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The Rapid Analysis of Heroin Drug Seizures Using Micellar Electrokinetic Chromatography with Short-End Injection

ABSTRACT: A simple and rapid method for the analysis of heroin seizures by micellar electrokinetic chromatography with short-end injection is described. Separations were performed using an uncoated fused silica capillary, 50 cm × 50 μm I.D. × 360 μm O.D. with an effective separation length of 8 cm. The system was run at 25°C with an applied negative voltage of –25 kilovolts. Injection of each sample was for 2 s at –50 mbar. UV detection was employed with the wavelength set at 210 nm. The background electrolyte consisted of 85:15 (water:acetonitrile, v/v) containing final concentrations of 25 mM SDS and 15 mM sodium borate, pH 9.5. Samples and standards were prepared in 0.1% v/v acetic acid and diluted in the run buffer containing 1 mg/ml of N,N-dimethyl-5-methoxytryptamine as an internal standard. Under these conditions a text mixture containing caffeine, paracetamol, morphine, codeine, heroin, and acetylcodeine was resolved within 1.5 min. The method was used to determine the concentration of heroin in heroin seizure samples, and the results were in good agreement with those obtained by a validated gas chromatographic method.

KEYWORDS: forensic science, short-end injection, micellar electrokinetic chromatography, heroin seizures

The term capillary electrophoresis (CE) describes a family of related techniques in which separations are carried out in narrow bore capillaries under the influence of an electric field. The separations obtained by CE are highly efficient and rapid and may be applied to both charged and neutral species. The potential of this technique for forensic analysis was first demonstrated by Weinberger and Lurie, who applied it to the analysis of a wide range of illicit drugs (1). Since that time, a number of papers have discussed the wider application of CE to forensic science (2–15).

The analysis of heroin and associated opiates in drug seizure samples is of great importance for forensic investigations, both for legal and criminal intelligence purposes (11). Micellar electrokinetic chromatography (MEKC) is one mode of CE that has been applied to the analysis of opiates in heroin seizure samples. This hybrid of electrophoresis and chromatography was first introduced by Terabe in 1984 (16) and allows for the separation of both neutral and charged molecules (17). Weinberger and Lurie (1) used MEKC to separate 18 illicit drugs, including opiate alkaloids, amphetamines, benzodiazepines, hallucinogens, and cannabinoids within 40 min. This was achieved using a 47 cm × 50 um i.d. (effective length 25 cm) capillary, an applied voltage of 20 kV, and an electrolyte containing 85 mM sodium dodecylsulfate (SDS), 8.5 mM phosphate, 8.5 mM borate, and 15% acetonitrile at pH 8.5. When applied specifically to the analysis of heroin seizures, this method was able to resolve the components of a heroin seizure within 14 min. Krogh et al. (18) used similar conditions and were able to separate a test mixture of heroin and amphetamine from structurally related compounds and adulterants in illicit drug seizure samples within 10 min. Trenarry and co-workers (19) reported a MEKC separation with a run-time of about 15 min using cetylammonium bromide (CTAB). Walker and co-workers (20) employed similar separation conditions to Weinberger and Lurie (1), but by using a shorter capillary and a lower SDS concentration, they were able to reduce the analysis time to less than 5 min.

Conventionally, one way to reduce analysis time is to apply a higher voltage or increased field strength (voltage/total length). This approach is limited due to joule heating (21). Problems arising from joule heating can result in high current production, generating temperature gradients and changes in solution viscosity, which result in band broadening (21). Prolonged applications of a very high voltage across a buffer solution can also cause electrolysis of the solution, altering its pH, and affecting the reproducibility of the system (22). While it is possible to reduce analysis time through the use of shorter capillaries, this approach is limited by the physical constraints of commercial CE instruments, where the minimum capillary length is fixed, typically around 30 cm (21,23).

In CE, injection is conventionally performed at the anode with detection at the cathode end of the capillary. If the normal polarity of the electrodes is reversed, such that the anode is at the detector (short) end of the capillary, samples and standards can then be introduced at the detector end, thus yielding a far shorter effective capillary length (24–28). This technique of short-end injection allows for the reduction in analysis time, voltage applied, increase in sensitivity, and also a decrease in buffer depletion effects (24,29). An increase in sensitivity is also generally observed due to diffusion processes being reduced, therefore minimizing band broadening and generating sharper peaks (24).

While short-end injection has found application in pharmaceutical analysis (24–28), to the best of our knowledge there has only

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been one previous report of this technique in the field of forensic science (30). Bjornsdottir and Hansen (30) used short end injection for the separation of 16 illicit drug substances, including heroin, in a non-aqueous separation medium within two min. However, the opiates codeine, ethylmorphine, and acetylcodeine could not be separated. In addition, this technique was not applied to real forensic samples.

In this paper, the application of short-end injection to forensic analysis is demonstrated by a rapid method for the quantitative analysis of heroin seizure samples.

Materials and Methods

Chemicals and Reagents

Solutions were prepared with de-ionised water from a Millipore Milli-Q system (Bedford, MA, USA). The heroin and acetylcodeine standards (99% + purity) were synthesized at Deakin University according to the U.S. Treasury Department (31). Morphine and codeine standards were obtained from Glaxo Smith Kline (Port Fairy, Victoria, Australia). Caffeine and sodium dodecylsulfate (SDS) were from BDH (Poole, England). Paracetamol was purchased from Riedel-de Haen (Seelze, Germany). N,N-dimethyl-5-methoxytryptamine and sodium tetraborate were purchased from Aldrich (Milwaukee, WI, USA). HPLC grade acetonitrile was used. All the buffers and samples were filtered with a 0.45 μm membrane filter prior to injection.

Instrumentation

Capillary electrophoresis was performed using a Hewlett Packard HP^{3D} System equipped with a diode array detector. An uncoated fused silica capillary, 50 cm × 50 μm I.D. × 360 μm O.D. with an effective separation length of 8 cm was used for all the experiments. The capillary was conditioned before use by successively washing for 30 min with 0.1 M NaOH, 15 min with deionised water, and 30 min with the run buffer. A one-min pre wash with the run buffer was performed prior to each injection, as was a one-min post run wash with 0.1 M sodium hydroxide. The running buffer was changed every ten injections.

Gas Chromatography was performed on an HP 5890 GC equipped with a Flame Ionisation Detector. An HP Ultra-1 capillary column with dimensions of 12 m × 0.2 mm × 0.33 μm was used for all experiments. The injector, detector, and oven temperature were 250°C, 300°C, and 260°C, respectively.

Sample Preparation

Samples and standards were prepared in 0.1% v/v acetic acid and diluted in the run buffer containing 1 mg/ml N,N-dimethyl-5-methoxytryptamine as an internal standard. Forensic samples were diluted (1:2 v/v) with the running buffer.

Electrophoretic Conditions

The run buffer consisted of 25 mM SDS and 15 mM sodium borate, pH 9.5, 15% acetonitrile. The system was run at 25°C with an applied negative voltage of –25 kilovolts, generating a field strength of 500 V/cm. The current did not exceed 40 μA. Injection of each sample was for 2 s at –50 mbar with a two-min run time. UV detection was employed with the wavelength set at 210 nm. All analyses were carried out in a temperature-controlled room.

Results and Discussion

Method Development

The key factors which control selectivity in MEKC are the identities of the micelles, buffer composition, and pH (21). Sodium dodecylsulfate (SDS) has been the most commonly used micelle for the separation of opiates by MEKC, generally in a phosphate/borate buffer at a pH in the range pH 9–10 (1,19,20,32,33). A preliminary investigation showed that complete resolution of the components of a 0.1 mg/ml opiate test mixture within 2.5 min was possible using 25 mM SDS, 20 mM phosphate, 10 mM borate, pH 9.5, and 15% acetonitrile. This was achieved using an effective capillary length of 8 cm and an applied voltage of –20 kV. However, both the retention time and peak area reproducibility were poor (Table 1). It was found that with the elimination of phosphate and an increased borate concentration, more acceptable reproducibility and greater resolution were achieved (Table 2).

TABLE 1—*Migration time and peak area reproducibility for a 0.1 mg/ml test mixture for a run buffer consisting of 25 mM SDS, 20 mM phosphate, 10 mM borate, pH 9.5, 15% acetonitrile. RSD calculated from 10 replicate injections.*

Analyte	Migration Time (min)	RSD of Migration Time for Intra-Day (%)	RSD of Migration Time for Between-Day (%)	RSD of Peak Area for Intra-Day (%)	RSD of Peak Area for Between-Day (%)
Caffeine	0.99	2.0	3.6	2.2	4.8
Paracetamol	1.22	2.6	3.1	2.2	5.3
Morphine	1.32	2.7	3.3	2.5	4.8
Codeine	1.39	2.6	4.2	2.1	5.9
Acetylcodeine	2.38	3.5	5.2	11.4	15.9
Heroin	2.51	4.4	5.9	3.3	6.1

TABLE 2—*Migration time and peak area reproducibility for a 0.1 mg/ml test mixture for a run buffer consisting of 25 mM SDS, 15 mM borate, pH 9.5, 15% acetonitrile. RSD calculated from 10 replicate injections.*

Analyte	Migration Time (min)	RSD of Migration Time for Intra-Day (%)	RSD of Migration Time for Between-Day (%)	RSD of Peak Area for Intra-Day (%)	RSD of Peak Area for Between-Day (%)
Caffeine	0.63	0.5	1.0	2.2	2.7
Paracetamol	0.72	0.4	1.0	3.5	3.9
Morphine	0.78	0.5	0.9	4.0	4.5
Codeine	0.81	0.9	1.1	2.3	3.9
Heroin	0.97	0.9	1.0	2.7	4.7
Acetylcodeine	1.04	0.8	1.1	2.8	4.4

The pH was investigated over the range 7.5–10. This parameter has a large effect on selectivity in capillary electrophoresis, not only due to the degree of ionisation associated with analytes, but also because of the effect of pH on the electro-osmotic flow (21). It was found that pH 9.5 was optimal for the separation of the test mixture. Operating at a higher pH resulted in poor resolution due to an increased electro-osmotic flow, while lower pH improved resolution at a cost to analysis time.

The separation mechanism associated with the addition of a surfactant is due to the partitioning of the analytes with the micellar phase in a chromatographic manner (34). Significant changes in migration times, resolution, and absorbance with varying SDS concentrations were observed. Increasing the SDS concentration from 10 mM to 40 mM prolonged the analysis time, however the resolution and peak shape improved due to the increased micelle interaction with the analytes. A final SDS concentration of 25 mM was employed as the best compromise between analysis time and resolution.

Organic modifiers contribute to alteration of selectivity, change in migration time, and modification of resolution (34). Generally, the maximum content of organic modifier that can be used is approximately 20% v/v. The selectivity change occurs by an alteration of the partition coefficient. In general, the addition of organic modifiers such as acetonitrile and methanol decrease the electro-osmotic flow, resulting in an increased elution range. The resulting separation is a compromise between effect of the solvent on electro-osmotic flow and partition coefficient (32). Methanol and acetonitrile were investigated for their effect on separations, with the latter exhibiting improved resolution and shorter migration time of the analytes within the test mixture. Increasing the concentration of acetonitrile from 5% to 15% enhanced the resolution of heroin and acetylcodeine, however above this level codeine and morphine were unresolved.

The final background electrolyte conditions were 25 mM SDS, 15 mM sodium borate, pH 9.5, 15% acetonitrile. These conditions were used for all subsequent experiments.

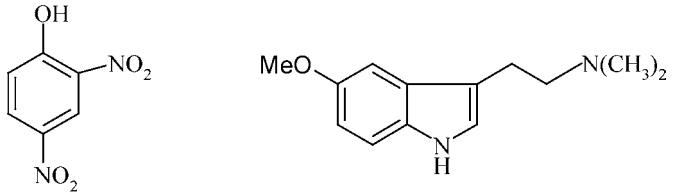
The final factor to be investigated was the applied voltage, which affects the speed and quality of a separation (21). The application of a higher voltage reduces analysis time due to increases in electro-osmotic flow and electrophoretic migration velocities. However, this approach to reduce analysis time is limited by the effects of joule heating (21). A voltage of –25 kV was found to provide an optimal separation, above which further increases in voltage resulted in non-baseline resolution of heroin and acetylcodeine.

Analysis of Heroin Seizures

Quantification using capillary electrophoresis depends on good peak area reproducibility. The most common source of imprecision is sample introduction; Altria has identified a number of factors which lead to variability in the volume introduced into a capillary using pressure difference (35). The incorporation of an internal standard will remove volume-related sources of error and lead to a consequent improvement in precision. The choice of a suitable internal standard depends on peak shape and migration time of the candidate standard (35).

Initially, 2,4-dinitrophenol (Fig. 1*a*) was used as an internal standard to prepare a series of calibrations for caffeine, paracetamol, codeine, morphine, heroin and acetylcodeine over the range 0.02 to 1.0 mg mL^{-1} for each analyte. Six heroin seizures were then diluted with the background electrolyte containing the internal standard and the heroin content of each was determined. As can be seen from Table 3, inaccurate results were obtained. While the reasons for this are not immediately clear, it may have been due to the solubility of 2,4-dinitrophenol, which required the addition of 0.1% acetic acid in order to dissolve in the run buffer.

Consequently, a range of potential internal standards was investigated, and the results are summarised in Table 4. N,N-dimethyl-5-methoxytryptamine (Fig. 1*b*) was selected as the internal standard on the basis of these results, as the other species either co-migrated with the analytes or had excessive migration times (Table 4). An electropherogram of a separation of a test mixture and a seized heroin sample containing the internal standard is presented in Figs. 2



a **b**

FIG. 1—*Choices of internal standards:* (a) *2,4-dinitrophenol and* (b) *N,N-dimethyl-5-methoxytryptamine.*

TABLE 3—*Quantification of heroin in heroin seizure samples. MEKC Conditions: 2,4-dinitrophenol (internal standard), UV absorbance at 210 nm, uncoated fused silica capillary 50 cm × 50 μm I.D. × 360 μm O.D., effective separation length 8 cm, background electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15% (v/v) acetonitrile, pH 9.5, 25°C, –25 kV, hydrodynamic injection: 2 s at –50 mbar, n = 2. GC Conditions: HP 5890, FID, 12 m × 0.2 mm × 0.33 μm HP Ultra-1 capillary column, oven temperature: 260°C, injector temperature: 250°C, detector temperature: 300°C.*

Sample	GC Results (% Diamorphine by Mass)	MEKC Results (% Diamorphine by Mass)	Difference (% Diamorphine by Mass)
1	11.9	11.9	0
2	51.6	66.4	–28.6
3	41.1	50.1	–21.8
4	59.4	72.7	–22.3
5	81.9	83.2	–1.5
6	79.7	77.6	2.6

TABLE 4—*Choice of internal standard.*

Internal Standard	Migration Time (min)	Comments
Imidazole	0.74	Co-migration
Tyramine	0.80	Co-migration
Dopamine	0.84	Co-migration
Serotonin	0.85	Co-migration
Hordenine	0.85	Co-migration
4-Ethylphenol	0.98	Co-migration
Ethylmorphine	1.09	Co-migration
N,N-dimethyl-5-methoxytryptamine	1.21	No co-migration, good peak shape
Barbitone	1.26	Increased migration time
4-hydroxy-3-methoxybenzoic acid	1.45	Increased migration time
2-Thiobarbituric acid	1.75	Excessive migration time
3,4,-dihydroxybenzoic acid	1.78	Excessive migration time
Dextromethorphan	2.80	Excessive migration time, poor peak shape

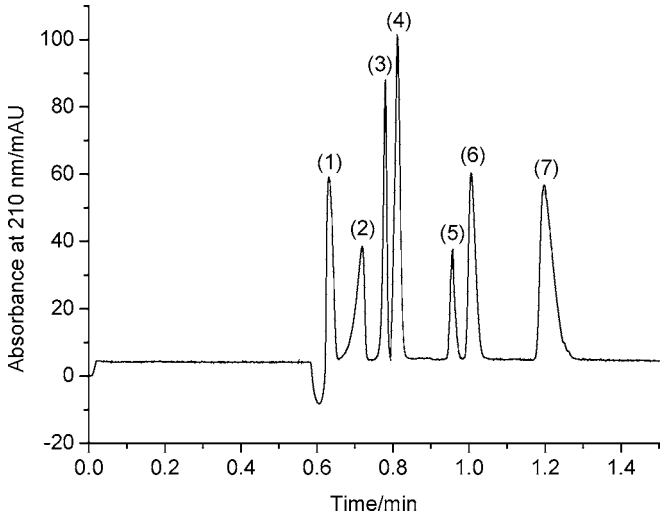


FIG. 2—*Electropherogram showing the separation of a 0.1 mg/ml test mixture containing (1) caffeine, (2) paracetamol, (3) morphine, (4) codeine, (5) heroin, (6) acetylcodeine, and (7) N,N-dimethyl-5-methoxytryptamine. UV absorbance at 210 nm, uncoated fused silica capillary 50 cm × 50 μm I.D. × 360 μm O.D., effective separation length 8 cm, background electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15% (v/v) acetonitrile, pH 9.5, 25°C, –25 kV, hydrodynamic injection: 2 s at –50 mbar.*

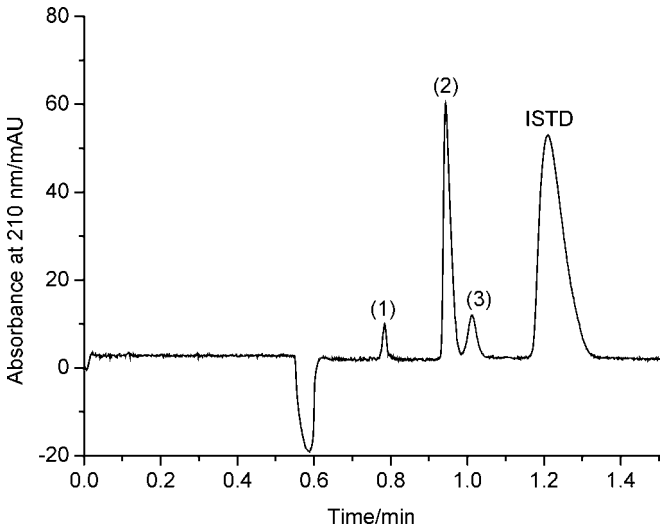


FIG. 3—*Electropherogram showing the separation of a seized heroin sample containing (1) morphine, (2) heroin, (3) acetylcodeine, ISTD = internal standard (N,N-dimethyl-5-methoxytryptamine). Sample prepared as per text. UV absorbance at 210 nm, uncoated fused silica capillary 50 cm × 50 μm I.D. × 360 μm O.D., effective separation length 8 cm, background electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15% (v/v) acetonitrile, pH 9.5, 25°C, –25 kV, hydrodynamic injection: 2 s at –50 mbar.*

TABLE 5—*Quantification of heroin in heroin seizure samples. MEKC Conditions: N,N-dimethyl-5-methoxytryptamine (internal standard), UV absorbance at 210 nm, uncoated fused silica capillary 50 cm × 50 μm I.D. × 360 μm O.D., effective separation length 8 cm, background electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15% (v/v) acetonitrile, pH 9.5, 25°C, –25 kV, hydrodynamic injection: 2 s at –50 mbar, n = 2. GC Conditions: HP 5890, FID, 12 m × 0.2 mm × 0.33 μm HP Ultra-1 capillary column, oven temperature: 260°C, injector temperature: 250°C, detector temperature: 300°C.*

Sample	GC Results (% Diamorphine by Mass)	MEKC Results (% Diamorphine by Mass)	Difference (% Diamorphine by Mass)
1	11.9	11.9	0
2	51.6	54.1	–4.8
3	41.1	43.1	–4.9
4	59.4	57.1	3.8
5	81.9	81.4	0.6
6	79.7	78.9	1.0

and 3, respectively. Although N,N-dimethyl-5-methoxytryptamine has been reported as being used as a drug of abuse, this is rare and not likely to be encountered in a heroin sample (36). The choice of this drug as an appropriate internal standard would be an issue only if this method was to be applied to the analysis of opiates in the presence of tryptamines or phenethylamines.

Re-analysis of the six heroin seizure samples using N,N-dimethyl-5-methoxytryptamine as an internal standard gave good agreement with results obtained using a validated GC method (Table 5). Analytical figures of merit for the quantification of heroin are presented in Table 6. Figures of merit for the other species are provided in order to illustrate that the methodology could be used for quantitation of other alkaloids and adulterants found in heroin seizure samples. This may be applied to profiling of drug seizures. It should be noted that to obtain the level of reproducibility illustrated in Tables 2 and 6, a few routine operations (23,28,37) were required. The capillary was flushed with buffer prior to analysis, and a post separation washing of the capillary with 0.1 M sodium hydroxide was also utilised. The background electrolyte was also replaced every ten injections to avoid the effects of buffer depletion.

In order to examine the effect of not using an internal standard, quantification of the heroin seizure samples without the incorporation of an internal standard was also performed (Table 7). As can be seen, the analysis without the use of the internal standard produced unacceptable results.

TABLE 6—*Analytical figures of merit.*

Analyte	Calibration Function*	Correlation Coefficient (r^2)	RSD of Peak Area Ratio for Intra-Day (%)†	RSD of Peak Area Ratio for Between-Day (%)†
Caffeine	y = 6.66x – 0.023	0.9990	0.7	1.6
Paracetamol	y = 5.34x – 0.195	0.9987	0.8	1.5
Morphine	y = 4.27x – 0.162	0.9991	0.9	1.6
Codeine	y = 5.11x – 0.162	0.9993	0.8	1.3
Heroin	y = 3.77x – 0.148	0.9992	1.3	1.3
Acetylcodeine	y = 4.69x – 0.208	0.9990	1.3	1.4

* y = peak area (mAu), x = concentration (M).
† RSD calculated from 10 replicates of a 0.1 mg mL^{-1} test mixture.

TABLE 7—*Quantification of heroin in heroin seizure samples without the employment of an internal standard. MEKC Conditions: UV absorbance at 210 nm, uncoated fused silica capillary 50 cm × 50 μm I.D. × 360 μm O.D., effective separation length 8 cm, background electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15% (v/v) acetonitrile, pH 9.5, 25°C, –25 kV, hydrodynamic injection: 2 s at –50 mbar, n = 2. GC Conditions: HP 5890, FID, 12 m × 0.2 mm × 0.33 μm HP Ultra-1 capillary column, oven temperature: 260°C, injector temperature: 250°C, detector temperature: 300°C.*

Sample	GC Results (% Diamorphine by Mass)	MEKC Results (% Diamorphine by Mass)	Difference (% Diamorphine by Mass)
1	11.9	17.3	–45.3
2	51.6	68.8	–33.3
3	41.1	46.8	–13.8
4	59.4	71.8	–20.8
5	81.9	98.2	–19.9
6	79.7	76.1	4.5

Conclusion

The use of the short-end injection technique facilitated the development of a rapid and accurate method for the analysis of heroin seizures by MEKC. The method requires no sample treatment other than dilution and addition of an internal standard to give reproducible results that are comparable with a validated GC method. The use of short-end injection capillaries reduces analysis time and verifies the potential for the development of portable capillary electrophoresis instrumentation that can be used in the field (38) for preliminary screening at a crime scene.

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References

1. Weinberger R, Lurie IS. Micellar electrokinetic capillary chromatography of illicit drug substances. Anal Chem 1991;63:823–7.
2. Hilhorst MJ, Somsen GW, de Jong GJ. Capillary electrokinetic separation techniques for profiling of drugs and related products. Electrophoresis 2001;22(12):2542–64. [PubMed]
3. Lemos NP, Bortolotti F, Manetto G, Anderson RA, Cittadini F, Tagliaro F. Capillary electrophoresis: a new tool in forensic medicine and science. Science and Justice 2001;41:203–10. [PubMed]
4. Thormann W, Lurie IS, McCord B, Marti U, Cenni B, Malik N. Advances of capillary electrophoresis in clinical and forensic analysis (1999–2000). Electrophoresis 2001;22(19):4216–43. [PubMed]
5. Chiarotti M, Fucci N. Comparitive analysis of heroin and cocaine seizures. J Chromatogr B 1999;733:127–36.
6. Thormann W, Wey AB, Lurie IS, Gerber H, Byland C, Malik N, et al. Capillary electrophoresis in clinical and forensic analysis: Recent advances and breakthrough to routine applications. Electrophoresis 1999;20:3203–36. [PubMed]
7. Altria KD. Overview of capillary electrophoresis and capillary chromatography. J Chromatogr A 1999;856:443–63. [PubMed]
8. Tagliaro F, Manetto G, Crivellente F, Smith FP. A brief introduction to capillary electrophoresis. Forensic Sci Int 1998;92:75–88.
9. Deyl Z, Miksik I, Tagliaro F. Advances in capillary electrophoresis. Forensic Sci Int 1998;92:89–124. [PubMed]
10. Thormann W, Caslavska J. Capillary electrophoresis in drug analysis. Electrophoresis 1998;19(16–17):2691–94. [PubMed]
11. Lurie IS. Application of micellar electrokinetic capillary chromatography to the analysis of illicit drug seizures. J Chromatogr A 1997;780:265–84. [PubMed]
12. Lurie IS. Application of capillary electrophoresis to the analysis of seized drugs. Int Lab 1996:21–28.
13. Tagliaro F, Turrina S, Smith FP. Capillary electrophoresis: priciples and applications in illicit drug analysis. Forensic Sci Int 1996;77(3):211–29. [PubMed]
14. Lurie IS. Analysis of seized drugs by capillary electrophoresis. In: Adamovics JA, Ed. Analysis of addictive misused drugs. New York: Marcel Dekker Inc, 1995;151.
15. Thormann W, Molteni S, Caslavska J, Schmutz A. Clinical and forensic applications of capillary electrophoresis. Electrophoresis 1994;15:3–12. [PubMed]
16. Terabe S, Otsuka K, Ichikawa K, Tsuchiya A, Ando T. Electrokinetic separations with micellar solutions and open-tubular capillaries. Anal Chem 1984;56:111–13.
17. Nishi H, Terabe S. Micellar electrokinetic chromatography; Perspectives in drug analysis. J Chromatogr A 1996;735:3–27. [PubMed]
18. Krogh M, Brekke S, Tonnesen F, Rasmussen KE. Analysis of drug seizures of heroin and amphetamines by capillary electrophoresis. J Chromatogr A 1994;674:235–40.
19. Trenerry VC, Wells RJ, Robertson J. The analysis of illicit heroin seizures by capillary zone electrophoresis. J Chromatogr Sci 1994;32:1–6.
20. Walker J, Krueger ST, Lurie IS, Marche HL, Newby N. Analysis of heroin drug seizures by micellar electrokinetic capillary chromatography (MECC). J Forensic Sci 1994;40(1):6–9.
21. Altria KD. Method development/optimisation. In: Altria KD, Ed. Capillary electrophoresis guidebook, principles, operations and applications. Totowa: Humana Press, 1996;29–48.
22. Kelly MA, Altria KD, Clark BJ. Approaches used in the reduction of buffer electrolysis effects for routine electrophoresis procedures in pharmaceutical analysis. J Chromatogr A 1997;768:73–80.
23. Hyotylainen T, Siren H, Riekkola ML. Determination of morphine analogues, caffeine and amphetamine in biological fluids by capillary electrophoresis with the marker technique. J Chromatogr A 1996;735:439–47. [PubMed]
24. Altria KD, Kelly MA, Clark BJ. The use of a short-end injection procedure to achieve improved performance in capillary electrophoresis. Chromatographia 1996;43(3):153–8.
25. Fabre H, Mesplet N. Robustness testing for a capillary electrophoresis method using the 'short-end injection' technique. J Chromatogr A 2000;897:329–38. [PubMed]
26. Bergholdt AB, Jorgensen KW, Wendel L, Lehmann SV. Fast chiral separations using sulfated β-cyclodextrin and short-end injection in capillary electrophoresis. J Chromatogr A 2000;875:403–10. [PubMed]
27. Euerby MR, Johnson CM, Cikalo M, Bartle KD. "hort-end injection" rapid analysis capillary electrochromatography. Chromatographia 1998;47:135–40.
28. Wan H, Holem A, Nagard M, Lindberg W. Rapid screening of pKa values of pharmaceuticals by pressure assisted capillary electrophoresis combined with short end injection. J Chromatogr A 2002;979:369–77. [PubMed]
29. Perrett D, Ross G. Rapid determination of drugs in biofluids by capillary electrophoresis. Measurement of antipyrine in saliva for pharmacokinetic studies. J Chromatogr A 1995;700:179–86. [PubMed]
30. Bjornsdottir I, Hansen HS. Fast separation of 16 seizure drug substances using non-aqueous capillary electrophoresis. J Biochem and Biophys Methods 1999;38:155–61.
31. Small LF, Lutz RE. Chemistry of the opium alkaloids. Vol. Supplement No.103 to the Public Health Reports, Part II. Washington: U.S. Government Printing Office, 1932;153–4.
32. Lurie IS, Chan KC, Spratley TK, Casale JF, Issaq HJ. Separation and detection of acidic and neutral impurities in illicit heroin via capillary electrophoresis. J Chromatogr B 1995;669:3–13.
33. Krogh M, Brekke S, Tonnesen F, Rasmussen KE. Application of micellar electrokinetic chromatography to pharmaceutical analysis. J Chromatogr A 1994;674:235–40.
34. Otsuka K, Terabe S. Micellar electrokinetic chromatography. In: Altria KD, Ed. Capillary electrophoresis guidebook, principles, operations and applications. Totowa: Humana Press, 1996;125–55.
35. Altria KD. Improved performance in capillary electrophoresis using internal standards. LC GC Europe 2002;15(9):588–94.
36. http://www.louwho.com/homewith/react.html.

37. Altria KD, Bestford J. Main component assay of pharmaceuticals by capillary electrophoresis: Considerations regarding precision, accuracy, and linearity data. J Capillary Electrophoresis 1996;3(1):13–23.
38. Kappes T, Schnierle P, Hauser PC. Field-portable capillary electrophoresis instrument with potentiometric and amperometric detection. Anal Chim Acta 1999;393:77–82.

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