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Separation and identification of designer drugs with capillary electrophoresis and on-line connection with ionspray mass spectrometry

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Abstract

The separation of methylenedioxyamphetamine-related designer drugs by capillary zone electrophoresis (CZE) and on-line detection with ionspray mass spectrometry (CZE–MS) is described. CZE–MS coupling permits the fast and reliable identification of this class of substances in the femtomole range. As methylenedioxyamphetamines have an asymmetric carbon atom, several cyclodextrins were investigated as buffer additives for the chiral separation of these compounds.

Keywords: Capillary electrophoresis–mass spectrometry; Enantiomer separation; Drugs; Methylenedioxyamphetamines; Amphetamines

1. Introduction

Designer drugs or synthetic addiction compounds of the second generation, to which fentanyles, phenyclidines, prodines (pethidine analogues) and amphetamines belong, are synthesized in illicit laboratories by varying the structure of well known pharmaceuticals or classical drugs. These chemically synthesized compounds have great potential for misuse. They are readily synthesized and therefore widespread on the illegal drug market [1]. In recent years, capillary zone electrophoresis (CZE) has been successfully applied to various substance classes including pharmaceutical compounds and drugs [2,3].

In this work, CZE was used to separate the methylenedioxyamphetamines (±)-3,4-methylenedioxyphenyl-2-propylamine (MDA, **1**), (±)-N-ethyl-3,4-methylenedioxyphenyl-2-propylamine (MDE, **2**) and (±)-N-methyl-3,4-methylenedioxyphenyl-2-propylamine (MDMA, **3**) (Fig. 1). To determine the pharmacological quality, metabolism and the manner of the drug effect, it is necessary to separate the enantiomers of these chiral compounds. Chiral separation was achieved with a β-cyclodextrin-modified buffer system. For the unambiguous identification and

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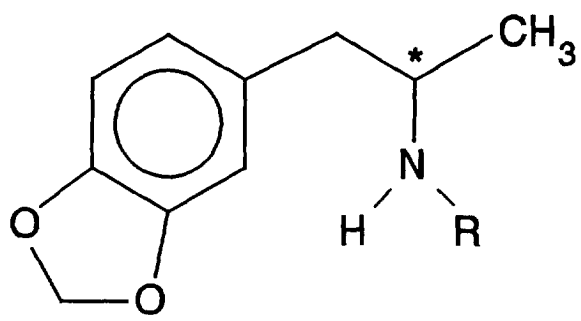

Fig. 1. Structure of the investigated methylenedioxyamphetamines: R = H, MDA (**1**); R = methyl, MDMA (**2**); R = ethyl, MDE (**3**).

determination of these drugs, on-line CZE–MS coupling was applied [4].

2. Experimental

CZE measurements were performed with a Model 100 capillary electrophoresis system (Grom, Herrenberg, Germany) connected to a Chromatopac C-6R A integrator (Shimadzu, Tokyo, Japan). For on-line CZE–MS coupling, the capillary electrophoresis system was coupled with an API III quadrupole mass spectrometer (Sciex, Toronto, Canada) via an ionspray interface. The pH of buffer solutions was checked with a model E 512 pH meter (Metrom Herisau, Germany).

Buffer solutions were prepared with reagents of analytical-reagent grade (Merck, Darmstadt, Germany), with the exception of cetyltrimethylammonium chloride (CTAC) (Fluka, Neu-Ulm, Germany). For CZE and CZE–MS separations, three different buffer systems were used: buffer A, 40 m*M* sodium formate–10 m*M* sodium chloride (pH 2.5); buffer B, 40 m*M* ammonium acetate–0.5 m*M* CTAC (pH 5.5); and buffer C, 50 m*M* sodium phosphate–10 m*M* β-cyclodextrin (β-CD)–5 m*M* triethylamine (pH 2.0).

Fused-silica capillaries (50 μm I.D. and 375 μm O.D.) with a polyimide cladding (Polymicro Technology, Phoenix, AZ, USA) were used for CZE separations. For CZE–MS coupling, capillaries of 50 μm I.D. and 180 μm O.D. were applied. A 5 mm length of the polyimide cladding was burnt off with a glowing wire to allow on-line UV detection. The detection length of the capillaries for CZE from injection to the UV detector was 0.5 m and the overall length for CZE and CZE–MS experiments was 0.7 m. Capillaries were pretreated by flushing for 10 min with 1 *M* sodium hydroxide, for 10 min with doubly distilled water and finally for 10 min with electrophoresis buffer. All buffers were prepared with doubly distilled water. The pH was adjusted by adding 1 *M* sodium hydroxide (buffers A and C) or 10% ammonia solution (buffer B). Buffers were degassed under reduced pressure in an ultrasonic bath and filtered with 0.2-μm filters before use. Between analyses, the capillary was rinsed successively with 50 μl each of 0.1 *M* sodium hydroxide, water and buffer. Samples were injected by raising the injection block 0.1 m for 15 or 20 s (gravity loading).

Samples (MDA, MDMA and MDE) were synthesized in our laboratories and dissolved as the hydrochlorides in doubly distilled water and acidified with 1 *M* hydrochloric acid. The final concentration was 70 μg/ml for CZE and either 30 or 3 μg/ml for CZE–MS separations. A liquid sheath flow system as described elsewhere was used for Ce–MS coupling [5].

3. Results

For the separation of basic methylenedioxyamphetamines, acidic to neutral buffers can be used. Fig. 2 shows the separation in sodium formate buffer at pH 2.5. At low pH values the separation depends on the differences in the mass of the compounds, since all the amines investigated can be expected to be fully protonated at pH <7 (pK_a = 9.9 for amphetamine). The observed migration order (MDA > MDMA > MDE) corresponds with increasing molecular mass. In buffer with pH 5–7 both the electrophoretic mobility μ_e of the compounds and the increasing electroosmotic flow μ_{eo} accelerate the separation (Eq. 1), but they reduce the resolution R (Eq. 2) because of the increasing average overall mobility m_{av}, hence MDA and MDMA are incompletely separated. Additionally, significant tailing

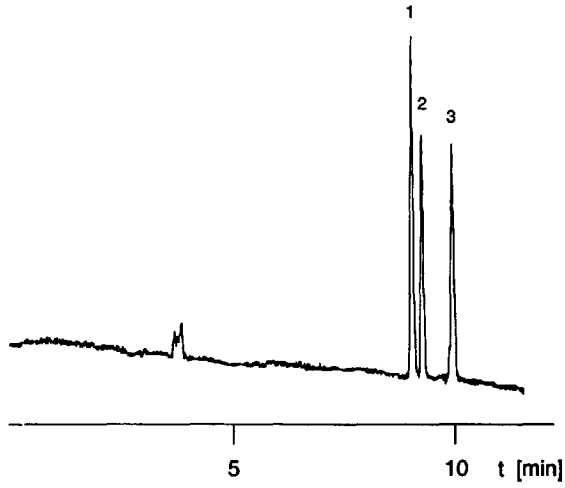

Fig. 2. CZE of methylenedioxyamphetamines in buffer A. Conditions: voltage, 20 kV; current 10 μA; hydrostatic injection, 20 s. Peak numbers correspond to compounds **1**, **2**, **3**.

caused by interaction of the sample ions with the capillary surface broadens the peaks at these pH values.

$$\mu_{ov} = \mu_{eo} = \mu_e \quad (1)$$

$$R = \frac{\Delta\mu_{ov}}{\mu_{av}} \frac{N^{1/2}}{4} \quad (2)$$

where μ_{ov} = overall mobility, $\Delta\mu_{ov} = \mu_{ov,1} - \mu_{ov,2} = \mu_{e,1} - \mu_{e,2}$, $\mu_{av} = \mu_{ov,1} + \mu_{ov,2}/2 = 1/2\mu_{e,1} + 1/2\mu_{e,2} + \mu_{eo}$, $\Delta\mu_{ov}$ = difference of overall mobility of the two compounds and μ_{av} = average overall mobility.

To solve this problem, a second system (buffer B) with a dynamic capillary modification was tested [6]. CTAC was added to an ammonium acetate buffer of pH 5.5 below the critical micelle concentration. This cationic detergent is attracted to the negatively charged capillary surface and, because of double layer formation of the detergents, a cationically charged surface is formed. In this case the sample molecules are detected at the anodic end. Fig. 3 shows the separation of the three amphetamines in 40 m*M* ammonium acetate buffer at pH 5.5 with a CTAC concentration of 0.5 m*M*. The buffer was also used for CZE–MS experiments, since in the absence of alkali metal ions we observed lower background noise and high ion-current signal

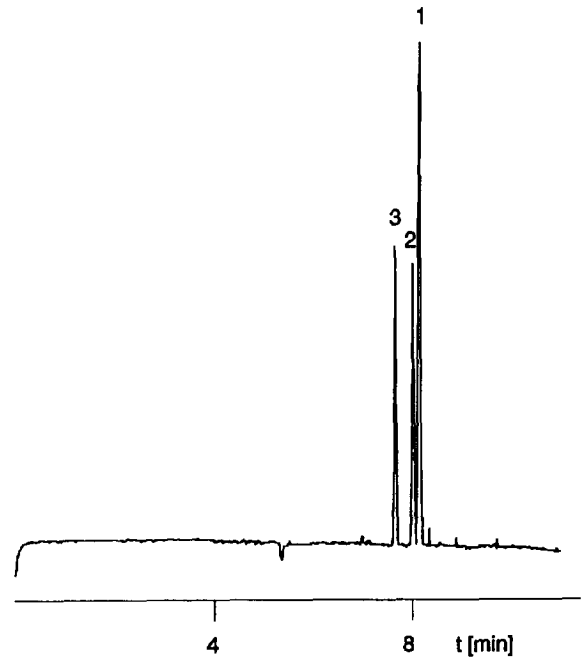

Fig. 3. CZE of methylenedioxyamphetamines in buffer B. Conditions: voltage, −15 kV; current −13 μA; hydrostatic injection, 20 s. Peak numbers correspond to compounds **1**, **2**, **3**.

intensities. Table 1 shows the overall electrophoretic mobilities μ_{ov}, the separation efficiency and the resolution of the amphetamines with buffers A and B. Although the number of plates (N/m) of the methoxyamphetamines in buffer B is higher than in buffer A, the resolution R of the compounds is greater in buffer A because of the larger differences in the overall electrophoretic mobility μ_{ov}. The migration order of the amphetamines in buffer B is reversed in comparison with buffer A because a high electroosmotic flow towards the anode carries the compounds towards this electrode.

Chiral drugs display stereoselectivity in their pharmacokinetic behaviour, pharmacological action and metabolism. Therefore, the use of chiral separation techniques is required. High-performance liquid chromatography (HPLC) and capillary gas chromatography are established techniques for the separation of chiral compounds on commercially available chiral stationary phases. Chiral separation by capillary electrophoresis is a

Table 1
Overall electrophoretic mobility μ_{ov}, number of plates (N/m) and resolution R of the CZE-separation of MDA, MDMA and MDE.

Separation buffer	Overall electrophoretic mobility, m_{ov}(m^2/V·s)[a]	Number of plates (N/m)	Resolution, R
Buffer A	Peak 1: $3.23 \cdot 10^{-8}$	235 470	Peak 2 (MDMA) from peak 1 (MDA): 3.03
	Peak 2: $3.15 \cdot 10^{-8}$	278 020	
	Peak 3: $2.93 \cdot 10^{-8}$	195 749	Peak 3 (MDE) from peak 2 (MDMA): 9.53
Buffer B	Peak 1: $-2.25 \cdot 10^{-8}$	384 701	Peak 2 (MDMA) from peak 1 (MDE): 6.21
	Peak 2: $-2.47 \cdot 10^{-8}$	331 742	
	Peak 3: $-2.55 \cdot 10^{-8}$	342 483	Peak 3 (MDA) from peak 2 (MDMA): 2.36

[a] A negative overall electrophoretic mobility μ_{ov} indicates detection at the anodic end of the capillary.

technique of increasing interest because of its simple instrumentation, high separation efficiency and the simple introduction of the chiral selector as a component of the buffer solution. As chiral selectors, various cyclodextrins, crown ethers, bile salts, chiral detergents and complexes are used [7].

Cyclodextrins (CDs) were investigated for the chiral separation of the methoxyamphetamines. Only the buffer containing β-CD (buffer C) permits the chiral separation (Fig. 4) of the three compounds investigated. Buffers with the same amount of α- or γCD (10 mM) showed no chiral discrimination of the investigated compounds. A β-CD phase was also successful in the chiral separation of the methoxyamphetamines by HPLC [8]. Quang and Kaledi [9] used chiral capillary electrophoresis with β-CD for the separation of basic amines and proposed a three-point-interaction mechanism between CD and the amines. The incorporation of the hydrophobic aromatic ring into the CD cavity and the interaction of the hydroxy and amino groups of the amines, e.g., ephedrine, with the C-2 and C-3 hydroxy groups by hydrogen bonding at the wider opening of the β-CD was assumed. Although the methoxyamphetamines are not able to form two hydrogen bonds because of the lack of a hydroxyl group, these compounds can be separated with a buffer containing β-CD. A reduced electroosmotic flow is necessary for the chiral separation of these compounds, because in this case resolution is not decreased by an additive summand (Eqs. 1 and 2). Therefore, a buffer of low pH and thus minimized electroosmotic flow is chosen for the chiral separation of the amphetamines.

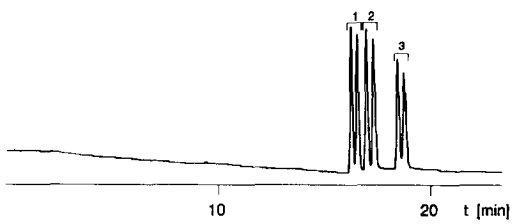

Fig. 4. Chiral separation of methylenedioxyamphetamines in buffer C. Conditions: voltage, 20 kV; current, 23 μA; hydrostatic injection, 20 s. Peak numbers correspond to compounds **1**, **2**, **3**.

Identification and characterization of unknown or complex samples can be achieved by the online connection of chromatographic or electrophoresis separation techniques with spectroscopic or mass spectrometric detection methods. CZE–MS has been shown to be a powerful tool for solving biochemical and bioanalytical problems. It is possible to separate and detect small sample volumes with this method. A great advantage of ionspray mass spectrometry (IS-MS) is the ability to introduce aqueous liquid samples under normal pressure and temperature. The

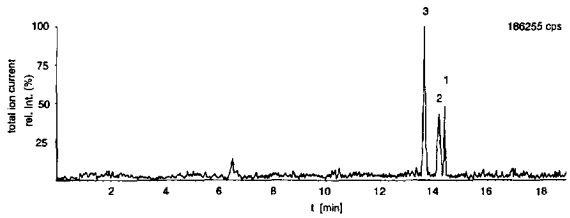

Fig. 5. Total ion current of the CZE–MS separation of methylenedioxyamphetamines in buffer B. Conditions: voltage, −10 kV; current −11 μA; hydrostatic injection, 15 s; MS buffer, methanol–0.1% formic acid (1:1). Peak numbers correspond to compounds **1**, **2**, **3**.

extremely low electroosmotic flow of a few nl/min in capillary electrophoresis is insufficient to maintain a stable ionspray. Therefore, an additional make-up flow of several μl/min is added coaxially to the analytical capillary (liquid-sheath method). Fig. 5 shows the total ion current of the CZE–MS separation of the methylenedioxyamphetamines with a concentration of 30 μg/ml of each compound. Buffer B is free of alkali metal ions, eliminating adduct ions and reducing background noise caused by the electrophoresis buffer in this low-mass range. The $[M + H]^+$ signals of the three methylenedioxyamphetamines appear in each spectrum (fig. 6) acquired from the three peaks of the total ion current. The base peak in each spectrum at 163.0 u results from elimination of the amino group of the methylenedioxyamphetamines. The degree of fragmentation and thus the decrease in the $[M + H]^+$

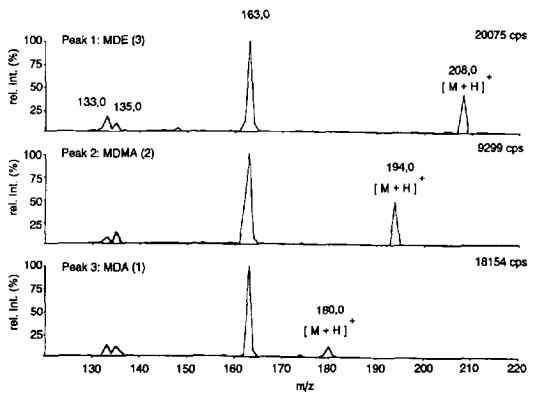

Fig. 6. Spectra of the total ion current (Fig. 5) of the separation of methylenedioxyamphetamines **1–3**.

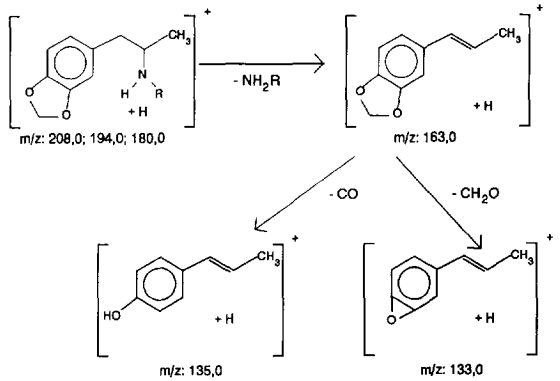

Fig. 7. Scheme of fragmentation of methylenedioxyamphetamines.

signal intensity are influenced by the orifice voltage. The elimination of the H_2CO and CO abstraction from the 163.0 u fragment results in fragments of 133.0 and 135.0 u, respectively. Such fragmentation (Fig. 7) is relatively unusual in IS-MS, which normally shows only molecule ions, multiple charged quasi-molecular ions and adduct ions.

The volume injected is 5.3 nl for a hydrostatic sampling time of 15 s and a 0.1 m height difference [10,11]. This is equivalent to 90 pg or 450 fmol when the sample concentration of each methylenedioxyamphetamine (with the average molecule mass assumed to be 200 u) is 30 μg/ml. Using the selected-ion monitoring (SIM) mode, a 10–100-fold increase in sensitivity can be achieved. Fig. 8 shows the CZE–MS separation of an amphetamine sample containing 3 μg/ml of each component. Only the $[M + H]^+$ signals (180.0, 194.0 and 208.0 u) were monitored (SIM). In this case the amount injected was 9 pg or 45 fmol.

4. Conclusion

The separation of methylenedioxyamphetamines by capillary electrophoresis was demonstrated with two different buffer systems. Chiral separation was achieved by an addition of β-CD to the buffer. CZE–MS in the low-femtomole range offers the possibility of the un-

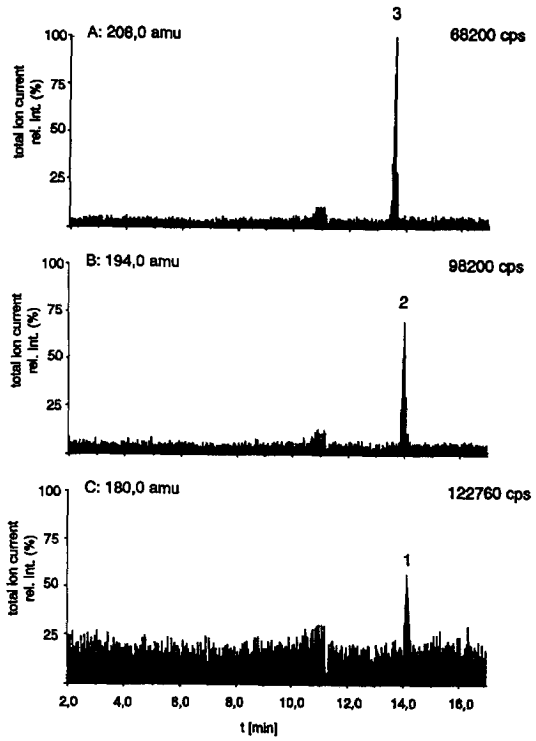

Fig. 8. Selected ion current (SIM) of the CZE–MS separation of methylenedioxyamphetamines **1–3** in buffer B. Conditions: voltage, −10 kV; current, −11 μA; hydrostatic injection, 15 s; MS buffer, methanol–0.1% formic acid (1:1). (A) 208.0 u (MDE, **3**); (B) 194.0 u (MDMA, **2**); (C) 180.0 u (MDA, **1**).

ambiguous identification of this class of substances in dilute samples (e.g. urine) without the necessity for pretreatment or concentration.

References

[1] C. Rösch, K.-A. Kovar, A. Rupp and L. Hermle, Pharmazie Unserer Zeit, 19 (1990) 99.
[2] S.F.Y. Li, Capillary Electrophoresis–Principles, Practice and Applications (Journal of Chromatography Library), Elsevier, Amsterdam, 1992.
[3] A. Wainright, J. Microcol. Sep., 2 (1990) 166.
[4] R.D. Smith, J.H. Wahl, D.R. Goodlett and S.A. Hofstadler, Anal. Chem., 65 (1993) 574A.
[5] R.D. Smith, J.A. Olivares, N.T. Nguyen and H.R. Udseth, Anal. Chem., 60 (1988) 436.
[6] Å. Emmer, M. Jansson and J. Roeraade, J. Chromatogr., 547 (1991) 544.
[7] I.E. Välko, H.A.H. Billiet, H.A.L. Corstjens and J. Frank, LC·GC Int., 6 (1993) 420.
[8] M. Brunnenberg and K.-A. Kovar, unpublished results.
[9] C. Quang and G. Khaledi, Anal. Chem., 65 (1993) 3354.
[10] D.J. Rose and J.W. Jorgenson, Anal. Chem., 60 (1988) 642.
[11] H.-J. Gaus, Thesis, Tübingen, 1994.