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Analysis of "Ecstasy" by capillary electrophoresis

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Abstract Capillary electrophoresis (CE) and it's applications in forensic toxicology are demonstrated by the investigation of amphetamine derivatives in "Ecstasy" tablets. The method is based on capillary zone electrophoresis (CZE) with a phosphate running buffer (pH 2.2). The drugs were dissolved in 0.01 N hydrochloric acid, diluted with distilled water and phenylephrine was added to the samples as an internal standard. A separation of the charged substances is obtained by the different velocities in the electric field. The qualitative determination can be made by the migration times or more accurately by the relative migration times. Other possibilities for identification are the UV-spectra using a diode array detector or the on-column standard addition procedure. By this procedure the sample solution is initially injected followed by the standard solution. Both are concentrated in the column before separation begins. CE shows clear advantages in comparison to high-performance liquid (HPLC) or gas chromatography (GC). The quantitative analysis was carried out using the internal standard phenylephrine.

The values obtained for 56 "Ecstasy" tablets and powder containing amphetamine or its derivatives were compared in this study after analysis with CE and HPLC. The results were in very good agreement. Because of it's speed, the high resolving power and the possibility of identification with the on-column standard addition, CE is a powerful alternative to HPLC or GC for the investigation of "Ecstasy" tablets.

Key words Ecstasy · Amphetamine · Methamphetamine · MDA · MDMA · MDE · Capillary electrophoresis · Capillary zone electrophoresis

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Introduction

"Ecstasy" is a scene designation for a drug which generally includes the amphetamine derivatives 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDE) and some rarer compounds such as N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) or 2,5-dimethoxy-4-bromamphetamine (DOB). The previous use of the term "Ecstasy" as a synonym for MDMA cannot be justified today.

The number of confiscations of "Ecstasy" tablets registered by the police increased drastically in recent years. In 1991 in Germany only 4000 tablets were confiscated but this increased to ca. 240000 in 1994 [1]. For 1995 a further increase is to be expected, so that a simple, rapid and unequivocal analysis method is necessary.

In the investigation of "Ecstasy" tablets it is important to identify the incorporated drugs. Because of the forensic relevance, many methods are available for analysis of amphetamine, methamphetamine, MDA, MDMA und MDE. To establish the identity of amphetamines there exist several thin-layer chromatography methods [2–5]. A quantification of the active substances is usually carried out using gas chromatography [5–9] or high-performance liquid chromatography methods [5, 10–13]. In recent years capillary electrophoresis and micellar electrokinetic chromatography (MEKC) have become increasingly more important in forensic toxicology [14–21]. This paper describes a capillary zone electrophoretic separation which permits a rapid qualitative and quantitative determination of amphetamine derivatives in tablets and other illicit drugs.

Materials and methods

Capillary electrophoresis (CE)

All separations were carried out on a P/ACE 5510 capillary electrophoresis system (Beckman, Palo Alto, Calif.). The optical unit

and the detector consisted of a deuterium lamp, operating at 200 nm, and a diode array detector (DAD). The capillary zone electrophoresis (CZE) was performed in 40 cm (effective length to the detector) untreated Beckman fused-silica tubes (total length 47 cm, 50 μm I.D. and 375 μm O.D.). Capillaries were treated before use by rinsing for 20 min with 0.1 M NaOH and before each sample for 1 min with running buffer. Further standard operating conditions, unless stated otherwise were as follows: effective voltage 500 V/cm (equivalent to 23.5 kV), temperature 20° C, sample introduction, low pressure (0.5 psi) 5 s.

Running buffer

Stock solutions of 100 mM KH_2PO_4 buffer were made in deionized and distilled water, filtered and degassed in an ultrasonic bath before use. The pH was adjusted to pH 2.2 with a stock buffer solution of 100 mM H_3PO_4.

High-performance liquid chromatography (HPLC)

A Hewlett Packard Series II 1090 liquid chromatograph with a Hewlett Packard Series II 1050 variable wavelength detector was used. Chromatographic separations were performed with a LiChrospher 60 RP-select B (5 μm, 250 × 4 mm) analytical column, (Merck, Darmstadt, Germany). The mobile phase was a mixture of methanol, acetic acid, triethylamine, deionized and distilled water (10:1.5:0.5:88) which was filtered and degassed before use. The column temperature was 40° C, the flow rate 1.5 ml/min, the injection volume 25 μl and detection was at 254 nm.

Standard and internal standard solutions

The standard solution used for qualitative and quantitative determination contained 50 μg/ml each of amphetamine, methamphetamine, MDA, MDMA and MDE. Quinine, cocaine, lidocaine, codeine, diydrocodeine were included at concentrations of 50–100 μg/ml. The internal standard stock solution contained 500 μg/ml phenylephrine.

Sample preparation

Tablets were homogenized to a fine powder and an aliquot of 100 mg was dissolved in 0.01 M HCl for 10 min in an ultrasonic bath. The filtered solution was diluted 1:50 with distilled water and the phenylephrine stock solution was added to the sample as internal standard at a final concentration of 50 μg/ml.

Results and discussion

Capillary zone electrophoresis (CZE)

For the investigation of compounds of forensic interest by capillary electrophoresis, the micellar electrokinetic chromatography (MEKC) method is commonly used. MEKC is a method which can separate non-ionic substances by using surfactants, in most cases sodium dodecyl sulfate (SDS), to promote interactions of the compounds with the micelles of surfactants [14]. This allows the separation of many different compounds in a very short time. Weinberger and Lurie [15] were the first to apply MEKC in a forensic drug screening method to separate 18 compounds in about 40 min. Wernly and Thormann [16] separated experimental mixtures of illicit drugs using MEKC in 25 min. Tagliaro et al. [17] found similar results with a separation of 20 compounds in ca. 50 min. These methods can also be used to separate many illicit drugs in a relative short time but the migration times for the determination of amphetamine or metamphetamine are too long. In Weinberger and Lurie's [15] investigation the migration time for amphetamine was about 12 min and for methamphetamine about 14 min. With the method proposed by Wernly and Thormann [16] the migration times were ca. 23 and 24 min and the method of Tagliaro requires about 38 min to determine amphetamine. These migration times are without doubt too long to investigate amphetamines in tablets, especially as there is no time advantage in comparison to HPLC methods. Krogh et al. [18] developed a MEKC separation method and succeeded in decreasing the migration times to ca. 9 and 10 min respectively. Walker et al. [19] were able to investigate heroin samples in 3 min with MEKC by shortening the capillary to an effective length of 20 cm. However under these conditions a separation of the five amphetamine derivatives is not possible. Trenerry et al. [20] separated the amphetamines by using a buffer containing cetyltrimethylammonium bromide in ca. 10 min.

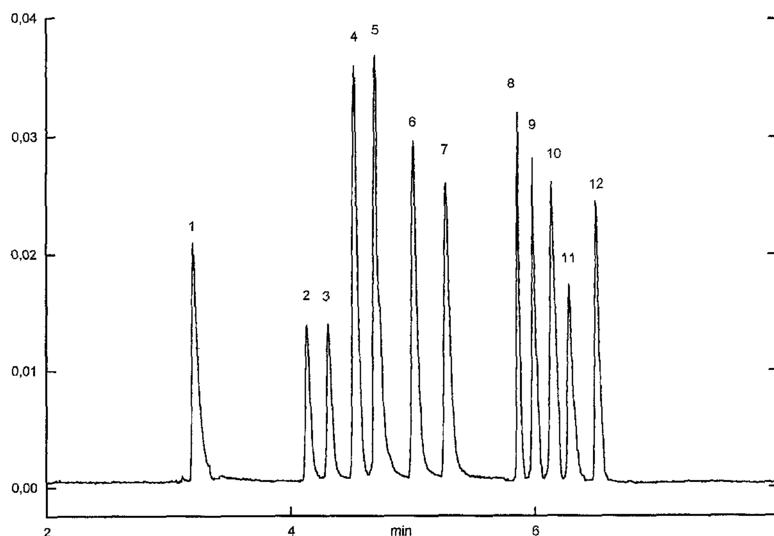

Fig. 1 Electropherogram of a standard mixture of amphetamine derivatives and other basic drugs (concentration range: 50–100 μg/ml for each compound). Conditions: fused silica capillary (47 cm total length, 40 cm to the detector, 50 μm I.D.); injection, 0.5 psi, 5 s; voltage, 23.5 kV; temperature, 20° C; buffer, 100 m*M* phosphate, pH 2.2; detector wavelength, 200 nm Key; *(1)* quinine, *(2)* amphetamine, *(3)* methamphetamine, *(4)* MDA, *(5)* MDMA, *(6)* phenylephrine (ISTD), *(7)* MDE, *(8)* cocaine, *(9)* lidocaine, *(10)* codeine, *(11)* dihydrocodeine, *(12)* methadone

Table 1 Within-day and day-to-day reproducibilities of migration time, relative migration time, corrected peak area and corrected peak area ratio as relative standard deviations (RSD)

	Within-day reproducibility (n = 6)				Day-to-day reproducibility (n = 6)			
	RSD [%] migration time	RSD [%] relative migration time	RSD [%] corr. peak area	RSD [%] corr. peak area ratio (ISTD/drug)	RSD [%] migration time	RSD [%] migration relative time	RSD [%] corr. peak area	RSD [%] corr. peak area ratio (ISTD/drug)
Amphetamine	1.36	0.08	0.89	1.30	1.41	0.17	4.81	1.23
Methamphetamine	1.37	0.07	1.26	1.04	1.44	0.15	4.60	1.39
MDA	1.23	0.22	1.07	0.94	1.51	0.09	4.67	1.57
MDMA	1.41	0.03	1.37	0.72	1.53	0.05	4.67	3.27
MDE	1.46	0.03	2.15	0.87	1.62	0.05	3.60	0.59
Phenylephrine (ISTD)	1.44		1.70		1.58		3.81	

By using CZE, the simplest form of CE because the capillary is filled only with buffer, it is possible to separate amphetamine derivatives found in "Ecstasy" tablets in less than 6 min. Figure 1 shows a standard electropherogram of a mixture of the amphetamines usually expected. Furthermore, basic illicit drugs such as cocaine, lidocaine, codeine, dihydrocodeine and methadone can be clearly identified in less than 8 min.

Qualitative analysis of "Ecstasy"

For the qualitative determination of amphetamine derivatives there are three possibilities:

1) Identification by migration time

Generally an identification is possible by migration time, but the within-day relative standard deviation (RSD) ranges from 1–2% (Table 1) which was at times unsatisfactory, because the difference between two compounds is about 0.1 min corresponding to ca. 2%. The day-to-day RSDs are even higher, as expected. The reproducibility of migration times depends on several operational factors such as pH, ionic strength, previous capillary treatment, applied voltage and age of the capillary tube [22]. The RSDs obtained by migration times can be reduced by using the relative migration times with reference to the internal standard. By using this method the within-day and the day-to-day RSDs could be reduced to less than 0.3% (Table 1).

2) UV-spectrum

A further method for substance identification is using the UV-spectrum. The Beckman P/ACE 5510 is equipped with a diode array detector which allows a spectrum in the desired wave length range to be made. Unfortunately the spectra of the amphetamine derivatives are very similar, so that a reliable identification is difficult. However a further safeguard could be attained with the qualitative determination with UV-spectrum of quinine in one sample. This sample was sold as "Ecstasy" on the drug market.

3) On-column standard addition method

The third and most convincing possibility for substance identification is offered by the on-column standard addition technique. In this method the sample is initially injected followed by injection of the standard solution and separation occurs after connecting the voltage. If the sample and standard are identical a zone is formed which moves with the same velocity in the direction of the detector and gives a single signal. Therefore the addition of the standard does not occur in the sample vessel as in

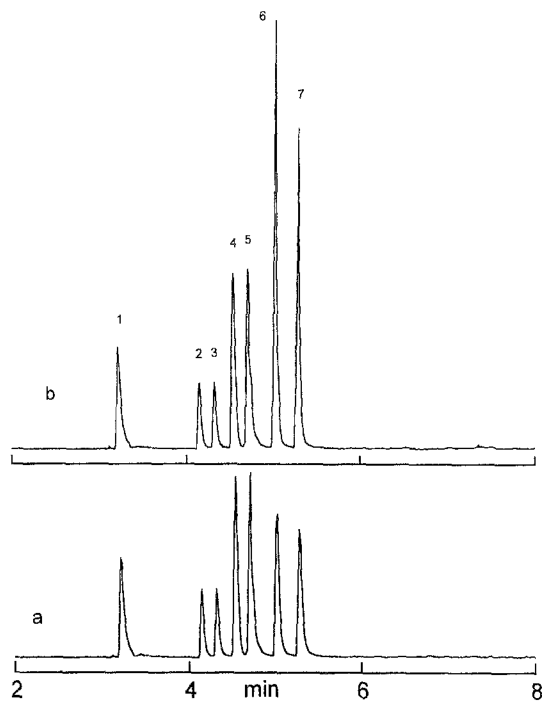

Fig. 2 Electropherograms of a) the standard mixture and b) a sample solution containing MDE and ISTD with standard addition. Conditions see Fig. 1

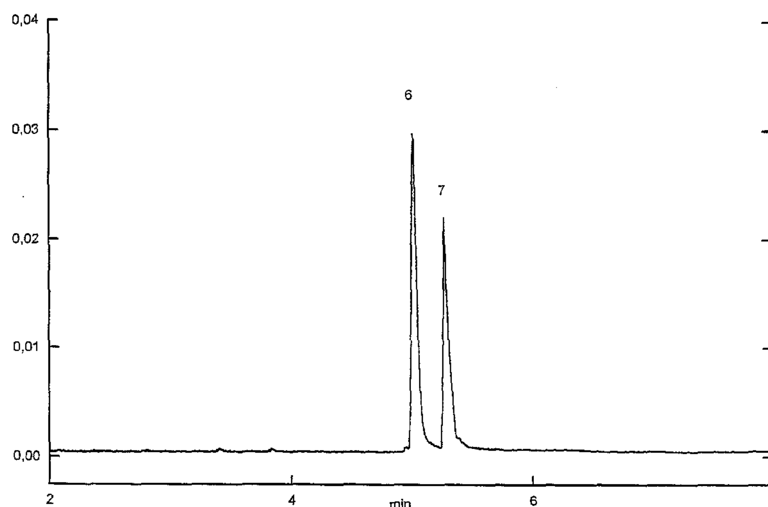

Fig. 3 Typical electropherogram of an "Ecstasy" tablet containing MDE with ISTD addition. Conditions see Fig. 1

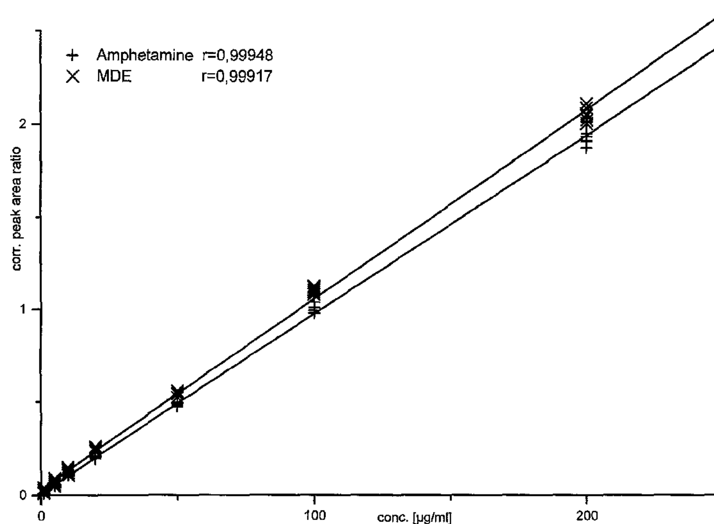

Fig. 4 Representative standard curves for amphetamine and MDE

chromatography methods (except thin-layer chromatography) but directly in the capillary so that a mixture of the sample with a standard is not necessary. Figure 2 shows a electropherogram of a sample containing MDE and an electropherogram of a standard mixture. A clear increase in peak size can be recognised in comparison to the standard.

It should be stressed that a second independent analysis procedure (eg GC/MS) is necessary for a clear qualitative determination of tablets and powder under investigation.

Quantitative analysis

Quantification of amphetamine derivatives in "Ecstasy" tablets was calculated using phenylephrine as internal standard (ISTD). The addition of an internal standard in capillary electrophoresis is necessary because variations can occur due to the very small injection volume (only a few nanoliters). As shown in Table 1 the within-day RSD could be reduced from 0.8–2.2% to 0.7–1.3% and the day-to-day RSD from 3.6–4.8% to 0.6–3.2% if the calculations were made using the internal standard. In capillary electrophoresis the calculation of the concentration of the active substances is commonly made by reference to the peak area corrected to migration time, simply by dividing the integrated peak area by migration time. This must be taken into consideration because the different dwell times, resulting from different migration velocities of the solutes, affect the peak area in the detection region. This is in contrast to chromatography techniques in which all solutes travel at the same rate when in the mobile phase [23]. Figure 3 shows a typical electropherogram of a sample containing MDE. As an example the standard curves for amphetamine and MDE (n = 6) based on corrected peak area ratios are shown in Fig. 4. Both curves were linear in a concentration range of 1–200 μg/ml with correlation coefficients of 0.9995 and 0.9992.

Comparison of HPLC and CE

To compare the CE method with the HPLC method a total of 56 "Ecstasy" tablets and powder containing amphetamine, MDMA and/or MDE were analysed with both techniques to control the accuracy of the results obtained by capillary electrophoresis. The comparison of the values

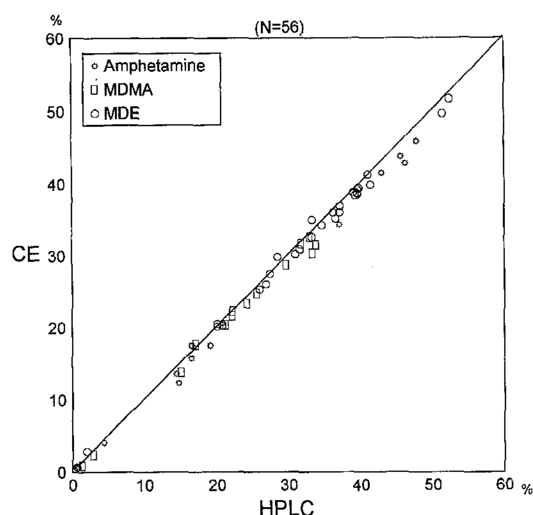

Fig. 5 Comparison of CE and HPLC; 56 tablets and powder were analysed with the previously mentioned CE and HPLC methods. See text for further discussion

obtained for active substances in the material of investigation is shown in Fig. 5. A very good agreement can be seen for both analysis methods so that capillary electrophoresis is a powerful alternative to HPLC for the analysis of "Ecstasy" tablets. Furthermore capillary electrophoresis has essential advantages because it is a very rapid method. Each run including rinsing of the capillary lasts a maximum of 7 min and can be interrupted at any stage. By the HPLC method the elution time of amphetamines is similar (MDE is eluted after ca. 11 min) but there is a waiting time of at least 17 min because caffeine is eluted after this time and is often found in tablets. A further post-run time of ca. 3 min. must also be included. Further advantages are the substance identification possible by the standard addition procedure and a very important fact is that the consumption of organic solvents is restrained.

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