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Analysis of illicit amphetamine seizures by capillary electrophoresis

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Abstract

A capillary electrophoretic (CE) method has been developed for the quantitation of complex seizures of amphetamines and related substances. Comparison with a currently used gas chromatographic (GC) method demonstrates that the CE method has the same order of repeatability, is faster, uses minimal organic solvent and has a resolving power which allows separations of certain compounds not attainable by the GC procedure.

1. Introduction

The majority of recent investigations on the utility of capillary electrophoresis (CE) have concentrated on the exploration of the outstanding separation potential of the technique. Less emphasis has been placed on the routine use of CE as a quantitative analytical tool [1].

Separation of a wide variety of illicit drug substances by micellar electrokinetic capillary chromatography (MEKC) was recently reported by Weinberger and Lurie [2]. However, the focus of that work was directed towards the separation potential of capillary electrophoresis (CE) rather than its use as a quantitative technique for use in drug analysis and profiling.

We recently reported the utility of MEKC for the separation and quantitation of illicit heroin seizures [3]. The buffer system developed by Weinberger and Lurie [2] was found to be unsuitable for the routine automated analysis of a large batch of samples but changing from sodium dodecyl sulphate to cetyltrimethylammonium bromide as the micellar additive overcame repeatability problems. The outstanding resolving power of CE enabled the separation of some complex heroin seizures far more rapidly than high-performance liquid chromatography (HPLC) and allowed detection and determination of some substances in the mixtures which were not achievable by HPLC. Furthermore, it was demonstrated that quantitative results obtained by CE were comparable to HPLC in both the quantitation and coefficient of variation determined for each constituent.

This work was subsequently extended to the analysis of seizures of cocaine and related substances [4]. It was found in this investigation that identical MEKC conditions could be used to quantitate both illicit heroin and cocaine seizures with only a alteration of UV detector wavelength

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being required to change from one analytical target to the other.

We have now focussed on the use of CE in the quantitative analysis of amphetamines. Our expectation that the electrophoretic buffer system utilised for heroin and cocaine would be equally applicable to amphetamines was not realised. A totally new buffer system had to be developed to enable reliable routine quantitation of the wide range of various amphetamines and related compounds commonly found in the analysis of illicit amphetamine seizures.

The CE analysis of some amphetamine derivatives of interest in the present work has previously been reported [5,6]. The range of amphetamine derivatives of primary interest for the analysis of illicit drug seizures was not fully explored in these reports. The chiral differentiation of some amphetamine derivatives by CE has also been reported [7].

This paper describes the development of a reliable method for identification and quantitation of common amphetamines and associated impurities found in illicit seizures found in Australia. A comparison of the results of quantitative analysis of major seizures by the GC method used in routine operational work in our laboratories [8] with that obtained on the same seizures by CE is also reported.

2. Experimental

2.1. Reagents

Amphetamine sulphate, methamphetamine hydrochloride, ephedrine, pseudoephedrine, norephedrine, pseudonorephedrine, methylenedioxyamphetamine, methylenedioxymethamphetamine, 4-methoxyamphetamine, 3,4-dimethoxyamphetamine, 4-bromo-2,5-dimethoxyamphetamine and caffeine were obtained from the Curator of Standards, Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, NSW 2073, Australia. Cetyltrimethylammonium bromide was obtained from Sigma (St. Louis, MO, USA), or from E. Merck (Kilsyth, Vic., Australia). All other chemicals and solvents were of AR grade or HPLC grade and were used without further purification.

2.2. Apparatus

Capillary electrophoresis

Qualitative and quantitative analyses were performed with 72 cm × 75 μm I.D. fused-silica capillary tubes (Isco, Lincoln, NE, USA), with an effective length of 50 cm to the detector window.

An Isco Model 3140 electropherograph (Isco) was used for all of the analyses. For quantitative work, the instrument was operated at −15 kV and at a temperature of 30°C. The sample solution was loaded onto the capillary under vacuum (vacuum level 2, 10.0 kPa s for Model Isco 3140 electropherograph). Under these conditions the detector response for caffeine was linear to 0.2 mg/ml and to 1.6 mg/ml for the amphetamines.

The compounds were detected at 254 nm at 0.01 AUFS. Electropherograms were recorded and processed with either the ICE Data Management and Control Software supplied with the Model 3140 electropherograph or a HP 3350A laboratory data system (Hewlett-Packard, Palo Alto, CA, USA). Peak-area ratios were used in the calculations.

Gas chromatography

The analyses were performed with a Varian 3400 gas chromatograph equipped with a Varian 8100 autosampler and a flame ionisation detector (Varian Associates, Walnut Creek, CA, USA), using a 25QC2/BP1 0.25 μm fused-silica capillary column (SGE Pty, Ringwood, Vic., Australia) operating in the split injection mode. Hydrogen was used as the carrier gas at a pressure of 12 p.s.i. and a split ratio of approx 50:1. The injector temperature was 230°C and detector temperature was maintained at 280°C. After injection the column temperature was maintained at 80°C for two min followed by temperature programming from 80°C to 200°C at 10°C/min and holding at the final temperature for 2 min. The data were analysed with a HP 3350A laboratory data system (Hewlett-Pac-

kard). Peak-area ratios were used in the calculations.

2.3. Preparation of standards, samples and buffers

Capillary electrophoresis work

Standards

Stock solutions of amphetamines and related substances were prepared weekly at 1 mg/ml by dissolving the substances in 0.01 *M* HCl. Caffeine was used as the internal standard and prepared in the same way at 1 mg/ml. The solutions were refrigerated before use. The stock solutions were diluted with de ionised water and filtered through a 0.45-μm cellulose acetate filter disc before analysis. A typical standard solution contains 0.4 mg/ml of amphetamine (or related substances) and 0.1 mg/ml of caffeine.

Samples

Sample solutions were prepared by mixing a weighed amount of sample (10–50 mg) with 1 ml of caffeine solution and diluting to 10 ml with 0.01 *M* HCl. The mixture was sonicated for 2 min, mixed thoroughly, filtered through a 0.45-μm cellulose acetate filter disc and refrigerated before use.

Buffers

CTAB buffers (0.025 *M*) were prepared by dissolving 0.92 g CTAB in 100 ml of 0.01 *M* sodium tetraborate. The pH of the solution is adjusted to 11.5 with 1 *M* NaOH. The running buffer was prepared by adding 88 ml of this solution to 1 ml of ethanolamine and 11 ml of dimethylsulphoxide. The solution was mixed thoroughly and filtered through a 0.45-μm PTFE filter disc before use.

Procedure for capillary preparation and handling

The capillary was filled with 1 *M* NaOH and allowed to stand for 1 h. This solution was replaced with 0.1 *M* NaOH, allowed to stand for a further hour and washed with deionised water before filling with the running buffer. The capillary was cleaned on a weekly basis by washing with 0.1 *M* HCl for 10 min, followed by successive washings with de ionised water, 0.1 *M* NaOH and deionised water before refilling with buffer.

For both qualitative and quantitative analyses, the capillary was flushed with running buffer for 2 min between analyses.

Gas chromatography work

Standards

Standard solutions were prepared by dissolving a known weight of substance (10–15 mg) in 2 ml of deionised water, basifying the solution with 5 drops of concentrated ammonia solution and extracting with methylene chloride (2×3 ml, 1×2 ml). The organic solution was dried over sodium sulphate, filtered and added to 1 ml of diphenylamine (4 mg/ml) in methylene chloride and made to 10 ml with methylene chloride. A typical standard solution contains 1 mg/ml of amphetamine (or related substances) and 0.4 mg/ml of diphenylamine.

Samples

Sample solutions were prepared by mixing a weighed amount of illicit substance (20–70 mg) with deionised water and proceeding as described for the preparation of standards.

3. Results and discussion

3.1. The search for suitable buffer mixtures

The successful combination of heroin and cocaine separations into a single quantitative method by CE encouraged the investigation of the suitability of the same method for the analysis of a range of amphetamines and related stimulants such as ephedrine and pseudoephedrine.

Separation of a mixture of norephedrine, pseudoephedrine, ephedrine, amphetamine and methamphetamine with the pH 8.6 CTAB buffer used previously and modified by addition of 10% acetonitrile as the micelle modifier gave satisfactory separations and peak shapes (albeit rather

broad) at low analyte concentrations. However, at higher analyte concentrations peak symmetry was lost and all peaks had a broad sawtooth shape. Although both separations and peak shapes were also satisfactory at low analyte concentrations when the organic modifier was either 10% dimethylformamide or 10% dimethylsulfoxide, the same problem of broad sawtooth-shaped peaks was again apparent at higher analyte concentrations.

Systematic variation of the pH of the buffer was now investigated. Electrophoretic reproducibility at pH values between 3 and 8 in CE is difficult because of the sensitivity of electroosmotic flow to small changes in buffer pH within this range. Therefore a move to more alkaline buffer conditions was first investigated. In a CTAB-phosphate–borate buffer at pH 10.5 with 7.5% acetonitrile, good separations of many amphetamine related substances were obtained. Pseudoephedrine migrated slower than ephedrine in this system but peak shapes showed a tendency to tail. Also the peak shape for *dl*-pseudonorephedrine was very poor at low analyte concentrations. However, at higher analyte concentrations, peudonorephedrine appeared as two partially resolved peaks. Reasons for this behaviour are not immediately apparent. There was little effect on analyte separation but some increase in peak sharpness on changing the amount of acetonitrile modifier from 0% to 12.5% in 2.5% increments. At acetonitrile concentrations above 12.5% separation efficiency deteriorated.

The change of buffer from phosphate–borate to borate at pH 10.5 with 0.05 *M* CTAB as micellar additive and acetonitrile as micelle modifier also gave satisfactory separations. Again this system caused peak tailing, particularly in the pseudoephedrine series and *d*-pseudonorephedrine appeared as a grossly distorted peak. The separations, peak widths and, in particular, the peak shape of pseudonorephedrine improved as the buffer pH was raised to 11.5 which, together with the use of acetonitrile as micelle modifier, appeared optimal for this particular system. However, accurate quantitative work could not be achieved with this buffer mixture.

Halving the concentration of CTAB to 0.025 *M* and replacing the 7.5% acetonitrile with 20% dimethylsulphoxide further improved the peak shapes of pseudonorephedrine and pseudoephedrine and the overall separation of the components. However, when standard mixtures of norephedrine, pseudonorephedrine, ephedrine, pseudoephedrine, amphetamine, methylenedioxyamphetamine, methamphetamine and methylenedioxymethamphetamine of 0.1, 0.25 and 0.5 mg/ml respectively were run nine times each, the linearity of the detector response for pseudonorephedrine and pseudoephedrine in particular were totally unsatisfactory. The peak shapes of pseudonorephedrine and pseudoephedrine changed dramatically after nine runs for the 0.1 mg/ml and 0.5 mg/ml solutions. Accordingly, the R.S.D. was poor for pseudoephedrine at low concentrations (standard concentration 0.1 mg/ml, R.S.D. 39.1%) and poor for pseudonorephedrine at higher concentrations (standard concentration 0.5 mg/ml, R.S.D. 32.2%).

We now sought to improve the linearity and peak shapes of pseudonorephedrine and pseudoephedrine by the inclusion of β-cyclodextrin into the buffer. Addition of 0.005 *M* β-cyclodextrin did give some peak sharpening and indications of better resolution. With 0.01 *M* β-cyclodextrin a reasonable resolution and good peak shapes for norephedrine, pseudonorephedrine, ephedrine and pseudoephedrine could be obtained. However, the separation of the remaining substances in the mixture diminished. As with the previous buffer, when the standard mixtures of norephedrine, pseudonorephedrine, ephedrine and pseudoephedrine of 0.1, 0.25 and 0.5 mg/ml respectively were run seven times each, the linearity of the detector response for pseudonorephedrine, norephedrine and pseudoephedrine in particular were still unsatisfactory and the R.S.D. for the instrument repeatability was poor at low concentrations (standard concentration 0.1 mg/ml, norephedrine R.S.D. 8.0%, pseudonorephedrine R.S.D. 5.3%, ephedrine R.S.D. 5.0%, pseudoephedrine R.S.D. 13.5%). Dimethyl-β-cyclodextrin was also unsatisfactory as a buffer additive in solving the problems with pseudonorephedrine and pseudoephedrine.

3.2. Ethanolamine containing buffers

The failure of "standard" buffer mixtures to give a stable and reproducible CE system for the whole range of amphetamines of interest led to the investigation of less traditional mixtures.

Pseudoephedrine and pseudonorephedrine, the substances which had caused the major problems, are 2-aminoethanol derivatives. We therefore argued that the errant CE behaviour exhibited by these substances might be suppressed if the CE buffer system was "flooded" by a compound with the same structural features.

This approach proved immediately successful and a stable and reproducible buffer system was rapidly developed which enabled not only the baseline separation of all amphetamines of interest but gave satisfactory detector linearity and R.S.D. on repeated injections for all compounds of interest, the problematical pseudoephedrines in particular. DMSO was found to be the micelle modifier of choice and the optimised CE system for amphetamine separation and quantitation is 0.025 *M* CTAB in a 0.01 *M* tetraborate buffer modified with 11% DMSO and 1% ethanolamine and adjusted to pH 11.5 with 1 *M* sodium hydroxide. The electropherogram of a standard mixture of amphetamine run in this buffer is shown in Fig. 1. The reproducibility of the system over 20 runs was excellent. The R.S.D. for instrument repeatability for each substance run seven times was also satisfactory. (Standard concentration 0.2 mg/ml, norephedrine R.S.D. 2.5%, pseudonorephedrine R.S.D. 3.1%, ephedrine R.S.D. 2.7%, pseudoephedrine R.S.D. 2.1%, amphetamine R.S.D. 1.0%, methylenedioxyamphetamine R.S.D. 0.9%, standard concentration 0.25 mg/ml, methamphetamine R.S.D. 3.4%, methylenedioxymethamphetamine R.S.D. 1.6%).

3.3. Less commonly encountered amphetamines

Although less commonly encountered as seizures in Australia, 4-methoxymethamphetamine (4-MMA) 3,4-dimethoxyamphetamine (3,4-DMA) and 4-bromo-2,5-dimethoxyamphetamine (4-Br-2,5-DMA) are, on occasion encountered as illicit drugs.

All three substances behave well in the ethanolamine buffer system. The detector response for these compounds was linear up to 0.5 mg/ml and the R.S.D. of quantitation after nine replicate injections was also satisfactory. (Standard concentration 0.25 mg/ml, 4-MMA R.S.D.

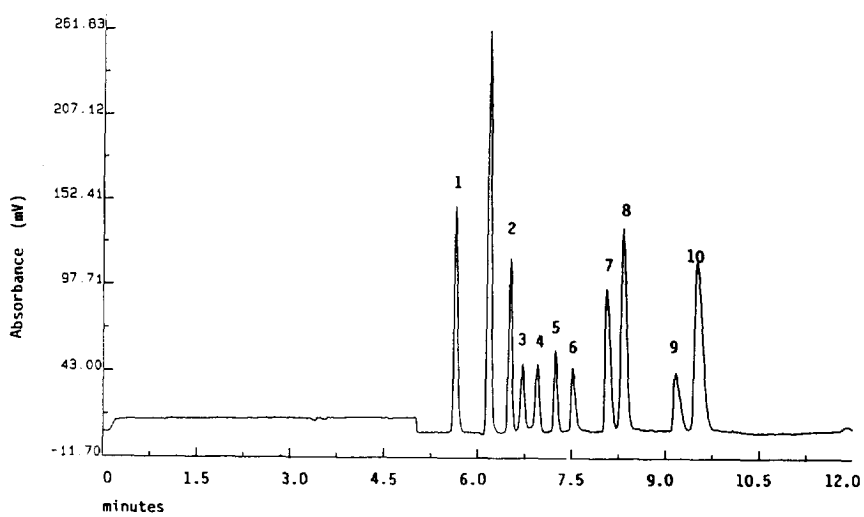

Fig. 1. Electropherogram showing the separation and peak shapes of *p*-aminobenzoic acid (1), caffeine (2), norephedrine (3), pseudonorephedrine (4), ephedrine (5), pseudoephedrine (6), amphetamine (7), methylenedioxyamphetamine (8), methamphetamine (9) and methylenedioxymethamphetamine (10) using a 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.

1.8%, 3,4-DMA R.S.D. 1.4%, 4-Br-2,5-DMA R.S.D. 4.7%).

3,4-DMA co-eluted with ephedrine and 4-MMA co-eluted with MDA. 4-Bromo-2,5-dimethoxyamphetamine appeared after MDMA. The substances which co-elute in this system can be readily differentiated by their different UV spectra if a diode-array UV detector is used to record results. The separation of methoxyamphetamines is shown in Fig. 2.

3.4. Comparison of quantitation of amphetamines by CE and GC

It now remained to establish that the new quantitative CE method developed for amphetamines was not only robust and repeatable but gave comparable results to the GC method which had been previously validated.

The R.S.D. for the GC method (see Experimental for details) was determined for the same eight amphetamine analogues that were evaluated in the CE method. As with the CE method, the R.S.D. was calculated from seven replicate injections. The R.S.D.s for instrument repeatability for the amphetamines by the GC method were similar to those determined for the CE method. The large R.S.D. for the GC procedure at low concentration coincided with poor peak shape. Both the peak shape and the R.S.D. improved at the two higher concentrations. The GC determination requires the amphetamine salts to be converted to the free base before injection onto the column. This adds an extra step and therefore more uncertainty to that method. A further drawback of the GC method was the co-elution of ephedrine and pseudoephedrine. A GC chromatogram of standards is shown in Fig. 3.

Results of the analysis of a series of illicit amphetamine and methamphetamine seizures by both CE and GC, each injected seven times, are reported in Table 1. The electropherogram for sample 90/54 is displayed in Fig. 4.

Both CE and GC give similar but non-identical results in our hands. There appears to be a difference in quantitation between the two methods, with no discernible systematic bias evident, which will be addressed in future by undertaking inter-laboratory quality assurance studies using both methods in parallel. The reasons for major differences between results from the CE method

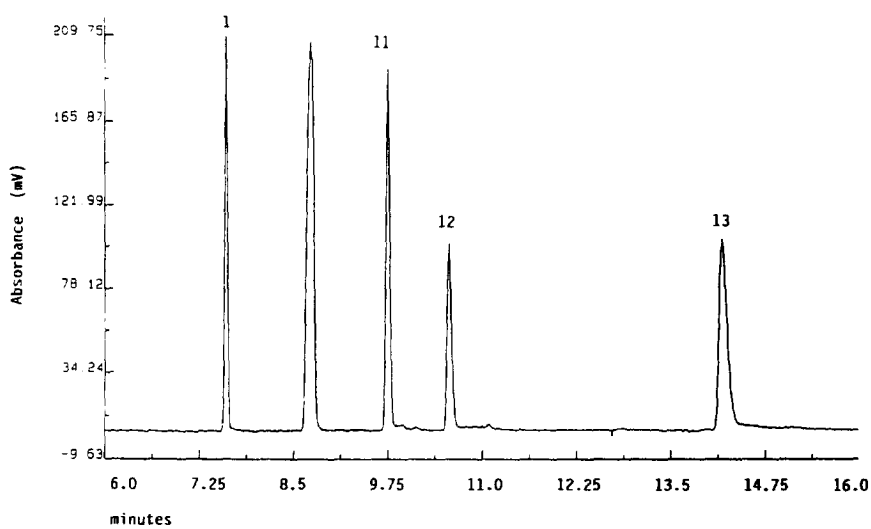

Fig. 2. Partial electropherogram showing the separation and peak shapes of *p*-aminobenzoic acid (1), 3,4-dimethoxyamphetamine (11), 4-methoxyamphetamine (12) and 4-bromo-2,5-dimethoxyamphetamine (13), using a buffer consisting of 1% ethanolamine/11% DMSO/88% 0.025 *M* CTAB, 0.01 *M* sodium tetraborate pH 11.5. *p*-Aminobenzoic acid was added as the internal standard.

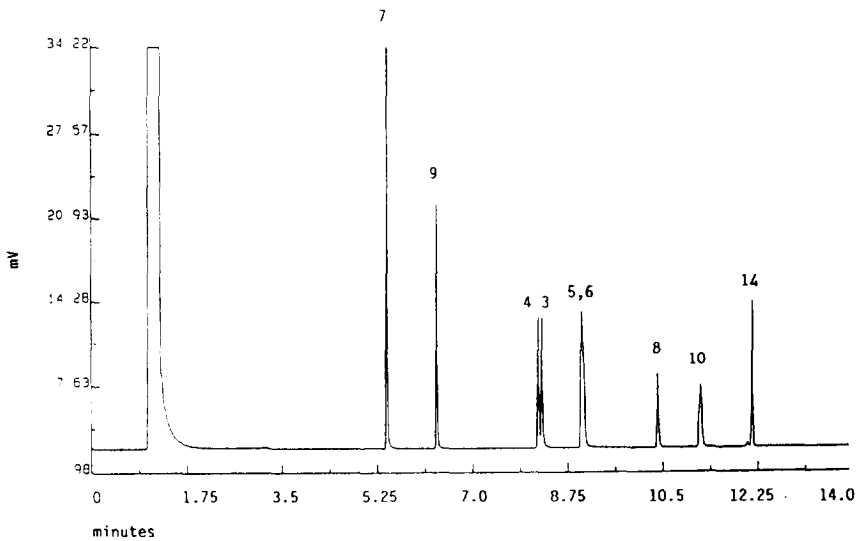

Fig. 3. Gas chromatogram showing the separation of amphetamine (7), methamphetamine (9), pseudonorephedrine (4), norephedrine (3), ephedrine (5), pseudoephedrine (6), methylenedioxyamphetamine (8), methylenedioxymethamphetamine (10) and diphenylamine (14). Diphenylamine was added as the internal standard.

and those from the GC procedure are not immediately apparent. However, recent quality assurance proficiency studies conducted between State and Commonwealth laboratories in Australia suggested that there is no discernible difference in the quantitation of heroin by CE when compared to HPLC or GC procedures respectively. Indeed, the CE procedure performed very well in comparison to other methods. A similar quality assurance proficiency

Table 1
Comparison of quantitative results presented as mean ± R.S.D. ($n = 7$) for CE and GC for a number of illicit amphetamine and methamphetamine seizures

Seizure	Conc. of A (%) (R.S.D., %)		Conc of MA (%) (R.S.D., %)		Other	
	CE	GC	CE	GC	CE	GC
Q92/684	23.7 (2.7)	22.0 (3.1)				
Q92/703	23.3 (2.1)	24.8 (0.4)				
93/126			7.8 (1.4)	8.9 (0.4)		
93/117			5.3 (4.0)	5.9 (0.4)		
93/118			6.0 (2.3)	5.9 (0.6)		
Q9033/3			19.7 (0.8)	20.7 (2.8)	57 (1.1)	66 (2.3)[a]
90/54	4.4 (2.4)	4.8 (1.0)			18.4 (1.3)	18.7 (2.9)[b]

A = amphetamine, MA = methamphetamine.
[a] Shown to be pseudoephedrine by CE.
[b] Shown to be ephedrine by CE.

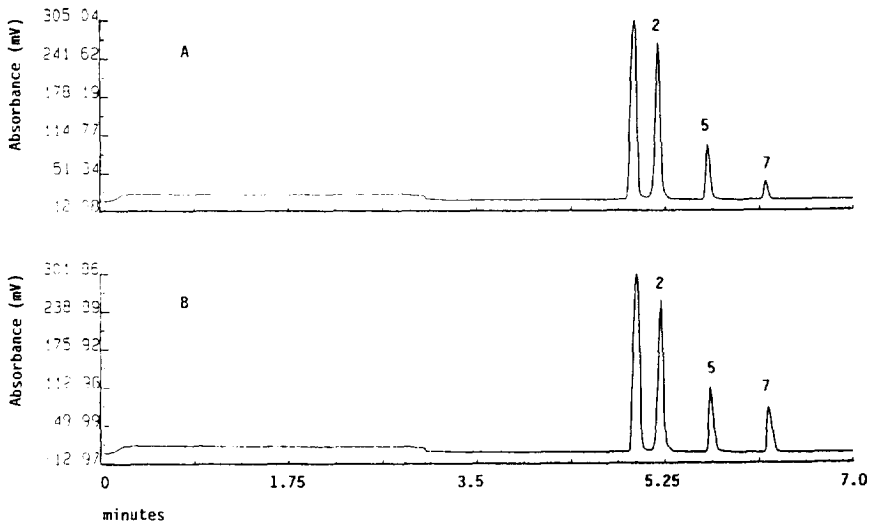

Fig. 4. Electropherogram of (A) sample 90/54 and (B) a standard solution showing caffeine (2), ephedrine (5) and amphetamine (7) using a buffer consisting of 1% ethanolamine/11% DMSO/88% 0.025 *M* CTAB, 0.01 *M* sodium tetraborate pH 11.5. Caffeine was present as the internal standard.

study will be performed in the amphetamine series to allow statistical evaluation of the new CE method against regularly used routine methods.

4. Conclusions

It has been demonstrated that CE is capable of giving quantitative data on amphetamine seizures which are comparable to those obtained by a fully validated GC method. However, the CE technique allows identification and quantitation of amphetamine derivatives which cannot be achieved by GC.

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References

[1] S.F.Y. Li, Capillary Electrophoresis: principles, practice and applications (J. Chromatogr. Library, Vol. 52), Elsevier, Amsterdam, 1992.
[2] R. Weinberger and I.S. Lurie, Anal. Chem., 63 (1991) 823.
[3] V.C. Trenerry, J. Robertson and R.J. Wells, J. Chromatogr. Sci., 32 (1994) 1.
[4] V.C. Trenerry, J. Robertson and R.J. Wells, Electrophoresis, 15 (1994) 103.
[5] Y.-M. Liu and S.-J. Sheu, J. Chromatogr., 600 (1992) 370.
[6] M. Krogh, S. Brekke, F. Tonnesen and K. Rasmussen, J. Chromatogr., 674 (1994) 235.
[7] I.S. Lurie, J. Chromatogr., 605 (1992) 269.
[8] Recommended methods for testing amphetamine and methamphetamine, United Nations Division of Narcotic Drugs, Vienna, United Nations, New York, 1987.