is a consequence of the fact that all compounds must elute at or before the t_{mc} in MECC. Due to the use of an organic modifier in this run, it was not possible to obtain a suitable t_{mc} marker; however, as is clear in Fig. 1 (and Table 2), this system is viable even for the highly hydrophobic cannabinoids. The use of lower micelle concentrations, more polar micelles (e.g., bile acids) and shorter capillaries would allow even more rapid screening.

Although not employed in this study, photodiode-array UV (PDA–UV) detection greatly increases specificity and discrimination of analysis. In addition, automatic library searches can be performed (similar to PDA–UV data obtained via HPLC).

On the surface, MECC is analogous to temperature programming GC and gradient elution HPLC for drug screening. However, temperature programming GC has greater peak capacity than MECC, because the latter technique is limited by the ratio of t_{mc}/t_0. However, MECC has a greater peak capacity than gradient elution HPLC, due to its higher efficiency. In addition, the peak capacity of CE can be greatly expanded if gradient elution is used in combination with MECC, as was performed by Sepaniak et al. [6].

Greater specificity of screening could be obtained by using two complementary separation techniques, e.g., MECC with either GC or HPLC. These three techniques have been previously shown to be orthogonal for the screening of anabolic steroids [7] (see below). Tagliaro et al. [8] have also demonstrated the complementary nature of MECC and capillary zone electrophoresis (CZE) for the identification of seventeen illicit drugs and related compounds that are ionized at pH 2.35; MECC with sodium dodecyl sulfate (SDS) at pH 9.2 gave a highly non-correlated separation compared to that obtained on a CZE

Table 1
Review of methodology for the MECC analysis of illicit drug seizures

Applications	Micelle	Additive	Buffer	Refs.
General analysis	85 m*M* SDS	15% Acetonitrile	8.5 m*M* Phosphate, 8.5 m*M* borate, pH 8.5	[5]
	100 m*M* SDS	20% Methanol	20 m*M* Borate, pH 9.24	[8]
Heroin	85 m*M* SDS	15% Acetonitrile	8.5 m*M* Phosphate, 8.5 m*M* borate, pH 8.5	[5]
	40 m*M* SDS	15% Acetonitrile	8.5 m*M* Phosphate, 8.5 m*M* borate, pH 8.5	[9]
	23.8 m*M* SDS	5% Acetonitrile	9.5 m*M* Phosphate, 9.5 m*M* borate, pH 9.0	[10]
	25 m*M* SDS	10% Methanol	10 m*M* Boric acid pH 9.18	[11]
	50 m*M* SDS		50 m*M* Glycine, pH 10.5	[12]
	45 m*M* CTAB	10% Acetonitrile	9.0 m*M* Potassium dihydrogen phosphate, 9.0 m*M* sodium tetraborate, pH 8.6	[13]
Heroin (acidic and neutral impurities)	45 m*M* SDS	6.9 m*M* β-CD SBE IV, 10% acetonitrile	9.0 m*M* Phosphate, 9.0 m*M* borate, pH 9.0	[17]
Opium	45 m*M* SDS	6.9 m*M* β-CD SBE, IV, 10% acetonitrile	9.0 m*M* Phosphate, 9.0 m*M* borate, pH 9.0	
	45 m*M* CTAB	10% Dimethyl-formamide	9.0 m*M* Potassium dihydrogen phosphate, 9.0 m*M* sodium tetraborate, pH 8.6	[22]
	43.8 m*M* CTAB	12.5% Acetonitrile	8.75 m*M* Potassium dihydrogen phosphate, 9.0 m*M* sodium tetraborate, pH 8.6	[23]
Cocaine	85 m*M* SDS	15% Acetonitrile	8.5 m*M* Phosphate, 8.5 m*M* borate, pH 8.5	[5]
	46.2 m*M* CTAB	7.5% Acetonitrile	9.25 m*M* Potassium dihydrogen phosphate, 9.25 m*M* sodium tetraborate, pH 8.6	[14]
Amphetamines	23.8 m*M* SDS	5% Acetonitrile	9.5 m*M* Phosphate, 9.5 m*M* borate, pH 9.0	[10]
	22 m*M* CTAB	11% Dimethyl-sulfoxide, 1% ethanolamine	8.8 m*M* Sodium tetraborate, pH 11.5	[15]
	22 m*M* CTAB	11% Acetonitrile, 1% ethanolamine	8.8 m*M* Sodium tetraborate, pH 11.5	[23]

(*continued on p. 268*)

Table 1 (*continued*)

Applications	Micelle	Additive	Buffer	Refs.
Amphetamines (enantiomers of GITC derivatives)	80 m*M* SDS	20% Methanol	8.0 m*M* Phosphate, 8.0 m*M* borate, pH 9.0	[24]
LSD	40 m*M* SDS	15% Acetonitrile	8.5 m*M* Phosphate, 8.5 m*M* borate, pH 8.5	[29]
Anabolic steroids	50 m*M* SDS	35% Acetonitrile	20 m*M* Borate, pH 8.15	[30]
	45 m*M* SDS	40% Acetonitrile	6.0 m*M* Phosphate, 6.0 m*M* borate, pH 9.0	[7]

system at pH 2.35. Interestingly, MECC was found to be significantly (but inversely) correlated with a CZE system at pH 9.24. The authors hypothesized that the basic drugs interacted with the micelle via both hydrophobic and hydrophilic interactions in explaining these observations.

The latter interaction occurs due to ion-exchange with the external negatively charged surfaces of the micelles. This effect, in MECC, depends on the charge of the analytes, however, in this case (in contrast to CZE), the positive charge of solutes decreases pair migration velocity because of stronger interactions with the micelles. The approach used by Tagliaro et al. [8] was advantageous in that the same instrumental hardware was used for both experiments. The major limitation of this combination is the screening of neutral solutes and compounds with similar charge-to-size ratios.

The reproducibility of migration times or relative migration times in MECC is of the utmost importance for screening applications. For a test drug mixture that contained acidic, neutral, basic and amphoteric compounds of forensic interest, the chromatographic precision was significantly dependent on the instrumentation used [8]. For example, the short-term imprecision of absolute migration times

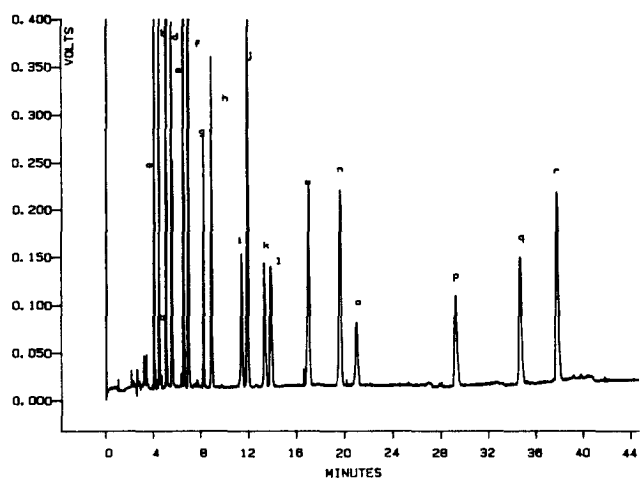


Fig. 1. MECC forensic drug screen. Conditions: capillary, 47 cm (25 cm length to detector)×50 μm I.D.; voltage, 20 kV; temperature, 40°C; buffer, 85 m*M* SDS–8.5 m*M* phosphate–8.5 m*M* borate–15% acetonitrile, pH 8.5; detector wavelength, 210 nm; sample concentration, 250 μg/ml of each drug. Key: (a) psilocybin, (b) morphine, (c) phenobarbital, (d) psilocin, (e) codeine, (f) methaqualone, (g) LSD, (h) heroin, (i) amphetamine, (j) librium, (k) cocaine, (l) methamphetamine, (m) lorazepam, (n) diazepam, (o) fentanyl, (p) PCP, (q) cannabidiol and (r) Δ^9-THC. Reproduced with permission from Ref. [5].

Table 2
Migration times of compounds of forensic interest relative to heroin

Compound	Relative migration time (min)
Benzoylecgonine	0.38
Psilocybin	0.46
Morphine	0.51
Dilaudid	0.54
Phenobarbital	0.58
Psilocin	0.62
O^6-Monoacetylmorphine	0.66
O^3-Monoacetylmorphine	0.72
Codeine	0.74
Methaqualone	0.78
Mescaline	0.92
LSD	0.93
LAMPA	1.00
Heroin	1.00 (8.91 min)
Acetylcodeine	1.14
Papaverine	1.24
MDA	1.27
Amphetamine	1.28
Librium	1.34
Cocaine	1.49
MDMA	1.55
Methamphetamine	1.56
Phentermine	1.56
Noscapine	1.69
cis-Cinnamoylcocaine	1.76
Lorazepam	1.91
Diazepam	2.21
trans-Cinnamoylcocaine	2.24
Fentanyl	2.36
Flurazepam	3.07
PCP	3.28
Pyrene[a]	3.72
Cannabidiol	3.90
Cannabinol	4.25
Δ^0-THC	4.25

[a] Added as a potential t_{mc} marker.
See text for discussion.
Reproduced with permission from Ref. [5].

was high when a non-automated instrument without sophisticated capillary temperature control was used, with R.S.D. values ranging from 2.0 to 8.6%. (However, the short term precision of relative migration times was considerably better, with values between 0.3 to 2.8%). When using an automated CE instrument with sophisticated temperature control, however, improved intra-day R.S.D. values of the relative migration time (in the range of 0.3–1.5%) were obtained. The long-term precision for relative migration times on this same instrument was also good, with values ranging from 0.8 to 2%. R.S.D. values of absolute migration times for both short- and long-term precision were only slightly higher.

3. Heroin

The analysis of heroin exhibits by MECC has been an area of great interest. Illicit heroin is typically a highly complex mixture containing various manufacturing by-products, impurities, adulterants and diluents, and, therefore, it represents a significant separation challenge.

The two most common approaches are to either analyze heroin, basic impurities, basic by-products and adulterants, or to analyze acidic and neutral impurities. cGC [2] and HPLC [3] have both been used for the analysis of basic solutes. HPLC is advantageous for those solutes that exhibit poor chromatographic behavior on GC, such as morphine, O^6-monoacetylmorphine, noscapine, aspirin and salicylic acid, while cGC is preferred for the analysis of acidic and neutral impurities (due to its superior resolving power [2]). However, derivatization is commonly required for cGC. MECC, with its high resolving power, its ability to analyze neutral as well as charged species in a single run and its ability to resolve compounds with similar charge-to-size ratios (e.g., acetylcodeine and O^3- and O^6-monoacetylmorphine), is well suited for the analysis of illicit heroin, assuming adequate sensitivity.

Weinberger and Lurie [5] demonstrated that MECC with an SDS micelle permits the separation of heroin, basic impurities, basic by-products and the adulterants, phenobarbital and methaqualone, in approximately one-third of the analysis time of HPLC, with superior resolution. In a subsequent study, Walker et al. [9] used a shorter capillary and a reduced micelle concentration to resolve a similar mixture of solutes in less than 3 min (see Fig. 2). This latter system was also found useful for the analysis of several additional adulterants (shown in Table 3).

Krogh et al. [10] used a similar system to separate various constituents in illicit heroin in a little over 10 min. It was additionally demonstrated that the addition of an organic modifier improved the resolution,

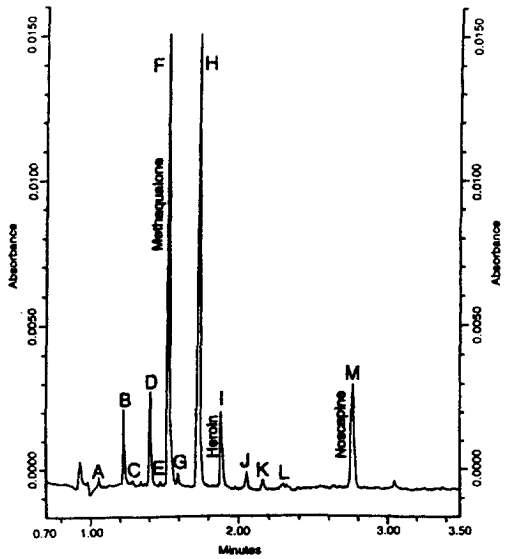


Fig. 2. Electropherogram of an illicit heroin sample. Conditions: capillary, 27 cm (20 cm length to detector)×50 μm I.D.; voltage, 20 kV; temperature, 30°C; buffer, 45 m*M* SDS–8.5 m*M* phosphate–8.5 m*M* borate–15% acetonitrile, pH 8.5; detector wavelength, 214 nm. Key: (A) phenacetin, (B) caffeine, (C) morphine, (D) O^6-monoacetylmorphine, (E) codeine, (F) methaqualone, (G) phenobarbital, (H) NPPB (internal standard), (I) heroin, (J) acetylcodeine, (K) papaverine (L) thebaine and (M) noscapine. Reproduced with permission from Ref. [9].

presumably by decreasing the osmotic flow and altering the partition coefficient. The authors also indicated that pH was an important variable in the optimization of the separation. This is not surprising, since many of the compounds had pK_a values and/or pK_b values in the 7–10 range and, therefore, they exhibited ionization changes in the pH range used in the experiment. Thus, the mobility of solutes in free solution, ion-pairing of cationic solutes with the negative SDS micelles, and ion-repulsion of phenolic groups bearing solutes, all greatly affected the apparent migration of the compounds of interest.

Yu et al. [11] used an SDS system with methanol instead of acetonitrile to separate heroin and related compounds in under 30 min, with similar selectivity.

For the systems above, phosphate–borate buffers were used at pH values between 8.5 and 10. In contrast, Hyotylainen et al. [12] employed a short capillary with a pH 10.5 glycine buffer and SDS to resolve heroin, morphine, codeine, O^6-monoacetylmorphine and caffeine in under 2 min. However, acetylcodeine, a major impurity present in virtually every illicit heroin sample, was not resolved from heroin using this system. It is of interest to note the selectivity changes that occurred using the phosphate–borate buffer compared with the glycine buffer. In contrast to the former system, O^6-mono-

Table 3
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 (I.S.)	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	Salicyclic 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

Reproduced with permission from Ref. [9].

acetylmorphine and codeine eluted after heroin using the pH 10.5 glycine buffer. Hyotylainen et al. [12] tested borate, CAPS and glycine buffers in the pH range of 10–11 and found significant differences in the separation, analysis time and peak shapes. Glycine is particularly useful for short capillaries, because it gives the lowest current.

Trenerry et al. [13] used the positively charged, micelle-forming, cetyltrimethylammonium bromide (CTAB), with acetonitrile as the organic modifier, to separate a mixture of heroin and related compounds in under 15 min (see Fig. 3). Of interest were the selectivity differences obtained using SDS compared with CTAB (compare Figs. 2 and 3). For example, codeine and O^6-monoacetylmorphine switched migration order, and noscapine was significantly better resolved from papaverine with SDS. These selectivity effects could be related to the differences in ion pairing and ion repulsion when switching from a negatively charged to a positively charged micelle.

However, Trenerry et al. [14,15] claimed that SDS systems are unsuitable for repeated analysis. The basis of their charge was a series of twenty repetitive injections they made on an SDS system where the migration times and peak shapes of several heroin-related solutes varied significantly [13]. However, they did not perform routine operations, such as flushing the capillary with run buffer between runs, replenishing run buffer after several runs, or capping the vials to prevent solvent evaporation. As the authors themselves pointed out, performing the above operations restored the original separation. In addition, after performing these steps, an additional wash with 0.1 *M* sodium hydroxide after 24 h of use maintained the quality of the separation. Twenty repetitive injections were made for a standard mixture of compounds in seized heroin using CTAB, and little variation in migration times were noted by the authors; however, they did not present any details of experimental results. For this study, pholcodine was used as an internal standard, and the capillary was flushed with run buffer for 2 min between runs. The capillary was also cleaned on a weekly basis, using 0.1 *M* HCl for 10 min, followed by deionized water, 0.1 *M* sodium hydroxide, and deionized water.

Walker et al. [9] obtained percentage R.S.D. values of approximately 0.5% for the migration times of heroin, methaqualone and noscapine for fifteen injections of an illicit heroin sample on an SDS system. For the same experiments, R.S.D. values for migration times of less than 0.28% were obtained [relative to the internal standard, N-propyl-*p*-hydroxybenzoate (NPPB)]. The capillary was rinsed with run buffer for 2 min between runs, fresh run buffer was used after every five injections, and stoppered buffer vials were used.

Krogh et al. [10] used an SDS system to analyze a test mixture of heroin and related compounds, and

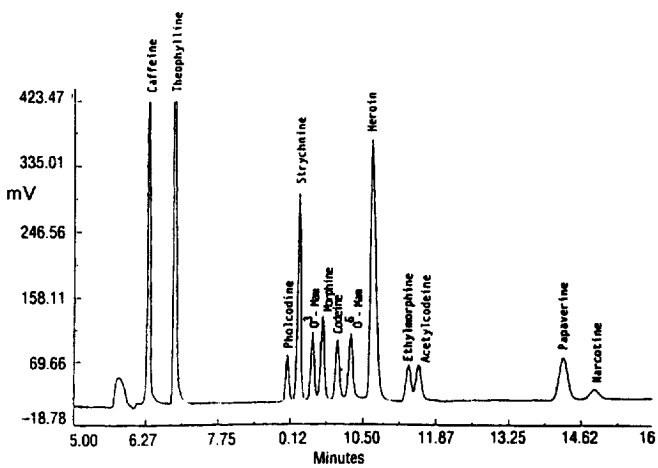


Fig. 3. Separation of heroin and related substances using a mixture of 10% acetonitrile and 90% of a buffer consisting of 0.05 *M* CTAB, 0.01 *M* potassium dihydrogen phosphate and 0.01 *M* sodium tetraborate, pH 8.6. UV detection at 280 nm. Reproduced with permission from Ref. [13].

obtained within-day R.S.D. values in the range of 2.5–4.0% and between-day R.S.D. values of 3.5–6.0% for absolute migration times. For relative migration times (relative to the internal standard, crystal violet), considerably better short term and long term precision values were obtained, i.e., R.S.D. values of 0.5–1.9 and 0.89–2.23%, respectively. These results were obtained despite using an instrument that provided no capillary temperature control. The significantly lower R.S.D. (%) values obtained by Walker et al. [9] for short term precision can be attributed to better temperature control. In general, the capillary was replaced after 500 analyses to maintain reproducibility.

Hyotylainen et al. [12] used an SDS run buffer and an instrument providing capillary temperature control to obtain excellent short-term precision for migration times; R.S.D. (%) values for heroin and related impurities and by-products were in the 0.33–0.50% range. Significantly lower R.S.D. values, in the range of 0.004–0.21%, were obtained using two carboxylic acids as migration indexes.

MECC using SDS or CTAB has also been shown to be viable for the quantitative analysis of seized heroin samples. Walker et al. [9] compared MECC with GC for the analysis of six illicit samples and fourteen synthetic heroin mixtures; internal standard methodology was used with previously described standards. Excellent agreement for heroin content (range 10–90%) was obtained between the two techniques, with differences of 3.9% or less. Krogh et al. [10] analyzed ten heroin seizures with heroin concentrations in the 12–51% range, and obtained R.S.D. values of between 2.0 and 4.3%. However, the methodologies presented were not viable for the simultaneous, quantitative determination of morphine, codeine, O^6-monoacetylmorphine, acetylcodeine, noscapine and papaverine. This is despite the fact that linearity has been demonstrated for most of these compounds down to (approximately) the 0.02 mg/ml level. This is not adequate for actual analyses, since these solutes are commonly encountered at levels of 5% or less, relative to heroin. Thus, starting concentrations considerably higher than 0.3 mg/ml of heroin or 0.5 mg/ml of sample would be required.

Trenerry et al. [13] used considerably higher sample concentrations (1.0–5.0 mg/ml) to demonstrate the viability of MECC for the simultaneous quantitation of heroin, O^6-monoacetylmorphine and acetylcodeine. As shown in Table 4, good agreement was found between MECC and HPLC for the determination of heroin and basic by-products and impurities in illicit samples. In addition, good precision was obtained via CE (albeit generally worse than HPLC) for solutes present at or above the 2% level. Better precision would have been obtained using UV detection at a more favorable wavelength (e.g., 210 nm). This would have required substituting the bromide counter-ion in CTAB with a lower UV cut-off counter-ion (e.g., chloride). This might also have allowed the determination of solutes such as morphine, codeine and noscapine, which can be present at levels below 1% relative to heroin. In addition, injection techniques, such as stacking or the use of bubble cell or Z cell technology, could also improve detection limits.

All the heroin analyses reported above were performed using single wavelength UV detection. The use of multiwavelength PDA–UV or rapid-scan UV detection would significantly improve specificity and/or the sensitivity of detection (as has been shown for HPLC-based heroin analyses [16]). In addition, confirmation of peak identity and purity by spectral analysis would help ensure the accuracy of quantitation. Library searches could be performed to screen for the scores of possible adulterants or diluents typically present in illicit heroin [16]. Commercial instrumentation is available to perform these protocols. Preliminary work in this laboratory with PDA–UV detection for heroin appears promising. In the best cases, useable UV spectra with as little as 1 milli absorbance unit full scale were obtained.

The utility of MECC for the analysis of acidic and neutral impurities in heroin is best illustrated by comparing an optimized HPLC separation with a "garden variety" MECC separation of the same solutes [5]. As shown in Figs. 4 and 5, an MECC system using SDS resolved approximately twice as many peaks as a four-solvent gradient HPLC system. However, an organic modifier was required to achieve more favorable partition coefficients between the hydrophobic heroin impurities and the SDS micelle. In the absence of an organic modifier, most of the peaks eluted at or near the probable position of t_{mc}. Acetonitrile was used since it gave a better peak distribution than that of methanol or tetrahydrofuran.

The separation illustrated in Fig. 5 was sub-

Table 4
Comparison of CZE and HPLC quantitation for several heroin seizures[a]

Sample	Morphine (%)		O^6-MAM[b] (%)		DAM[b] (%)		AC[b] (%)	
	CZE	HPLC	CZE	HPLC	CZE	HPLC	CZE	HPLC
91/267	Absent		2.2	1.9	74.6	76.1	6.5	6.5
C.V. (%)[b]			4.1	2.1	0.7	1.6	2.8	1.3
1-52	2.8	3.0	24.6	26.2	43.0	43.9	3.3	3.7
C.V. (%)[b]			1.9	0.1	2.3	0.2	3.6	0.3
1-68	Absent		1.3	0.6	86.7	86.6	4.0	4.4
C.V. (%)			3.1	11.7	0.9	1.0	2.3	1.1
1-65	Absent		3.3	2.8	78.7	81.9	4.7	5.0
C.V. (%)			3.0	1.4	1.7	0.4	3.4	0.8
85/1141	Absent		3.5	3.2	73.4	73.1	8.5	8.5
C.V. (%)			1.7	3.8	0.5	1.4	1.1	1.1
85/1219	Absent		0.9	0.7	36.7	33.2	4.0	3.8
C.V. (%)			6.7	7.1	0.7	0.8	3.3	1.3
85/1222	Absent		1.0	1.1	13.7	13.8	2.2	2.2
C.V. (%)			5.0	4.5	2.6	3.1	3.6	4.5
85/10218	Absent		0.2	0.3	26.1	27.1	2.7	2.7
C.V. (%)			10.0	16.6	1.3	3.1	3.0	2.2
85/10220	Absent		0.5	0.6	27.3	26.8	2.9	2.8
C.V. (%)			10.0	5.0	0.8	0.6	4.1	1.4
S91/274	Absent		2.3	2.0	75.9	78.7	6.6	6.8
C.V. (%)			4.8	1.5	1.3	0.4	1.7	0.9

[a] The internal standard was pholcodine.
Average of nine determinations for each sample.
[b] Abbreviations: coefficient of variation, C.V.; O^6-MAM, O^6-monoacetylmorphine: DAM, diacetylmorphine (heroin) and AC, acetylcodeine.
Reproduced with permission from Ref. [13].

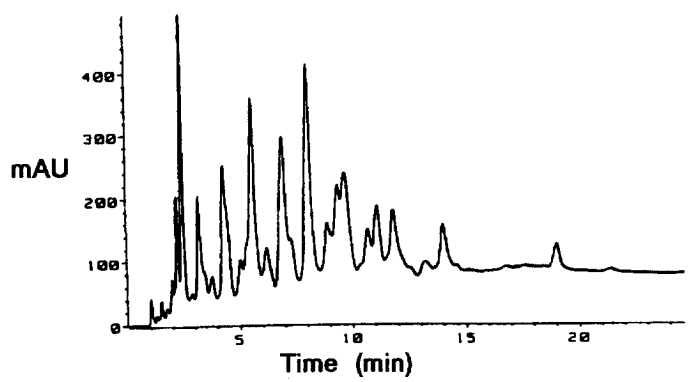


Fig. 4. HPLC separation of acidic and neutral impurities in illicit heroin samples. Conditions: injection size, 50 μl; column, 11.0 cm×4.7 mm Partisil 5-ODS-3; phosphate buffer (pH 2.2). Initial conditions: 13% methanol, 8.9% acetonitrile, 6.7% tetrahydrofuran (THF) and 71.1% phosphate buffer. Final conditions: 21.7% methanol, 14.5% acetonitrile, 10.8% THF and 53% phosphate buffer; gradient, 15 min linear gradient, hold for 5 min at final conditions; flow-rate, 1.5 ml/min; detector wavelength, 210 nm. Reproduced with permission from Ref. [5].

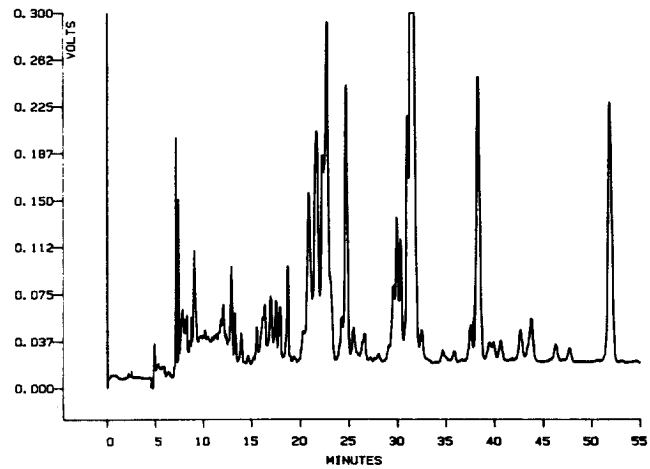


Fig. 5. MECC separation of acidic and neutral heroin impurities. Conditions are the same as in Fig. 1, except that the length to the detector is 50 cm, the voltage is 30 kV and the temperature is 50°C. Reproduced with permission from Ref. [5].

sequently improved by varying the experimental parameters [17]; separations carried out at 30°C (instead of 50°C) minimized degradative reactions such as hydrolysis. As a compromise between speed and resolution, a lower SDS concentration was used. As shown in Fig. 6, the addition of a neutral or charged cyclodextrin (CD) to the run buffer resulted in different migration times and increased resolution. Furthermore, the addition of a neutral CD (β-cyclodextrin) to a run buffer containing SDS resulted in reduced migration times and selectivity changes (cf. Fig. 6a–b); this was consistent with the findings of Nishi et al. [18]. The addition of a negatively charged CD [β-cyclodextrin sulfobutyl ether IV (β-CD SBE IV)] resulted in increased migration times, especially for the more hydrophobic (later-eluting) compounds (cf. Fig. 6a and c). Although similar selectivity changes occurred with either CD, there were significant increases in resolution with the negatively charged CD, especially for the later-eluting solutes. The selected concentration of 7.5 m*M* β-CD SBE IV was a compromise between speed of

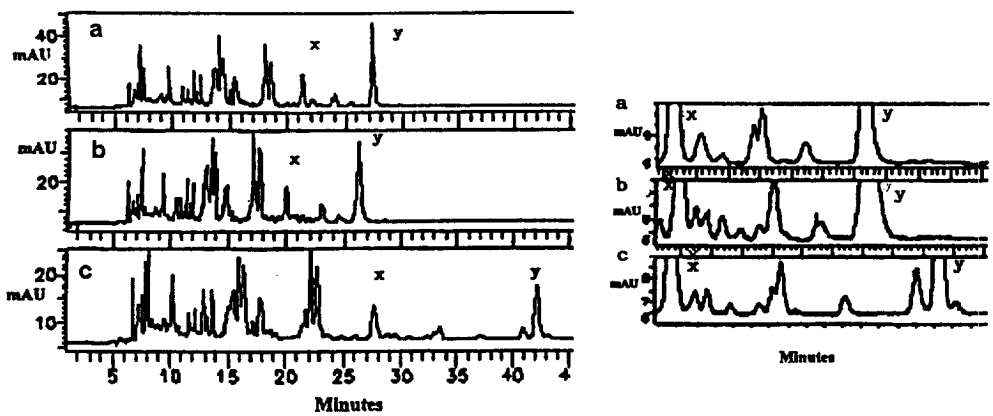


Fig. 6. Effect of CD on the separation of an acidic extract of a Southwest Asian heroin base sample: (a) 42.5 m*M* SDS, (b) 42.5 m*M* SDS+6.5 m*M* β-CD, (c) 42.5 m*M* SDS+β-CD SBE IV. A 78-cm (56 cm to the detector)×50 μm capillary at 30°C was used with a voltage of 30 kV. In addition to SDS and CD, the run buffer contained 8.5 m*M* phosphate and 8.5 m*M* borate at pH 9.0 plus 15% acetonitrile. UV detection was at 210 nm. Reproduced with permission from Ref. [17].

analysis and resolution. It is of interest to note that the migration time window t_{mc}/t_0 appears to significantly increase with the addition of the negatively charged CD. Although it is difficult to measure t_{mc} in a MECC system containing organic modifiers and a charged CD, peak y in Fig. 6 would appear to be a reasonable guess for this parameter. In this instance, t_{mc} would represent a combination of the time of the micelle and the time of the charged CD, where β-CD SBE IV is a polydispersive entity with an average degree of substitution of four. The increased peak capacity is a result of both strong inclusion complexes with the CD and the high counter-mobility of β-CD SBE IV (which has an average charge of −4.) As shown in Fig. 7, a further improvement in the separation is obtained by lowering the amount of acetonitrile in the run buffer. This occurs because the decrease in the organic modifier increases the k' values to a range where the charged CD has a greater effect. The structures of various solutes identified in Fig. 7 are shown in Table 5.

Since acidic and neutral impurities in illicit heroin represent a highly complex mixture, with solutes generally present at levels below 0.5% relative to heroin, techniques to increase the selectivity and/or sensitivity are desirable [17]. For refined samples, a sample equivalent to 100 mg of heroin is first subjected to liquid–liquid extraction. The isolated impurities are then reconstituted in 50 μl of the injection solvent, consisting of run buffer diluted ten to one with acetonitrile–water (10:90, v/v). The use of an injection solvent with a lower conductivity than the run buffer allows for a four-fold increase in the injection amount. Multi-wavelength PDA–UV detection increased the specificity and/or sensitivity of analysis. Laser-induced fluorescence (LIF) detection (using a UV krypton–fluoride laser with excitation at 248 nm) allowed for the selective and sensitive detection of phenanthrene-like impurities (e.g., see compounds 11–13 in Table 5). LIF detection can offer detection limits greater than two orders of magnitude better than those of UV detection. For acetylthebaol, the limit of detection via LIF is 1.8 ng/ml, which is 500 times more sensitive than with UV detection. Similarly, MECC with conventional fluorescence detection affords an approximately twenty-fold increase over UV detection for the phenanthrene impurities [5]. Peak-enriched electropherograms were obtained using both fluorescence detection schemes.

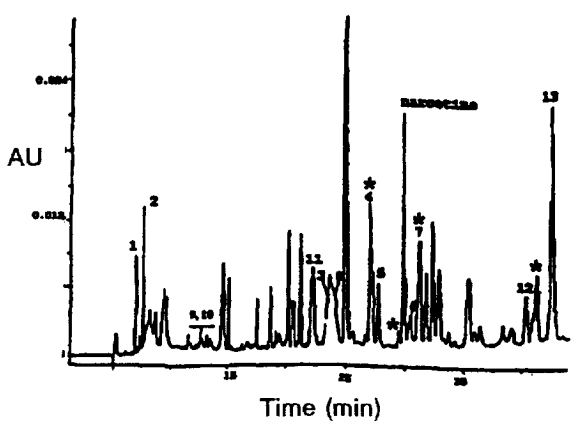


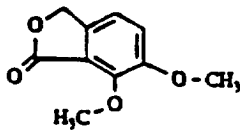
Fig. 7. A 57-cm (50 cm to the detector)×50 μm capillary at 30°C was used with a voltage of 21 kV. The run buffer contained 9.0 m*M* phosphate, 9.0 m*M* borate, 45.0 m*M* SDS, 6.9 m*M* β-CD SBE IV and 10% acetonitrile. The identities of the numbered compounds are shown in Table 5. An asterisk refers to peaks containing unknown compounds with phenanthrene moieties. Reproduced with permission from Ref. [17].

Short- and long term precision measurements for relative migration times and relative peak areas for an acid–neutral extract of illicit heroin demonstrate the viability of MECC for intelligence gathering purposes. R.S.D. values of under 0.6 and 1.4% were obtained for short- and long term relative migration time values, respectively (relative to compound 11, Table 5). Typically, R.S.D. values of approximately 4% were obtained for both short- and long term relative peak areas.

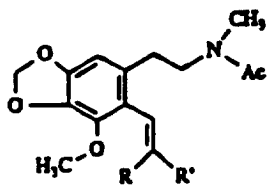
4. Opium

Opium, a complex mixture containing morphine, codeine, thebaine, noscapine, papaverine and various other alkaloids, is particularly amenable to MECC analysis. GC assays require derivatization of morphine [2], while thebaine and noscapine exhibit poor chromatographic performance [19]. HPLC allows the direct analysis of these solutes; however, it lacks sufficient resolution for opium [20]. Free solution CE is not amenable for analysis of opium, since many of the compounds have similar charge-to-size ratios [21].

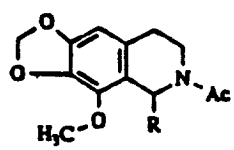
Table 5
Some acidic and neutral acetylated rearrangement products of opium alkaloids



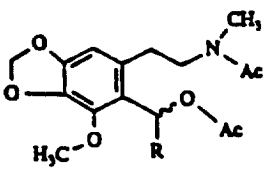
1.



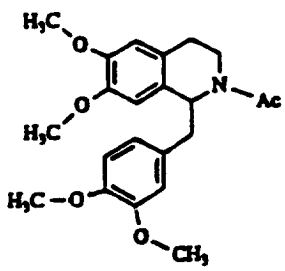
2. R=H, R'=COOH
6. "E", R-R'=1.
7. "Z", R-R=1.



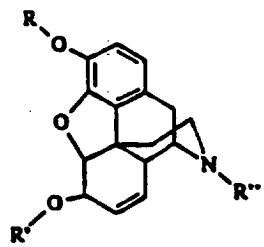
3. R=1.



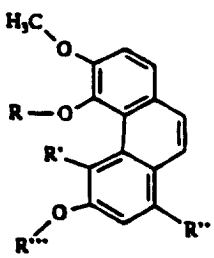
4 erythro, R=1.
5 threo, R=1.



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

Reproduced with permission from Ref. [17].

Bjornsdottir and Hansen [21] used a mixture of SDS and the neutral surfactant, Tween 20, at pH 4.0 to resolve morphine and codeine in under 10 min. However, hydrophobic solutes (such as thebaine, noscapine and papaverine) eluted as broad peaks long after the t_0 (i.e., 14.7 min). For this reason, the authors investigated alternate MECC approaches.

Using an MECC run buffer at a higher pH (where greater osmotic flow was generated) resulted in an improved separation. Our laboratory obtained a good separation of opium using the same conditions used for the separation of acidic and neutral impurities in heroin (see Fig. 8); as before, SDS was used in combination with β-CD SBE IV to increase the migration time window.

An excellent separation of nine opium alkaloids was obtained by Trenerry et al. [22] using CTAB. Morphine, codeine, salutaridine, oripavine, cryptopine, narceine, thebaine, papaverine, noscapine and an internal standard (pholcodine) were resolved in under 8 min, using dimethylformamide as an organic modifier. Good separations were also obtained for other organic modifiers, including methanol, acetonitrile, dimethylsulfoxide (DMSO) and hexamethylphosphoramide. Not unexpectedly, systems containing 27.5% methanol or 12.5% acetonitrile displayed different selectivities than those containing 10% dimethylformamide. This can be attributed to the different partition coefficients between the micelle and the various organic modifiers. Dimethylformamide was the modifier of choice when considering overall resolution, speed of analysis and reproducibility of separation.

It is also of interest to note that different selectivities were observed using CTAB and SDS. With CTAB, morphine eluted after codeine, followed by

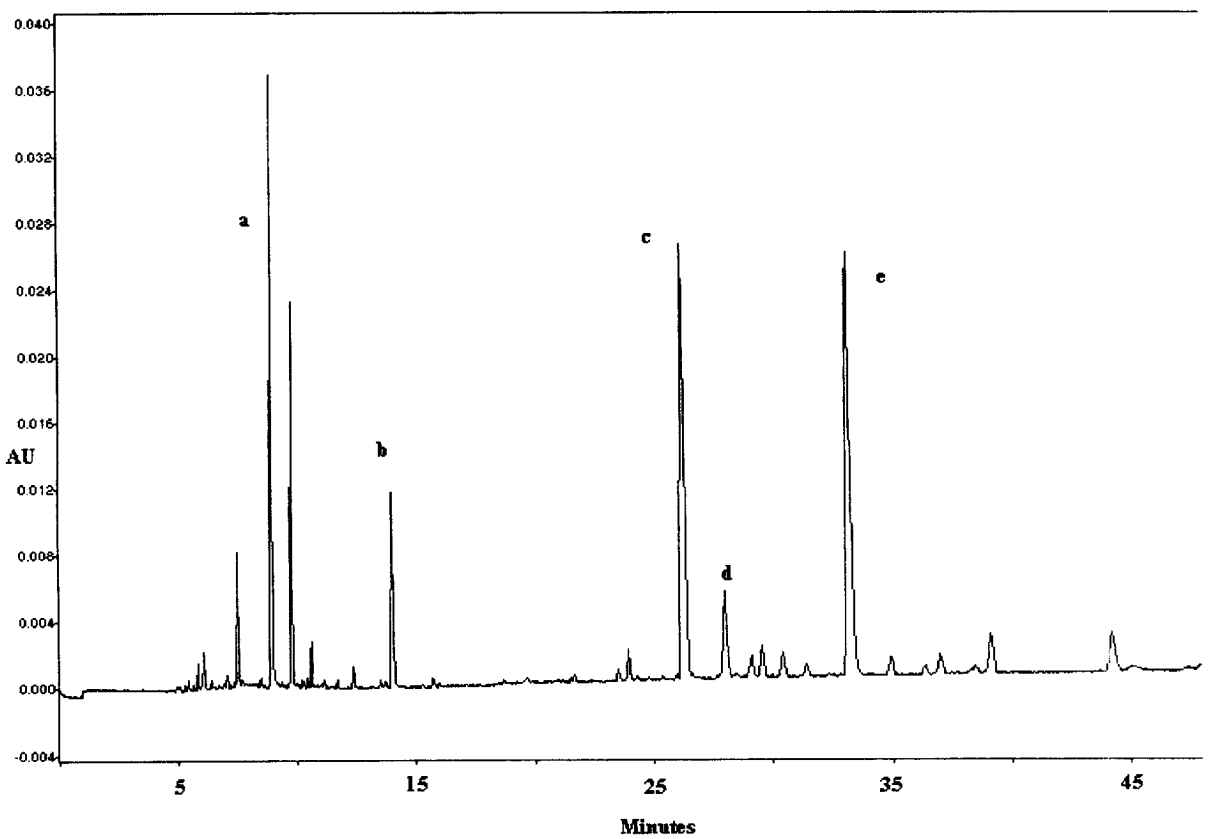


Fig. 8. MECC of opium sample with the same conditions as in Fig. 8. Key: (a) Morphine, (b) codeine, (c) papaverine, (d) thebaine and (e) noscapine.

thebaine, papaverine and noscapine. For SDS, the migration order was morphine, codeine, papaverine, thebaine and noscapine (see Fig. 8).

Samples analyzed using CTAB included crude morphine, poppy straw and opium. In general, there was good agreement between MECC and HPLC (see Table 6), with MECC taking only half the run time of HPLC. However, MECC was less precise than HPLC (see Table 6), and a higher sample concentration was required for good precision. A good separation was maintained when a solution containing 15 mg/ml of crude morphine was injected onto the capillary. Morphine quantitation was not reported for this higher concentration, probably due to detector overload. Obviously, better precision would be obtained for less concentrated injections, if run buffer modifiers with lower UV cut-offs were used. As Trenerry et al. [22] point out, a disadvantage of dimethylformamide is its high UV cut-off, which requires the use of higher UV wavelengths for analysis.

In a subsequent paper, Vandepeer et al. [23] replaced dimethylformamide with acetonitrile so that PDA–UV detection could be performed without the UV cut-off limits imposed by the former solvent. Opium alkaloids present in crude morphine extracts were identified by matching UV spectra with those in a library. As the authors noted, peak purity determinations could also be carried out using PDA–UV detection. This would be extremely valuable for performing quantitative analyses on complex mixtures derived from opium. Although not employed by the authors, the use of multi-wavelength detection would also be highly desirable to maximize selectivity and/or sensitivity. Substituting acetonitrile for dimethylformamide and chloride for bromide would also allow the use of lower wavelengths for increased overall sensitivity.

5. Cocaine

Seized cocaine is usually analyzed via GC and/or HPLC [2,3]. However, cocaine can exhibit peak tailing when analyzed by HPLC [5]. CE is advantageous because of the low solvent usage, reduced solvent disposal problems and less expensive column costs compared with those of HPLC. In addition, the

Table 6
Comparison of the quantitative results (%) and the C.V. (%) for area calculation for MEKC and HPLC for samples of crude morphine, poppy straw, opium and opium dross using a buffer consisting of 10% DMF and 90% 0.05 *M* CTAB and a 1:1 (v/v) mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6

Sample	Codeine (%)		Morphine (%)		Oripavine (%)		Thebaine (%)		Papaverine (%)		Narcotine (%)	
	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC
1-70	3.9	3.6	85.4	81.6	1.6	1.4	2.0	2.0	–	–	–	–
C.V. (%)[a]	9.0	0.6	4.3	0.3	4.1	0.8	6.4	0.9	–	–	–	–
C.V. (%)[b]	1.8	–	–	–	0.9	–	1.0	–	–	–	–	–
1-44C	5.5	5.9	62.4	58.2	1.6	1.9	1.4	1.4	–	–	–	–
1-49	1.0	0.8	67.3	61.9	–	–	–	–	–	–	–	–
1-54E	1.5	1.4	95.5	93.2	0.4	0.4	–	–	–	–	–	–
Poppy straw[c]	0.1	0.1	0.9	0.9	0.01	0.01	0.05	0.05	–	–	–	–
Poppy straw[d]	0.1	0.1	1.2	1.2	0.01	0.01	0.02	0.02	–	–	–	–
Opium[e]	5.3	4.7	11.5	9.9	–	–	2.0	1.7	0.5	0.4	0.4	0.6
Dross[e]	1.7	1.6	2.9	2.9	–	–	0.2	0.2	0.6	0.6	0.9	0.9

C.V. (%) data obtained from seven replicate injections of standard solutions.
[a] 11.4 mg/10 ml of 0.01 *M* HCl.
[b] 51.5 mg/10 ml of 0.01 *M* HCl.
[c] Soxhlet extraction.
[d] Lime-water extraction.
[e] Acetic acid extraction.
Reproduced with permission from Ref. [22].

use of chlorinated solvents (typically employed in GC) is avoided. Illicit cocaine, which contains mainly basic compounds along with various neutral adulterants and diluents, is amenable to analysis by MECC.

Weinberger and Lurie [5] demonstrated the feasibility of using MECC with SDS for the analysis of illicit cocaine. Cocaine is well separated from benzoylecgonine and *cis*- and *trans*-cinnamoylcocaine, with good peak shapes, in under 15 min. Benzoylecgonine, which is negatively charged at the pH of the run buffer (8.5), is repelled from the micelle and, therefore, migrates near the t_0. Shorter run times are achievable by lowering the SDS concentration and the capillary length.

Trenerry et al. [14] used a system similar to one they employed for illicit heroin to analyze cocaine samples using CTAB. A very satisfactory separation of cocaine, benzoylecgonine, *cis*- and *trans*-cinnamoylcocaine, procaine, lidocaine, tetracaine and an internal standard (pholcodine) was obtained in under 12 min. Good reproducibility for relative migration times was obtained for this mixture (for twenty repetitive injections). Similar selectivity was obtained for the separation of cocaine, benzoylecgonine and *trans*-cinnamoylcocaine as was observed using SDS. Benzoylecgonine migrates just after the t_0 on both systems. Since benzoylecgonine is negatively charged in the CTAB system, it would be expected to interact strongly with the positively charged micelle and, therefore, to have a considerably longer migration time relative to t_0. The shorter migration time actually observed for benzoylecgonine can be attributed to its migration in free solution in the same direction as that of osmotic flow. (Note that in the CTAB system, the polarity is reversed due to the positive charge of micelles).

The authors obtained good agreement between CE and GC for the quantitative determination of cocaine. The CE-based quantitations were generally slightly lower than those obtained by GC; however, CE afforded excellent and comparable precision to GC, with % C.V.s of under 2.5%. In a single analysis study of four samples, cocaine quantitations were checked by CE, GC and HPLC; good agreement was noted between CE and GC, with somewhat poorer agreement between both of the latter two techniques with HPLC (see Table 7). However, some difficulty was experienced in assaying low levels of solutes such as benzoylecgonine and *trans*-cinnamoylcocaine, probably due to a low signal-to-noise ratio. Multi-wavelength detection, or the use of bubble cell or Z cell technology, or stacking techniques, could significantly improve the limits of detection. Although 230 nm is a good choice for the sensitive detection of cocaine and benzoylecgonine, *cis*- and *trans*-cinnamoylcocaine are detuned at this wavelength. A better choice for these latter solutes would be 277 nm, which would not only provide improved sensitivity but also good selectivity [16].

Table 7
Comparison of quantitative values obtained for cocaine seizures by MECC, GC and HPLC[a]

Sample	Cocaine (%)		
	MECC	GC	HPLC
93/002	29.4	28.9	29.0
93/003	69.7	68.0	72.0
93/004	82.2	79.7	88.0
92/938	71.7	69.8	76.0

[a] Results are from a single analysis of each sample by each method.
Reproduced with permission from Ref. [14].

6. Amphetamines

At present, the most commonly encountered illicitly prepared phenethylamines are amphetamine, methamphetamine, methylenedioxyamphetamine and methylenedioxymethamphetamine. The chiral resolution of these compounds can be important for both legal and intelligence gathering purposes. The analysis of amphetamines and related compounds (e.g., norephedrine, norpseudoephedrine, ephedrine and pseudoephedrine) can be problematic when using GC [15]. Salts usually have to be converted to the free base prior to analysis and, even then, poor peak shapes are typically observed at low sample concentrations. Prior derivatizations are usually performed for the chiral separation of phenethylamines using GC [2]. CE, which offers superior resolution and speed of analysis compared to HPLC, is amenable to both achiral and chiral separations for these solutes. Due to the close similarities of their charge-to-size ratios, an electrokinetic technique such as

MECC is suitable for the achiral separation of phenethylamines and related compounds. For chiral separations, MECC can be used with either a chiral micelle, combinations of chiral/chiral or chiral/achiral selectors, or by resolving diastereomers resulting from the prederivatization of enantiomers with chiral reagents (similar to GC).

Compounds structurally related to amphetamine, including phenylephrine, ethylphenylephedrine, phenylpropanolamine, ephedrine, amphetamine and methamphetamine, were well resolved in under 12 min using an SDS system previously employed for heroin analysis [10]. Within-day R.S.D. values for migration times relative to an internal standard (crystal violet) varied from 0.26 to 1.59%. As was previously observed for heroin in the same study, better precision would have been obtained using an instrument that provided capillary temperature control. Quantitative analysis was performed on ten different amphetamine seizures; the amphetamine contents varied from 10–83%, with relative standard deviations in the 2.0–4.3% range ($n=6$).

Trenerry et al. [15] obtained a good separation of amphetamines, related compounds and internal standards (para-aminobenzoic acid and caffeine) in under 10.5 min using CTAB (see Fig. 9). The addition of 2-aminoethanol to the run buffer was necessary in order to obtain a stable and reproducible system. This CE system is also applicable for less commonly encountered amphetamines, such as 4-methoxyamphetamine, 3,4-dimethoxyamphetamine and 4-bromo-2,5-dimethoxyamphetamine. The use of DMSO as an organic modifier improved peak shapes; however, since DMSO absorbs significant UV light at low wavelengths, sensitivity was limited. For the determination of the major amphetamine present, a wavelength of 254 nm (which was used in this study) appears to be adequate. For the separation of methylenedioxymethylamphetamine (MDMA) and methylenedioxyethylamphetamine (MDEA) using PDA–UV detection, Vandepeer et al. [23] replaced DMSO with acetonitrile; this allowed the acquisition of UV spectra over a more complete range.

The authors [15] report excellent migration time reproducibility over twenty runs for the separation of the more common amphetamines and related compounds. The R.S.D. values for peak areas for seven injections of this same mixture varied between 0.9 and 3.4%. Similar reproducibility was observed for the less common amphetamines. Quantitative results

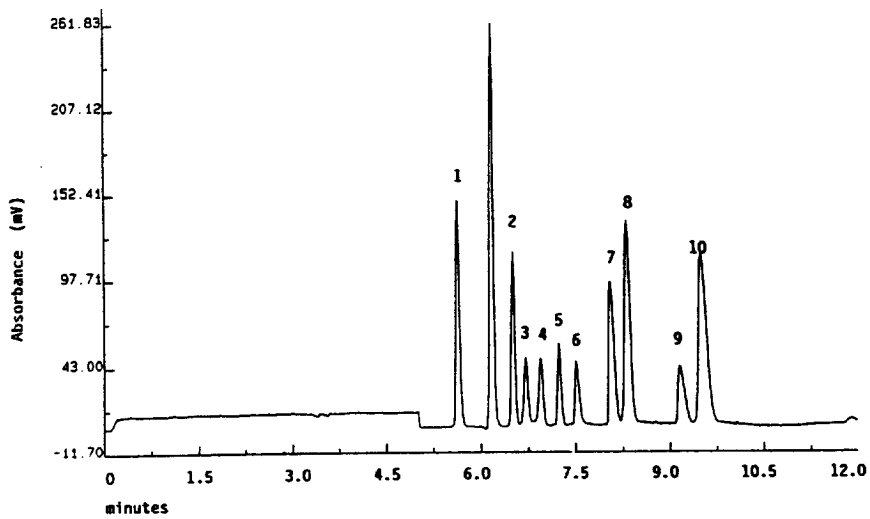


Fig. 9. Electropherogram showing the separation and peak shapes of *p*-aminobenzoic acid (1), caffeine (2), norephedrine (3), norpseudoephedrine (4), ephedrine (5), pseudoephedrine (6), amphetamine (7), methylenedioxyamphetamine (8), methamphetamine (9) and methylenedioxymethamphetamine (10), using a buffer consisting of 1% ethanolamine–11% DMSO–88% 0.025 *M* CTAB–0.01 *M* sodium tetraborate, pH 11.5. *p*-Aminobenzoic acid and caffeine were added as the internal standards. Reproduced with permission from Ref. [15].

following CE analysis were compared with values obtained by GC; fair agreement was obtained between the two techniques, with no systematic bias being evident.

Lurie [24] was unable to resolve the enantiomers of amphetamine and methamphetamine using the chiral micellar agent, sodium taurocholate, either with or without the neutral CD, heptakis (2,6-di-O-methyl)-β-CD). However, in a separate work not directed towards the analysis of seized drugs, Aumatell and Wells [25] separated the enantiomers of methamphetamine, methyldimethoxyethylamphetamine and methyldimethoxymethamphetamine using a mixture of sodium taurodeoxycholate (STDC) and hydroxypropyl-β-CD. However, most of the common amphetamines and related compounds were not resolved using this system. The use of N-dodecoxycarbonylvaline, a recently reported chiral micelle that is available in both enantiomeric forms, or a similar micelle, appears most promising for the resolution of these solutes [26].

A separation of the 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) derivatives of the enantiomers of amphetamines and related compounds was obtained using SDS [24]. The neutral diastereomers formed after derivatization exhibited good chromatographic properties on analysis by MECC. The separation of a standard mixture and a seized methamphetamine exhibit are shown in Fig. 10.

During method development, the following trends were observed:

1. The addition of organic modifier (methanol, acetonitrile or tetrahydrofuran) vastly improved the separation, both because of an increase in the migration time window and a decrease in the partition coefficient.
2. Because of changes in partition coefficients, significant selectivity differences were observed with different organic modifiers, including changes in migration orders.
3. For the modifier of choice (methanol), selectivity significantly changed with concentration. The greatest overall resolution was observed with 20% methanol. At higher concentrations, decreased sensitivity was observed.
4. Lowering the temperature from 50 to 30°C resulted in improved separations because of higher peak efficiencies and more favorable partition coefficients.
5. Lowering the voltage to moderate values (20 to 15 kV) resulted in improved resolution despite

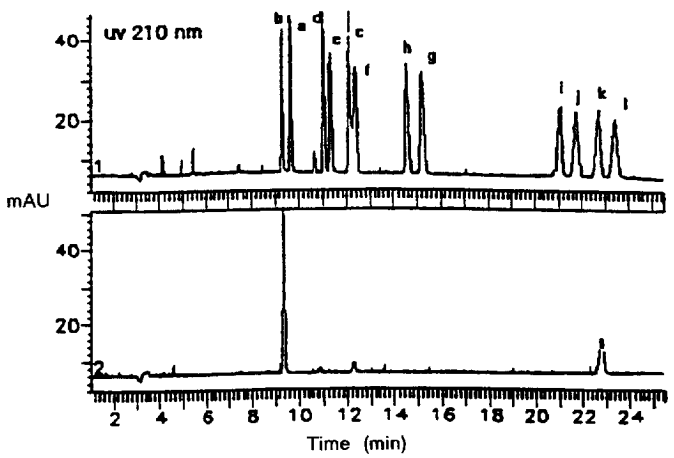


Fig. 10. MECC separation of phenethylamine–GITC derivatives. Standard mixture (top); seized exhibit (bottom). Conditions: capillary, 48 cm×50 μm I.D. (length to the detector, 26 cm); voltage, 20 kV; temperature, 30°C; buffer, 80 m*M* SDS–8.0 m*M* borate–8.0 m*M* phosphate–20% methanol, pH 9.0. Key: (a) (+)-Norpseudoephedrine, (b) (−)-ephedrine, (c) (−)-norephedrine, (d) (−)-norpseudoephedrine, (e) (+)-ephedrine, (f) (+)-pseudoephedrine, (g) (+)-norephedrine, (h) (−)-pseudoephedrine, (i) (−)-methamphetamine, (j) (−)-amphetamine, (k) (+)-methamphetamine and (l) (+)-amphetamine. Reproduced with permission from Ref. [29].

decreased efficiency, due to increased selectivity. The change in selectivity with applied potential can be described in terms of Joule heating effects, which influence the partition coefficients. At higher voltage values, peaks may not be detected because of excessive Joule heating.

6. Increasing the SDS concentration from 50 to 100 m*M* resulted in increased resolution due to increased peak efficiency and selectivity.

7. LSD

HPLC has traditionally been the method of choice for the analysis of this thermally degradable solute, especially for quantitative determinations [2,27]. However, HPLC analyses of LSD is subject to interferences from dyes typically present in illicitly prepared tablets or blotter paper. In order to separate LSD from iso-LSD, an electrokinetic technique such as MECC is suitable.

The benchmark of any LSD separation technique is the resolution of LSD (i.e., lysergic acid diethylamide) from LAMPA (lysergic acid methylpropylamide). At best, these solutes are only partially resolved on HPLC [28]. In order to determine the efficacy of MECC for LSD analysis, a single blotter paper dosage was extracted with methanol, spiked with LAMPA, and resolved using CE using the same conditions as those previously employed for heroin [9,29]. As shown in Fig. 11, LSD, LAMPA and iso-LSD were well resolved in under 5 min. However, additional work needs to be done to establish quantitative procedures. Finally, although LSD, LAMPA and iso-LSD have significant UV chromophores, the use of He–Cd lasers with excitation at 325 nm would provide greatly improved limits of detection.

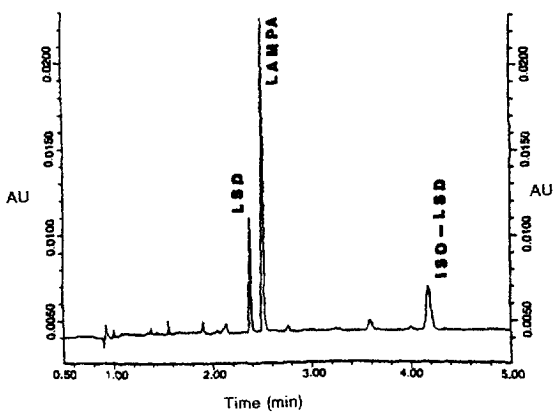


Fig. 11. Electropherogram of an LSD exhibit spiked with standard LAMPA. For MECC conditions see Fig. 2. Reproduced with permission from Ref. [29].

8. Anabolic steroids

The analysis of anabolic steroids and their corresponding esters are complicated by their sheer numbers and close structural similarities. Methodologies are required for screening as well as quantitation of these solutes in a single run. Temperature programming GC, gradient elution HPLC, and MECC are all viable choices; however, since anabolic steroids and their corresponding esters are neutral solutes, and all solutes must elute by the t_{mc}, MECC is well suited for their analyses.

Vindevogel and Sandra [30] used a Plackett-Burman statistical design to optimize an MECC separation for the testosterone esters present in the commercially available preparation "Sustanon 250". Testosterone propionate, phenylpropionate, isocaproate and decanoate were all well resolved in under 15 min, using SDS. One remarkable aspect of this system was the high organic content present in the run buffer, i.e., 35% acetonitrile. A legitimate question is whether micelles can still exist at this concentration of organic modifier. The authors make several good arguments for the presence of micelles, with the best being that separation is observed for the neutral solutes. However, as Vindevogel and Sandra [30] also point out, the ultimate proof of whether micelles are present must be established by physical methods.

A similar system was used by Lurie et al. [7] for the analysis of anabolic steroids and corresponding esters in various dosage forms, including tablets, aqueous suspensions and oil preparations. In this same study, a comparison is made between MECC, gradient HPLC, and temperature programming GC for the analysis of these solutes. HPLC and GC gave essentially equivalent resolution, with MECC having a slightly lower resolving power. In the latter case,

solubility considerations for MECC limited the use of lower percentages of acetonitrile (which would have given more favorable k' values and an increase in overall resolution). A major limitation of GC is the thermal degradation of certain anabolic steroids, e.g., clostebol, clostebol acetate, stanozolol, danazol and dromostanolone. Retention data for MECC, HPLC and GC is given in Table 8. Principal component analysis of the data for seventeen steroids indicates that the three separation techniques are orthogonal. This is important for increased specificity if multiple techniques are to be used for screening purposes. Using PDA–UV detection for MECC analyses would also increase the specificity for screening purposes. In this vein, automatic library searches could be performed.

Table 8
Retention of anabolic steroids relative to testosterone

Compound	MECC	HPLC	GC
Fluoxymesterone	0.925	0.78	1.50
Boldenone	0.964	0.74	1.05
Nandrolone	0.979	0.84	0.91
Methandrostenolone	0.985	0.86	1.12
Testosterone	1.00[a]	1.00[b]	1.00[c]
Methyltestosterone	1.02	1.17	1.05
Methandriol	1.06	1.25	0.89
Stanolone	1.06	1.25	0.89
Boldenone acetate	1.12	1.46	1.27
Stanozolol	1.16	1.69	1.68
Testosterone acetate	1.17	1.76	1.21
Nandrolone propionate	1.22	1.88	1.29
Danazol	1.23	1.52	[d]
Clostebol acetate	1.24	1.90	[d]
Testosterone propionate	1.26	2.01	1.43
Methandriol 3 acetate	1.26	2.13	1.10
Testosterone isobutyrate	1.35	2.17	1.54
Nandrolone phenylpropionate	1.44	2.25	2.28
Testosterone cypionate	1.64	2.63	2.19
Testosterone enanthate	1.69	2.60	1.92
Methandriol dipropionate	1.81	2.98	1.70
Nandrolone decanoate	2.06	2.87	2.26
Boldenone undecylenate	2.20	2.73	2.62
Testosterone undecanoate	2.36	3.18	2.56
Oxymetholone	[e]	[f]	1.28
Oxandrolone	–	–	1.17
Testosterone isocaproate	–	–	1.77
Testosterone decanoate	–	–	2.36

[a] Retention time 13.9 min.
[b] Retention time 9.2 min.
[c] Retention time 13.8 min.
[d] Breaks down GC.
[e] Does not chromatograph MECC.
[f] Breaks down HPLC.
Reproduced with permission from Ref. [7].

The run-to-run variability of migration times (retention times) and peak areas on MECC analysis agreed well with values obtained for HPLC. R.S.D. values of under 0.8 and 1.9% were obtained for retention times and peaks areas, respectively. Improved values could have been obtained using a reference standard. Quantitative analyses were performed on various preparations, including tablets, aqueous suspensions and oil samples. Good agreement was obtained between MECC, HPLC and GC. An external standard method was used for MECC and HPLC, while internal standard methodology was used for GC. Although single wavelength detection at 240 nm was used, multiwavelength detection would be preferred due to the diversity of the UV spectral properties of the various steroids.

9. Conclusions

Due to its great resolving power, flexibility, applicability to quantitative analysis, and ability to analyze solutes over a large migration range, MECC is well suited for forensic drug analysis. Satisfactory results can be obtained in most instances using either SDS or CTAB (which, furthermore, are complementary approaches). Advances in injection schemes and detection devices will continue to develop the potential of MECC for fingerprinting of drug samples.

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