ELSEVIER

Journal of Chromatography A, 979 (2002) 345–352

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analysis of illicit amphetamine seizures by capillary zone electrophoresis

Véronique Piette*, Frans Parmentier

Laboratory of Drug Analysis, Scientific Institute of Public Health—Louis Pasteur, Rue Juliette Wytsman 14, B-1050 Brussels, Belgium

Abstract

Capillary zone electrophoresis was applied for the determination of amphetamine and related substances in seized drugs. A buffer made of 0.1 *M* phosphoric acid adjusted to pH 3.0 with triethanolamine was selected. With this background electrolyte, triethanolamine is adsorbed to the capillary wall and the electroosmotic flow is reversed. This gives rise to peaks with good symmetry, high efficiency and reproducible migration times. The separation of the different analytes was performed in a fused-silica capillary thermostatted at 25 °C and the applied voltage was 25 kV. Under these experimental conditions, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethamphetamine, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butamine and ephedrine were resolved within 8 min and without interference from adulterants usually found in illicit powders. Their identification by the migration time was confirmed by their UV spectra recorded with a diode array UV detector (190–350 nm). The selected method was then applied to identify these substances in illicit tablets known as ''Ecstasy'' and the MDMA determined in these samples according to a laboratory validation procedure.

Keywords: Forensic analysis; Drugs; Amphetamines; Ephedrine; Ecstasy

1. Introduction

Amphetamine and related substances are reported among the most used illicit drugs for their psychostimulant effects [1]. Their use produces an elevation of mood (euphoria) and a sense of increased self-esteem and mental and physical capacity. It seems likely that the major mechanism by which amphetamine-like drugs produce their effects is their capacity to release newly synthesized dopamine from intraneuronal stores [2,3]. In addition to the more common toxic effects (tremor, confusion, hallucinations, hypertension and cardiac arrhythmias), the developed tolerance and physical dependence produced by the use of these substances constitute the main reasons for their legal restriction.

In order that these drugs are effectively controlled, selective analytical methods suitable for their unambiguous identification and determination in illicit samples are necessary.

In forensic drug testing programs, two independent methods are required to report a sample as positive [4]. Presently, gas chromatography coupled to mass spectrometry (GC–MS) is considered the method of choice for a confirmation [5,6]. Indeed, this is a highly sensitive and specific method which identifies drugs without ambiguity, but the equipment and the maintenance are relatively costly and

*Corresponding author. Tel.: +32-2-642-5145; fax: +32-2-642-5327.

E-mail address: veronique.piette@iph.fgov.be (V. Piette).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01405-X

need qualified operators. Several other analytical methods are described in the literature for the screening and quantitation of amphetamines such as radioimmunoassays [7], enzyme-multiplied immunoassay technique [8,9], fluorescence polarization immunoassay [8], competitive binding immunoassay [10] or thin-layer chromatographic methods [11]. Other chromatographic methods have been developed for the identification and quantitation of amphetamines in biological samples: GC coupled with several detection methods [electron-capture detection (ECD), flame ionization detection (FID), nitrogen–phosphorus detection (NPD)] [12] and reversed-phase liquid chromatography coupled with UV detection with or without a derivatization step [13,14]. More recently, high-performance capillary electrophoresis (HPCE) has become a complementary analytical tool to classical GC and high-performance liquid chromatography (HPLC).

Besides these chromatographic techniques, HPCE provides very high efficiency, short analysis times, low operational cost and fast method development [15]. The analysis of drugs, in both pharmaceutical preparations and body fluids, represents one of the most rapidly growing application areas of HPCE which is ideally suited to the analysis of such as polar metabolites [16].

Specific applications in the field of illicit or controlled drugs appeared 10 years ago with the use of micellar electrokinetic capillary chromatography (MEKC) for the study of heroin, cocaine and amphetamine using sodium dodecyl sulfate (SDS) [17–22] or cetyltrimethylammonium bromide (CTAB) [23–25] as surfactants. This separation technique was also applied for the toxicological investigation of biological fluids (urine, blood) and hair [26–32]. Studies using capillary zone electrophoresis (CZE) were presented for the quantitative determination of cocaine and heroin in illicit powders [33,34] and for the investigation of amphetamine derivatives in seized drugs using phosphate buffers at low pH [35–40] including their enantiomeric separation [32,41–43]. Recently, electrochromatography was applied to cannabinoids and methamphetamine [44,45].

In HPCE the use of acidic buffers is particularly favourable for the resolution of basic compounds in uncoated fused-silica capillaries, since these compounds are present in cationic form and the electroosmotic flow (EOF) is minimized [16].

The suppression of the EOF and of analyte interactions with the capillary wall is also favourable and can be obtained by modifying the surface of the capillary by dynamic coating with hydrophylic polymers [40,46–49], by permanent coating with polyacrylamide [50–53] or by the use of alkylammonium ions in the buffer [54–59]. In this situation, these cationic components can be adsorbed to the capillary wall, leading in most cases to a reversal of the EOF [60–64].

The usefulness of 0.1 *M* phosphate buffer adjusted to pH 3.0 with triethanolamine was previously demonstrated for the HPCE analysis of basic drugs and especially their enantioseparation [60,61,64]. Triethanolamine was found to be a particularly suitable buffer co-ion for cationic analytes giving rise to peaks with good symmetry, high efficiency and reproducible migration times.

In this study, the latter buffer was investigated to improve the separation of amphetamine (A), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethamphetamine (MDEA), *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butamine (MBDB) and ephedrine (E) in order to determine these substances (cf. Fig. 1) in illicit tablets known under the generic name ‘‘Ecstasy’’.

2. Experimental

2.1. Apparatus

All experiments were performed on a Beckman P/ACE 5500 CE instrument (Beckman Instruments, Fullerton, CA, USA) equipped with an autosampler, a UV diode-array detector (190–600 nm) and a temperature-control system (15–50 °C). A Digital Model 5510 PII-266 computer (Digital Equipment, Maynard, MA, USA) was used for instrument control and data handling using P/ACE Station software version 1.0. (Beckman). Electropherograms were printed on a HP LaserJet 6L printer (Hewlett-Packard, Boise, USA). A column cartridge was obtained from Beckman. The pH of the buffer solutions was

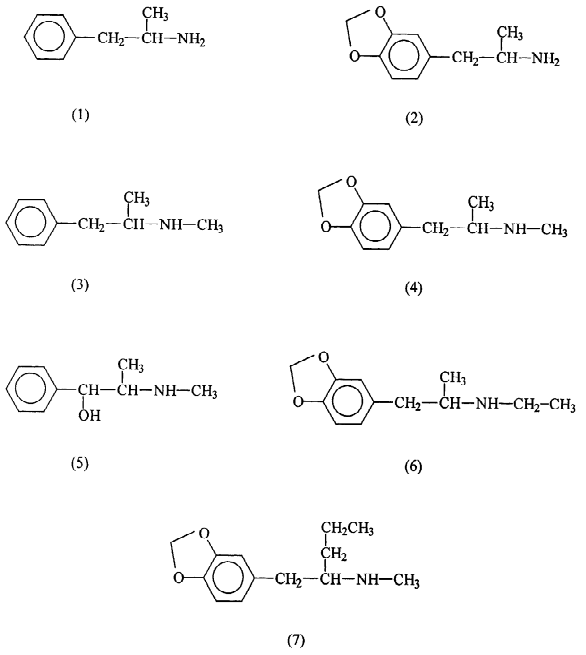

Fig. 1. Amphetamine and related substances: (1) amphetamine (A), (2) 3,4-methylenedioxyamphetamine (MDA), (3) methamphetamine (MA), (4) 3,4-methylenedioxymethamphetamine (MDMA), (5) ephedrine (E), (6) 3,4-methylenedioxyethamphetamine (MDEA), (7) *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butamine (MBDB).

measured with a Metrohm pH meter Model 713 (Metrohm, Herisau, Switzerland).

2.2. Chemicals

Phosphoric acid (85%) and triethanolamine were of analytical-reagent grade quality from Merck (Darmstadt, Germany). Water used for solution preparation was of Milli-Q quality (Millipore, Bedford, MA, USA).

Amphetamine sulfate, methamphetamine hydrochloride, ephedrine hydrochloride, paracetamol, caffeine and phenylephrine hydrochloride were obtained from Federa (Brussels, Belgium). 3,4-Methylenedioxyamphetamine and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butamine were isolated from authenticated casework samples. Benzylic alcohol (0.01% methanolic solution), 3,4-methylenedioxymethamphetamine hydrochloride and 3,4-methylenedioxyethamphetamine were purchased from Sigma (St. Louis, MO, USA). Illicit ''Ecstasy'' tablets were provided by different sources.

2.3. Standard solutions and sample preparation

Each reference solution was obtained by dissolving one compound in water at a concentration of 50 μg/ml. A reference mixture was prepared with the seven different compounds (A, MA, MDA, MDMA, MDEA, MBDB, E) each at 50 μg/ml in water.

For MDMA quantification, a stock solution of phenylephrine in water (150 μg/ml) was used as an internal standard. Two standard stock solutions containing 60.0 mg of MDMA in 100 ml were prepared in water. Various aliquots (7, 9, 11, 13 ml) of the first standard stock solution were taken, 20 ml of the internal standard added and then diluted to 100 ml with water. The same preparation was done with 10 ml of the second standard stock solution.

The ''Ecstasy'' tablet was ground in a mortar with a pestle to a fine and homogeneous powder. Two separate masses of 25 mg of this powder were dissolved in 20 ml of water, sonicated for 15 min and diluted to 25 ml with water. For each tablet extract, a first aliquot of 10 ml was diluted to 50 ml with water, a second aliquot of 10 ml was added to 10 ml of the internal standard solution and diluted to 50 ml with water and a third aliquot of 0.8 ml was added to 3.2 ml of the reference mixture and homogeneised in order to constitute the spiked sample. A 10-ml volume of the internal standard solution was diluted to 50 ml with water and was used as the blank solution. Sample solutions were filtered through a Polypure polypropylene membrane filter (0.2 μm) from Alltech (Laarne, Belgium) before use.

2.4. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused-silica capillaries, 47 cm length (40 cm to the detector)×50 μm I.D., provided by Supelco (Bellefonte, PA, USA). Before use, the capillary was treated successively with basic solutions (1 *M* NaOH followed by 0.1 *M* NaOH), water and running buffer. The latter consisted of 0.1 *M* phosphoric acid adjusted to pH 3.0 with tri-

ethanolamine (0.084 *M*) and was filtered through a Polypure polypropylene membrane filter (0.2 μm) from Alltech before use. At the beginning of each working day, the capillary was washed with separation buffer for 15 min and after each injection a washing of the capillary with buffer for 2 min was performed. The injections were made at the anodic side and the applied voltage was +25 kV (normal polarity mode). The reversed polarity mode (−25 kV) was used to measure the anodic electroosmotic mobility μ_{EOF} ($I \approx 58$ μA) with benzylic alcohol (0.01% methanolic solution) as neutral marker to visualize the electroosmotic flow breakthrough. Injections were made using the hydrodynamic mode (3447.38 Pa) for a period of 5 s. The diode array UV detection (at the cathodic side) was performed from 190 to 350 nm and the capillary was thermostatted at 25 °C.

The resolution (R_s) and plate number (N) were calculated according to the standard expressions based on peak width at half-height [65]. The asymmetry factor (A_s) was determined using the expression: $A_s = W/2F$ where W is the width of peak at 5% of height and F is the distance between the front edge and the top of the peak at 5% of peak height. Peak areas were normalised by the correction of the peak area with the corresponding migration time.

3. Results and discussion

3.1. Selected buffer composition and pH

All reported analysis were performed at 25 °C using a buffer made of 0.1 *M* phosphoric acid adjusted to pH 3.0 with triethanolamine (cf. Section 2.4). At this pH, triethanolamine was adsorbed to the capillary wall and gave rise to a reversal of the EOF, as observed with other alkylammonium ions added to the running buffer, such as short-chain tetraalkylammonium ions [63] or polyamines, like spermine [66]. It should be noted that this reversal of the EOF observed with triethanolamine was strongly pH-dependent since it was only obtained at a pH of around 3 [67]. At pH 3.0, the electroosmotic mobility, μ_{EOF}, had a low and fairly constant value of $-4.0 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ (EOF toward the anode), leading to highly reproducible migration times (t_m) [61]. In this situation, the possibilities of interactions between the cationic analytes (migrating toward the cathode) and the capillary wall were minimized leading to an improvement in peak symmetry and efficiency. For example using the selected electrophoretic technique, for A: $N = 230\,385$ plates, $A_s = 1.01$ and for MA: $N = 223\,458$ plates, $A_s = 1.02$.

3.2. Separation of amphetamine and related substances

Fig. 2 presents the different electropherograms obtained under the selected conditions (UV detection at 190 nm) for the reference solutions of A, MA, MDA, MDMA, E, MBDB, MDEA and for caffeine and paracetamol. No interferences from these two adulterants usually found in illicit powders could be observed at the migration times of the other analytes. These compounds migrating slowly at the chosen pH are usually studied at higher pH using sodium dodecyl sulfate as surfactant in the buffer [68]. For the analysis of the studied substances, the chosen method proved to be selective. Indeed, the sugars

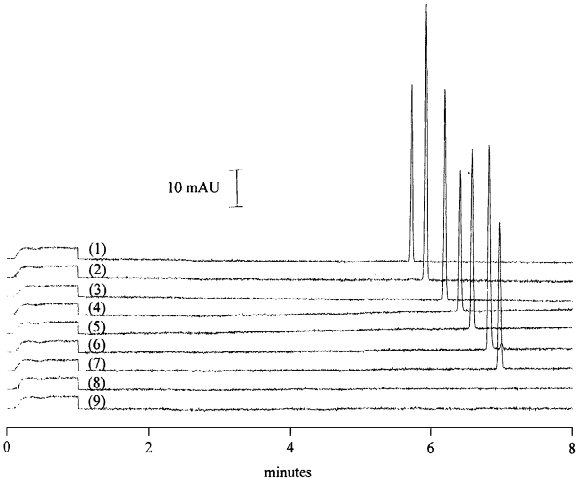

Fig. 2. Electropherograms of reference compounds: (1) A, (2) MA, (3) MDA, (4) MDMA, (5) E, (6) MBDB, (7) MDEA, (8) caffeine, (9) paracetamol. Electrolyte: 0.1 *M* phosphoric acid adjusted to pH 3.0 with triethanolamine. Detection: UV at 190 nm. Other conditions as described in Section 2.

and the salts are not detectable by UV detection and do not interfere at all.

Under the conditions selected, good results with respect to the repeatability of migration times were obtained, as illustrated by the relative standard deviation (RSD) values obtained for the migration time of A (t_m=5.70 min) and MDMA (t_m=6.35 min): respectively, 0.26 and 0.23% (n=10).

A reference mixture containing A, MA, MDA, MDMA, E, MBDB and MDEA was prepared as described in Section 2.3 and injected using the same HPCE method. Fig. 3 shows the separation of the seven substances in this mixture performed within 8 min. Closely related compounds like MDA and MDMA which differ only by a methyl group were baseline separated. The observed resolution was not less than 2.4 (between MBDB and MDEA) and reached 5.1 between MA and MDA.

3.3. Identification of compounds in illicit tablets

The first parameter for identification was the migration time of the peak(s) observed in the electropherogram of the diluted ''Ecstasy'' sample extract (prepared as described in Section 2.3) in comparison with the electropherogram of the reference mixture. The migration times of the reference compounds in the mixture illustrated in Fig. 4A were: 5.69 min for A, 5.88 min for MA, 6.14 min for MDA, 6.34 min for MDMA, 6.55 min for E, 6.83 min for MBDB and 6.97 min for MDEA. The electropherogram of the ''Ecstasy'' sample presented in Fig. 4B showed a peak corresponding to the migration time of MDMA. The spiked sample (prepared as described in Section 2.3) was then injected: the obtained electropherogram is presented in Fig. 4C. A clear increase in one peak size could be recognised in comparison to the reference mixture (cf. Fig. 4A) and could confirm the identification of MDMA in the sample.

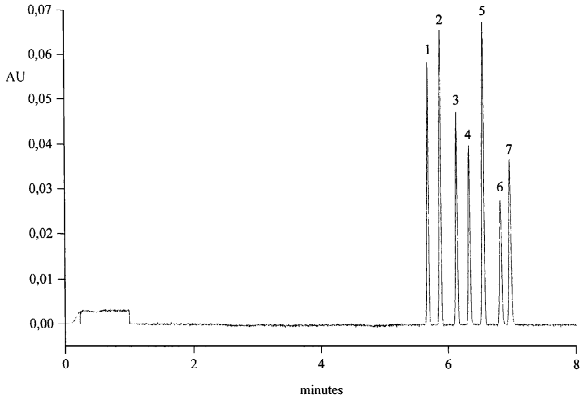

Fig. 3. Separation of amphetamine and related substances in the reference mixture: (1) A, (2) MA, (3) MDA, (4) MDMA, (5) E, (6) MBDB, (7) MDEA. Electrolyte: 0.1 *M* phosphoric acid adjusted to pH 3.0 with triethanolamine. Detection: UV at 190 nm. Other conditions as described in Section 2.

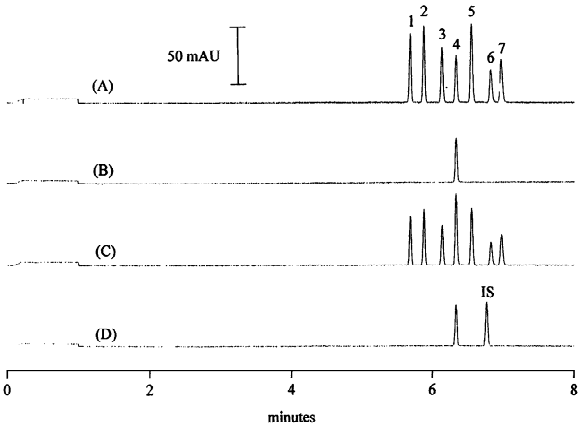

Fig. 4. Identification in illicit tablets: (A) mixture of reference compounds, (B) ''Ecstasy'' sample, (C) ''Ecstasy'' sample spiked with the reference mixture, (D) ''Ecstasy'' sample with the internal standard (I.S.). Electrolyte: 0.1 *M* phosphoric acid adjusted to pH 3.0 with triethanolamine. Detection: UV at 190 nm. Other conditions as described in Section 2.

In order to have the unambiguous identification of the studied illicit compounds, the UV spectra, recorded at migration time with the diode array UV detector (190–350 nm), were analysed for the sample peak and for the reference compound in the same electrolyte. The comparison of the two spectra observed for the studied ''Ecstasy'' sample gave a similarity index of 0.9945 (cf. Fig. 5) and confirmed the identification of MDMA.

In fact in the selected electrolyte two types of UV spectra were observed. Amphetamine, methamphetamine and ephedrine exhibited two absorption maxima; MDA, MDMA, MDEA and MBDB exhibited three absorption maxima. The last analytes possess the same chromophore (benzodioxane) and their on-line recorded UV spectra cannot be consid-

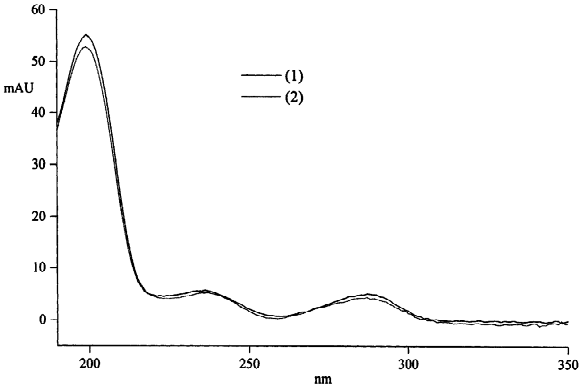

Fig. 5. Comparison of UV spectrum (190–350 nm) at migration time of (1) reference MDMA, (2) ''Ecstasy'' sample peak.

ered a diagnostical utility to distinguish the individual dioxyamphetamines. The same holds true for the phenylalkylamino compounds examined. Therefore, the combination of the specific electrophoretic behaviour, the migration times and the UV spectra constitutes the most reliable basis for the identification of each compound. Moreover, it should be stressed that another independent analysis procedure (GC–MS) was also always performed.

3.4. Quantification of MDMA in Ecstasy tablets

In order to determine MDMA in ''Ecstasy'' tablets, a calibration curve was established using different MDMA concentrations according to a laboratory validation procedure.

As described in Section 2.3 a first mass of the reference compound was done to prepare four concentrations corresponding to 70, 90, 110 and 130% of the mean expecting concentration of the sample (30% MDMA). A second mass of the reference MDMA was used to prepare the concentration corresponding to 100%. Phenylephrine was added as an internal standard as described in Section 2.3 in order to overcome the error associated with a variation in injection volume. Duplicate injections of all concentrations including the blank solution were performed.

Linear regression analysis of calibration data (70, 90, 110 and 130%) was performed by plotting the ratio of normalised peak area to that of the internal standard (y, arbitrary units) versus the analyte concentration (x, μg/ml) using the least squares method to obtain slope, intercept and correlation coefficient. All the calculations for this laboratory short validation were performed with a formatted Excel worksheet. The obtained straight line $y=18.1215x+0.0033$ passed through the origin and the coefficient of determination (r^2) was 0.9984. The linearity was confirmed by an analysis of variance (ANOVA); the control of the lack-of-fit was performed by a F-test; the intercept was not significantly different from 0. The g-function ($g<0.01$), the quality coefficient (QC<2.0%) and the theoretical detection limit were also deducted from the calibration curve [69,70].

The accuracy was confirmed by the result obtained with the 100% solution prepared by the second mass of the reference compound and by the injection of the blank solution. The amount of MDMA in the tablet was determined using the ratio of the normalised peak area to that of the internal standard observed for the sample and the slope and intercept data of the calibration line. The assay of MDMA in the tablet was then expressed as percentage of mass and for example was calculated to be 23.5% in the presented ''Ecstasy'' sample (cf. Fig. 4D). The repeatability ($r=2.8$SD) [71] was evaluated by means of the duplicate preparation of the sample (RSD<2.0%). The specificity of the method was confirmed by the injection of adulterants and sugars as described in Section 3.2 and with the identification confirmed by the UV spectrum as described in Section 3.3.

4. Conclusions

A CZE system using a buffer made of 0.1 *M* phosphoric acid adjusted to pH 3.0 with triethanolamine and diode array detection was applied for the analysis of amphetamine derivatives. At this selected pH, triethanolamine was adsorbed to the capillary wall and gave rise to a constant EOF reversed toward the anode. The resulting highly reproducible migration times, good peak symmetry, high efficiency and resolution were the principal advantages of this method. Baseline separation of A, MA, MDA, MDMA, E, MBDB and MDEA was performed. The identity of these analytes could be easily confirmed in seized drugs by this independent,

rapid and selective method which is an attractive alternative to GC–MS for the qualitative and quantitative determination of amphetamine and related compounds in ''Ecstasy'' samples.

References

[1] Belgian Information REITOX Network, Belgian National Report On Drugs 2001, Scientific Institute of Public Health, Brussels, 2002.
[2] A. Gilman, T. Rall, A. Nies, P. Taylor, Goodman and Gilman's The Pharmacological Basis of Therapeutics, Pergamon Press, Oxford, 1990.
[3] R. Baselt, Drug Effects On Psychomotor Performance, Biomedical Publications, Foster City, CA, USA, 2001.
[4] UNDND, Recommended Methods For Testing Amphetamine and Methamphetamine, United Nations Division of Narcotic Drugs, United Nations, New York, 1987.
[5] M. Lehrer, Clin. Lab. Med. 10 (1990) 271.
[6] B. Gan, D. Baugh, R. Liu, A. Walia, J. Forensic Sci. 36 (1991) 1331.
[7] D. Baker, M. Murphy, P. Shepp, V. Royo, M. Caldarone, B. Escoto, S. Salamone, J. Forensic Sci. 40 (1995) 108.
[8] W. Ruangyuttikarn, D. Moody, J. Anal. Toxicol. 12 (1988) 229.
[9] A. Dasgupta, S. Saldana, G. Kinnaman, M. Smith, K. Johansen, Clin. Chem. 39 (1993) 104.
[10] A. Wu, S. Wong, K. Johnson, J. Callies, D. Shu, W. Dunn, S. Wong, J. Anal. Toxicol. 17 (1993) 241.
[11] P. Martel, D. Adams, D. Jones, C. O'Donnel, Clin. Chem. 32 (1986) 915.
[12] R. Taylor, S. Le, S. Philip, N. Jain, J. Anal. Toxicol. 13 (1989) 293.
[13] B. Farrell, T. Jefferies, J. Chromatogr. 272 (1983) 111.
[14] M. Lambrechts, K. Rasmussen, J. Chromatogr. 331 (1985) 339.
[15] S. Li, Capillary Electrophoresis—Principles, Practice and Applications, Journal of Chromatography Library, Vol. 52, Elsevier, Amsterdam, 1994.
[16] K. Altria, Analysis of Pharmaceuticals By Capillary Electrophoresis, Chromatographia CE Series, Vol. 2, Vieweg, Braunschweig, 1998.
[17] R. Weinberger, I. Lurie, Anal. Chem. 63 (1991) 823.
[18] M. Krogh, S. Brekke, F. Tonnesen, K. Rasmussen, J. Chromatogr. A 674 (1994) 235.
[19] J. Walker, S. Krueger, I. Lurie, H. Marché, N. Newby, J. Forensic Sci. 40 (1994) 6.
[20] I. Lurie, K. Chan, T. Spartley, J. Casale, H. Issaq, J. Chromatogr. B 669 (1995) 3.
[21] I. Lurie, Int. Lab. 26 (1996) 21.
[22] I. Bjornsdottir, S. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 687.
[23] V. Trenerry, R. Wells, J. Robertson, J. Chromatogr. Sci. 32 (1994) 1.
[24] V. Trenerry, J. Robertson, R. Wells, Electrophoresis 15 (1994) 103.
[25] V. Trenerry, J. Robertson, R. Wells, J. Chromatogr. A 708 (1995) 169.
[26] P. Wernly, W. Thormann, Anal. Chem. 64 (1992) 2155.
[27] F. Tagliaro, C. Poiesi, R. Aiello, R. Dorizzi, S. Ghielmi, M. Marigo, J. Chromatogr. 638 (1993) 303.
[28] J. Hudson, M. Golin, M. Malcolm, Can. Soc. Forensic Sci. J. 28 (1995) 137.
[29] R. Taylor, A. Low, R. Reid, J. Chromatogr. B 675 (1996) 213.
[30] F. Tagliaro, S. Turrina, F. Smith, Forensic Sci. Int. 77 (1996) 211.
[31] F. Tagliaro, S. Turrina, P. Pisi, F. Smith, M. Marigo, J. Chromatogr. B 713 (1998) 27.
[32] F. Tagliaro, G. Manetto, S. Bellini, D. Scarcella, F. Smith, M. Marigo, Electrophoresis 19 (1998) 42.
[33] J. Walker, H. Marché, N. Newby, E. Bechtold, J. Forensic Sci. 41 (1996) 824.
[34] A. Krawczeniuk, V. Bravenec, J. Forensic Sci. 43 (1998) 738.
[35] M. Frost, H. Köhler, G. Blaschke, Int. J. Legal Med. 109 (1996) 53.
[36] F. Sadeghipour, E. Varesio, C. Giroud, L. Rivier, J.L. Veuthey, Forensic Sci. Int. 86 (1997) 1.
[37] P. Esseiva, E. Lock, O. Gueniat, M. Cole, Sci. Justice 37 (1997) 113.
[38] S. Cherkaoui, E. Varesio, P. Christen, J.L. Veuthey, Electrophoresis 19 (1998) 2900.
[39] A. Di Pietra, R. Gotti, E. Del Borrello, R. Pomponio, V. Cavrini, J. Anal. Toxicol. 25 (2001) 99.
[40] I. Lurie, M. Bethea, T. McKibben, P. Hays, P. Pellegrini, R. Sahai, A. Garcia, R. Weinberger, J. Forensic Sci. 46 (2001) 1025.
[41] S. Cladrowa, R. Hirz, E. Kenndler, A. Rizzi, J. Chromatogr. A 710 (1995) 339.
[42] Z. Wang, A. Huang, Y. Sun, J. High Resolut. Chromatogr. 20 (1997) 298.
[43] E. Varesio, J.Y. Gauvrit, R. Longeray, P. Lanteri, J.L. Veuthey, Electrophoresis 18 (1997) 931.
[44] I. Lurie, R. Meyers, T. Conver, Anal. Chem. 70 (1998) 3255.
[45] I. Lurie, C. Bailey, D. Anex, M. Bethea, T. McKibben, J. Casale, J. Chromatogr. A 870 (2000) 53.
[46] P. Righetti, F. Nembri, J. Chromatogr. A 772 (1997) 203.
[47] S. Ermakov, L. Capelli, P. Righetti, J. Chromatogr. A 744 (1996) 55.
[48] Y. Rawjee, G. Vigh, Anal. Chem. 66 (1994) 619.
[49] I. Björnsdottir, S. Hansen, Chirality 7 (1995) 219.
[50] R. McCormick, Anal. Chem. 600 (1988) 2322.
[51] K. Jinno, Y. Han, M. Nakamura, J. Cap. Electrophoresis 3 (1996) 139.
[52] K. Assi, A. Abushoffa, K. Altria, B. Clark, J. Chromatogr. A 817 (1998) 83.
[53] A. Balchunas, M. Sepaniak, Anal. Chem. 59 (1987) 1466.
[54] V. Trenerry, C. Ward, J. Cap. Electrophoresis 3 (1996) 271.
[55] P. Lukkari, H. Vuorela, M. Riekkola, J. Chromatogr. A 652 (1993) 451.
[56] S. Pedersen, K. Rasmussen, T. Tilander, J. Chromatogr. A 807 (1998) 285.

[57] K. Altria, K. Assi, S. Bryant, B. Clark, Chromatographia 44 (1997) 367.
[58] R. Colombara, M. Tavares, S. Massaro, J. Rubim, J. Cap. Electrophoresis 3 (1996) 147.
[59] S. Piperaki, S.G. Penn, D.M. Goodall, J. Chromatogr. A 700 (1995) 59.
[60] I. Bechet, Ph. Paques, M. Fillet, Ph. Hubert, J. Crommen, Electrophoresis 15 (1994) 818.
[61] M. Fillet, I. Bechet, P. Chiap, Ph. Hubert, J. Crommen, J. Chromatogr. A 717 (1995) 203.
[62] S. Li, D.K. Loyd, J. Chromatogr. A 666 (1994) 321.
[63] C. Quang, M.G. Khaledi, J. Chromatogr. A 692 (1995) 253.
[64] M. Fillet, I. Bechet, Ph. Hubert, J. Crommen, J. Pharm. Biomed. Anal. 14 (1996) 1107.
[65] The European Pharmacopoeia, Part 2.2.2, 3rd ed., Council of Europe, Strasbourg, 1996.
[66] T. Schmitt, H. Engelhardt, J. Chromatogr. A 697 (1995) 561.
[67] M. Fillet, P. Hubert, J. Crommen, Electrophoresis 18 (1997) 1013.
[68] C. Vogt, S. Conradi, E. Rohde, J. Chem. Educ. 74 (1997) 1126.
[69] L. Rodriguez, A. Campana, C. Unares, M. Ceba, Anal. Lett. 26 (1993) 1243.
[70] P. Vankeerberghen, J. Smeyers-Verbeke, Chemometr. Intell. Lab. Systems 15 (1992) 195.
[71] ISO Standard 5725-2, International Organization for Standardization, Geneva, 1994.