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Analysis of drug seizures of heroin and amphetamine by capillary electrophoresis

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Abstract

Capillary electrophoresis has been used to separate heroin and amphetamine from structurally related compounds and commonly occurring adulterants in drug seizures. The method was based on micellar electrokinetic chromatography with a running buffer of pH 9.0 containing sodium dodecyl sulphate as surfactant and acetonitrile as organic modifier. The drugs were dissolved in running buffer containing crystal violet. Crystal violet was used to calculate relative migration times for drug identification and as internal standard for quantitative analysis. Both qualitative and quantitative analysis was shown to be reproducible. Because of the speed and resolving power of the method it is a powerful alternative to the high-performance liquid chromatographic and gas chromatographic methods in current use for the analysis of illicit drugs.

1. Introduction

Seizures of clandestinely manufactured drugs such as heroin and amphetamine can be highly complex. Heroin is produced by a variety of batch processes from a variable natural product and thereafter altered for trafficking purposes. The physical appearance varies widely, ranging from almost pure-white heroin hydrochloride to crude and impure heroin containing manufacturing impurities and various adulterants. Illicit amphetamine varies in colour from white to pink to yellow to brown depending upon the type and amount of impurities and adulterants. Illicit heroin and amphetamine may consist of a mixture of neutral, acidic and basic compounds that can be non-polar and/or polar.

Many methods are available for analysis of illicit heroin and amphetamine, and approaches to select a technique appropriate to the sample being examined are available to national narcotics laboratories [1,2]. At least two independent analytical parameters should be used to establish the identity of the drug, and infrared spectroscopy and thin-layer chromatography are widely used for this purpose [3]. Quantitation is usually carried out by gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC). These methods are in general able to separate heroin from other opiates, amphetamine from structurally related drugs and common adulterants [3,4].

Capillary electrophoresis and micellar electrokinetic chromatography (MEKC) has attracted much attention as an efficient separation technique in many areas. MEKC provides the possibility to separate both neutral and charged molecules in a single run [5–7]. Owing to its speed and high resolving power, MEKC has

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been shown to be well suited for drug screening [8].

Heroin and amphetamine are two of the most widely abused drugs in Norway. A method which is able to analyse heroin and amphetamine seizures more rapidly and with greater resolving power than the HPLC and GC methods in use is highly desirable. A method based on MEKC for analysis of heroin and amphetamine seizures was developed and evaluated through analysis of a series of drug seizures collected over the last 2 years.

2. Experimental

2.1. Equipment

Capillary electrophoresis was performed using a Dionex Capillary Electrophoresis System I (Dionex, Sunnyvale, CA, USA). This apparatus features automated sampling and execution of the electrophoretic run. For these experiments it was equipped with a 375 μm O.D. × 50 μm I.D. fused-silica capillary of 50 cm effective separation length. Automated gravity injection was performed for all runs. The sample was held 5 cm above the level of the downstream buffer for 10 s. All separations were run at 20 kV. The current did not exceed 40 μA. On-column UV detection was employed with the wavelength set at 214 nm. The detector signals were collected and analysed using The Dynamax HPLC Method Manager Programme (Rainin, Woburn, MA, USA) and a Macintosh LC computer.

The column was etched with 0.1 *M* NaOH for 30 min at the start of each day. Water was used to rinse the column and then the running buffer was introduced and allowed to equilibrate with the silica capillary for 10 min. Samples were then injected every 13 min with new running buffer automatically filled into the source vial, destination vial and column. The flush time of new running buffer between injections was 2 min.

2.2. Chemicals

Sodium dodecyl sulphate (SDS) from Sigma (St. Louis, MO, USA) was used as received. Sodium dihydrogenphosphate, sodium monophosphate and sodium hydroxide were of analytical grade from E. Merck (Darmstadt, Germany). Crystal violet was from Sigma. Deionized water from a Milli-Q system (Millipore, MA, USA) was used to prepare all buffers. HPLC-grade acetonitrile was supplied by Rathburn (Walkerburn, UK). The drug standards used to prepare the test solution were supplied either by The Norwegian Medicinal Depot (Oslo, Norway) or as gifts from The National Institute of Forensic Toxicology (Oslo, Norway). Drug seizures were supplied by The Bureau of Crime Investigation (Oslo, Norway).

2.3. Running buffer

The running buffer was prepared by mixing 25 m*M* SDS, 10 m*M* NaH_2PO_4, 10 m*M* $Na_2B_4O_7$, adjusted to pH 9.0, 5% acetonitrile.

The running buffer was degassed and filtered through a 0.45-μm membrane filter prior to use.

2.4. Test solution

The test solution used for method development contained 0.1 mg/ml nicotinamide, caffeine, paracetamol, phenacetin, phenemal, morphine, 6-monoacetylmorphine, codeine, procaine, heroin, acetylcodeine, papaverine and noscapine dissolved in running buffer. Crystal violet used as internal standard was added to the test solution at a concentration of 0.05 mg/ml.

2.5. Sample preparation

Drug seizures were homogenized to a fine powder and dissolved in running buffer at a concentration of 0.5 mg/ml. To the running buffer were added 0.05 mg/ml of crystal violet. The sample solution was filtered through a 0.45-μm syringe filter before analysis.

2.6. Quantitative analysis

Crystal violet was used as internal standard for quantitative measurements. The standard curves, accuracy and precision of the method were evaluated by analysis of the drug standards and

drug seizures in running buffer. The calibration graphs were based on peak-area ratios relative to the internal standard.

3. Results and discussion

3.1. MEKC

The test solution used to develop the running buffer was a mixture of the major alkaloids found in most heroin samples and adulterants. Crystal violet was added as internal standard. The criteria used to develop the running buffer were separation of all components in the test solution in as short an analysis time as possible, preferably less than 15 min. In addition, accurate and reproducible migration times and quantitative analysis should be obtained.

Several reports have shown the effect of surfactants, surfactant concentration and pH on the MEKC separation of various classes of drugs [5–7], and a general strategy for improving separation in MEKC has been presented [9]. Weinberger and Lurie [8] separated bulk heroin on a 25 cm × 50 μm I.D. capillary using 85 m*M* SDS, 8.5 m*M* phosphate, 8.5 m*M* borate, 15% acetonitrile, pH 8.5, as running buffer. At 40°C and 20 kV the last-eluted compound in the sample, noscapine, had a migration time of 13.5 min. A model mixture of eighteen drugs were separated with this system in 40 min. Wernly and Thormann [10] separated model mixtures of illicit drugs in 25 min with 75 m*M* SDS, 6 m*M* $Na_2Ba_4O_7$, and 10 m*M* Na_2HPO_4 (pH about 9.1) as running buffer.

In order to separate the compounds in the test solution in less than 15 min a MEKC separation with SDS as surfactant was investigated. The selectivity was optimized by altering the pH and by addition of organic modifier to the buffer. Short analysis times were obtained with low surfactant concentrations and low ion strength buffers. The electroosmotic flow decreases as the buffer concentration increases [11]. The desired resolution was obtained with an effective column length of 50 cm. The pH was found to be a critical factor affecting resolution of the present test mixture and an adequate separation was obtained at pH 9. With the exception of opiates with a phenolic group the migration times of the alkaloids increased by lowering the pH. The phenolic group of, *e.g.*, morphine is partly ionized at pH 9.0, which causes a decreased interaction with the micelle. Morphine is therefore the first eluted of the opiates.

Fig. 1 shows the effect of organic modifier and SDS concentration on the separation of the compounds in the test mixture. Complete separation of all components in the test mixture was not possible without addition of organic modifier. Both noscapine and papaverine as well as caffeine and paracetamol were unresolved with-

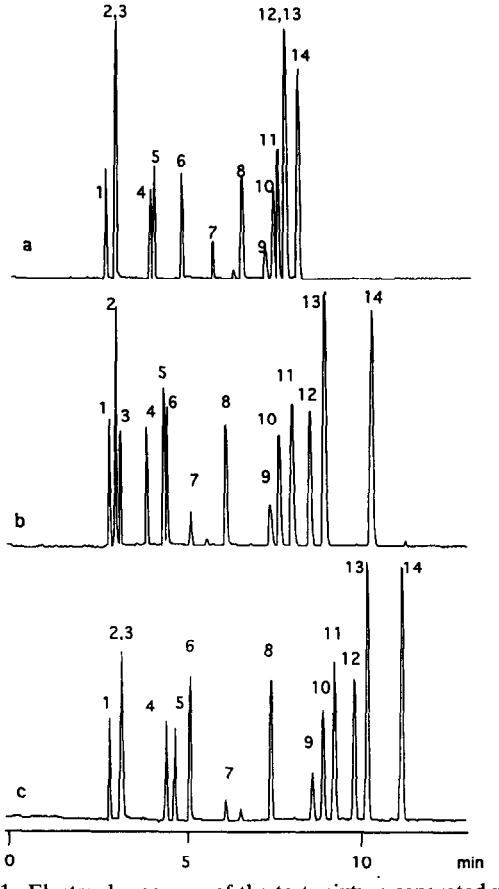

Fig. 1. Electropherograms of the test mixture separated with a running buffer consisting of 25 m*M* SDS, 10 m*M* NaH_2PO_4, 10 m*M* $Na_2B_4O_7$, pH 9.0 (a), 25 m*M* SDS, 10 m*M* NaH_2PO_4, 10 m*M* $Na_2B_4O_7$, pH 9.0, 5% acetonitrile (b), and 50 m*M* SDS, 10 m*M* NaH_2PO_4, 10 m*M* $Na_2B_4O_7$, pH 9.0, 5% acetonitrile (c). Further details see text. Peak numbers as in Table 1.

out organic modifier. The addition of acetonitrile greatly improved resolution and complete separation was achieved in 10 min with 25 m*M* SDS and 5% acetonitrile. The improved resolution obtained by addition of organic modifier was due to a decrease in the electroosmotic flow [11] and to the alteration of the partition coefficient. A decreased resolution was observed for phenemal and morphine and an increased resolution was observed for caffeine and paracetamol under the given conditions. Increasing the SDS concentration to 50 m*M* prolonged the analysis time and the resolution of caffeine and paracetamol was lost. Both caffeine and paracetamol have been found in recent drug seizures, while phenemal is more rarely detected. Priority was therefore given to the resolution of caffeine and paracetamol. The running buffer consisting of 25 m*M* SDS, 10 m*M* NaH_2PO_4, 10 m*M* $Na_2B_4O_7$, pH 9.0, and 5% acetonitrile was therefore selected for analysis of the drug seizures. This running buffer was also able to separate drugs structurally related to amphetamine, as shown in Fig. 2. The number of theoretical plates was in the range 120 000–290 000.

3.2. Analysis of illicit heroin and amphetamine

For the analysis of illicit samples, an internal standard, crystal violet, was added to the sample solution for calculation of relative migration times. Crystal violet was also used as internal standard in quantitative analyses. The selection of the internal standard was based on the requirement that there should be no possibility of its presence in any drug seizure. Several substances were tested and crystal violet was selected because it is not found in any drug seizure, it is highly soluble in the running buffer and it is eluted from the column with a longer migration time than the basic drugs found in illicit samples. The migration time of crystal violet was similar to Sudan III, which is often used as tracer for the micelle but is poorly soluble in the running buffer. Substances present in a drug seizure should therefore be eluted with a migration time shorter or equal to that of crystal violet.

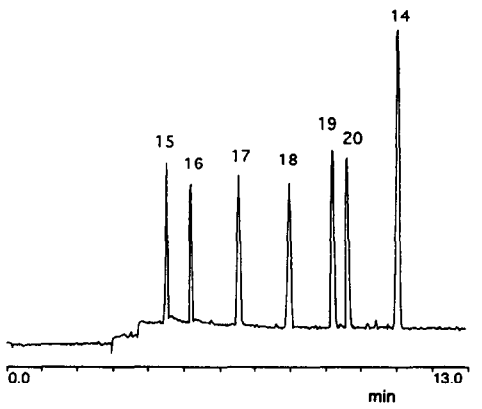

Fig. 2. Electropherogram of drugs similar in structure to amphetamine. Running buffer: 25 m*M* SDS, 10 m*M* NaH_2PO_4, 10 m*M* $Na_2B_4O_7$, pH 9.0, 5% acetonitrile. Further details see text. Peak numbers as in Table 1.

Table I shows the migration times for substances tested relative to crystal violet and their within-day and between-day coefficient of variations. In addition to a positive identification by at least another analytical method, these relative migration times were used to verify identity. The migration reproducibility depends on several operational factors such as ionic strength of the buffer, age of capillary, previous capillary treatment, applied voltage and external capillary temperature [12]. With the apparatus used in this study no control over the capillary temperature was possible and the migration times decreased as the room temperature increased. The within-day relative standard deviations (R.S.D.s) on the migration times were in the range 2.5–4.0% and the between-day R.S.D. values were 3.5–6.0% when the room temperature varied in the range 20–23°C. However, by using relative migration times instead of absolute migration times identification problems were reduced. As shown in Table I, reproducibility of the relative migration times was satisfactory with a within-day R.S.D. in the range 0.5–1.9% and a between-day R.S.D. ranging from 0.89 to 2.23%. The capillary was in general replaced after 500 analyses to maintain reproducibility.

Fig. 3 shows electropherograms of a typical heroin seizure and a typical amphethamine seizure. In general, no problems were encountered in identification of the illicit drugs even if some heroin seizures were highly complex.

Table 1
Relative migration times and within-day relative standard deviations and between-day relative standard deviations

Peak No.	Drug	Relative migration time	Within-day R.S.D. (%) ($n = 6$)	Between-day R.S.D. (%) ($n = 5$)
1	Nicotinamide	0.280	1.90	2.04
2	Caffeine	0.295	1.84	2.03
3	Paracetamol	0.310	1.82	1.96
4	Phenacetin	0.378	1.60	2.01
5	Phenemal	0.422	1.66	2.23
6	Morphine	0.439	1.54	1.58
7	6-monoacetylmorphine	0.495	1.55	2.23
8	Codeine	0.589	1.41	2.30
9	Procaine	0.714	0.96	2.16
10	Heroin	0.378	0.98	1.91
11	Acetylcodeine	0.772	0.94	1.73
12	Papaverine	0.823	0.69	1.25
13	Noscapine	0.867	0.49	0.89
14	Crystal violet	1.000	–	–
15	Phenylephrine	0.403	0.88	–
16	Etilefrine	0.461	1.02	–
17	Phenylpropanolamine	0.574	1.59	–
18	Ephedrine	0.713	0.67	–
19	Amphetamine	0.827	0.39	–
20	Methamphetamine	0.865	0.26	–

3.3. Quantitative analysis

The standard curves were based on peak-area measurements relative to crystal violet. Standard curves were set up for morphine, heroin, codeine, papaverine, paracetamol, metamphetamine and amphetamine. All standard curves were linear in the concentration range 0.02–0.5 mg/ml with correlation coefficients (r) in the range 0.997–0.999. The method was validated by analysis of ten heroin seizures and ten amphetamine seizures. The content of heroin in these seizures was in the range 12–51% and the content of amphetamine was in the range 10–83%. The relative standard deviations were in the range 2.0–4.3% ($n = 6$). It is known that small changes in electroendosmosis can lead to changes in peak area. To overcome this problem peak areas can be normalized to one of the migration times in each experiment [13]. The

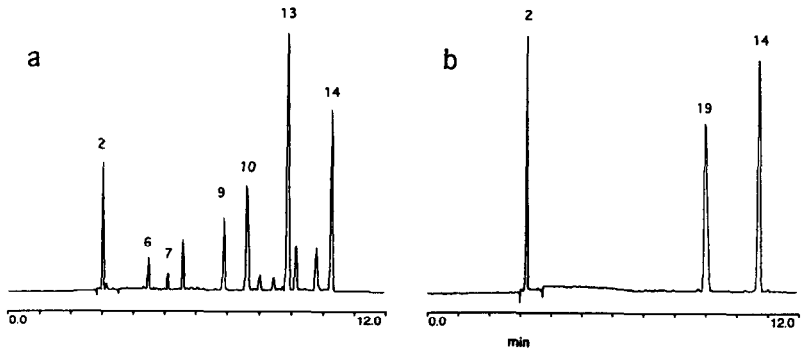

Fig. 3. Electropherograms of a heroin seizure (a) and of an amphetamine seizure (b). Running buffer: 25 mM SDS, 10 mM NaH_2PO_4, 10 mM $Na_2B_4O_7$, pH 9.0, 5% acetonitrile. Further details see text. Peak numbers as in Table 1.

relative standard deviations obtained in this investigation were considered satisfactory without using normalized peak areas.

4. Conclusions

The advantages of using capillary electrophoresis for the analysis of illicit drugs with MEKC is the speed and resolving power of the method. The drug seizures are dissolved in the running buffer and new samples were injected every 13 min. Capillary electrophoresis is a valuable complement to the HPLC and GC methods in current use for the analysis of illicit drugs.

Acknowledgements

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