Enantiomeric identification of chiral drugs, adulterants and impurities by capillary electrophoresis-ESI-mass spectrometry (CE-ESI-MS)

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Abstract

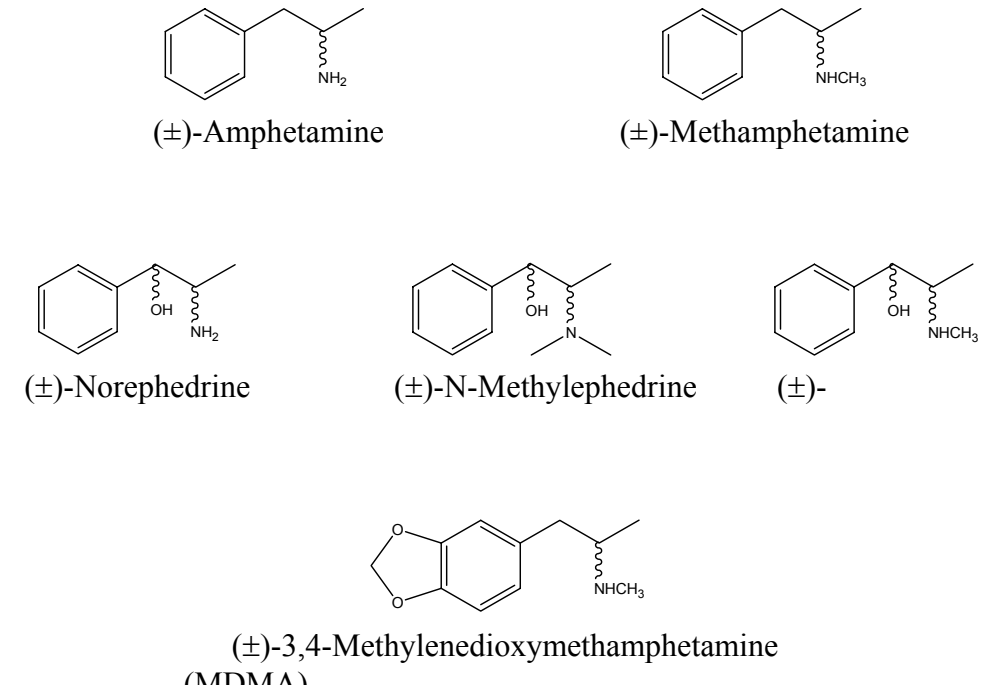
Most of the illicit drugs and some adulterants occur as optical isomers with different psychotropic activities. Especially for illicit methamphetamine and Ephedra alkaloid samples not only the enantioselective determination of the active substance but also the enantioselective determination of the chiral impurities is important for intelligence purposes with respect to the discrimination of different production batches.

Capillary electrophoresis is often used for chiral analysis because high enantiomeric resolution is achieved by simply adding polar cyclodextrins to the running buffer. To obtain higher sensitivity and selectivity, capillary electrophoresis coupled to mass spectrometry was applied in this work for the chiral separation and unambiguous identification of drugs and adulterants belonging to different families of compounds. To overcome the problem of contaminating the mass spectrometer with non-volatile cyclodextrins and to avoid ion suppression, mixtures of chiral selectors were employed only at low concentrations.

The running buffer consisted of 1 mol/l formic acid containing 1 mmol/l 2,3-diacetyl-6-sulfato-beta-cyclodextrin and 10 mmol/l 2-hydroxypropyl-beta-cyclodextrin. Dry nitrogen gas was delivered at 4 l/min at 250°C. The pressure of nebulizing nitrogen gas was set at 4 psi. The sheath liquid was isopropanol/water (50/50, v/v) at a flow rate of 3 µl/min. Enantiomeric separation of the most important beta-phenylethylamines, methadone and tetramisole was achieved at 20°C within 30 minutes using a high voltage of +25 kV. The developed CE-ESI-MS method allows the chiral identification of a wide range of drugs and adulterants. It was also possible to discriminate between different batches of illicit methamphetamine samples by comparing their chiral impurities.

1. Introduction

Most of the illicit drugs and some adulterants occur as optical isomers which have different psychotropic activities and partially fall within the scope of different regulations of the German narcotics act. The enantioselective determination of the active substance can give some advice as to the synthetic route and the precursors used in the illicit production of synthetic drugs [9]. Therefore, it is important to have a flexible and sensitive analytical method for the chiral separation and unambiguous identification of drugs and adulterants belonging to different families of compounds. The model analytes which were selected for this work are shown in Figure 1.



(±)-Amphetamine (±)-Methamphetamine

(±)-Norephedrine (±)-N-Methylephedrine (±)-

(±)-3,4-Methylenedioxymethamphetamine (MDMA)

(±)-Synephrine (±)-Methadone (±)-

Figure 1: Structural formulas of the selected chiral model analytes

The most common clandestine synthesis route to produce (+)-methamphetamine is the reduction of (+)-pseudoephedrine or (-)-ephedrine [6,7,8,9], which are often extracted from Ephedra sinica ("Ma Huang") or from pharmaceutical formulations containing (+)-pseudoephedrine. Ephedra sinica can contain not only (-)-ephedrine and (+)-pseudoephedrine but also (-)-norephedrine and traces of other Ephedra alkaloids [10]. Therefore, especially for illicit methamphetamine and Ephedra alkaloid samples, not only the enantioselective determination of the active substance but also the enantioselective determination of the chiral impurities is important for intelligence purposes with respect to the discrimination of different production batches [1].

Capillary electrophoresis is often used for chiral analysis because high enantiomeric resolution is achieved by simply adding polar cyclodextrins to the running buffer [1,2,3,6,7,8,9,]. To obtain higher sensitivity and selectivity, capillary electrophoresis coupled to mass spectrometry was applied in this work for the chiral analysis of drugs, adulterants and impurities. To overcome the problem of contaminating the mass spectrometer with non-volatile cyclodextrins and to avoid ion suppression, mixtures of derivatized cyclodextrins as chiral selectors were employed only at low concentrations [4,5].

2. Experimental

2.1 Chemicals

(±)-Amphetamine sulphate was purchased from Merck (Darmstadt, Germany). (±)-Tetramisole hydrochloride, (±)-synephrine, (±)-norephedrine hydrochloride, (-)-ephedrine hydrochloride, (-)-N-methylephedrine and (+)-N-methylephedrine were purchased from Sigma-Aldrich (Taufkirchen, Germany). (+)-Ephedrine hydrochloride, 2-hydroxypropyl-beta-cyclodextrin and formic acid solution (1 mol/l in water) were from Fluka (Buchs, Switzerland) and (±)-methamphetamine hydrochloride and (±)-3,4-methylenedioxymethamphetamine hydrochloride from Lipomed (Arlesheim, Switzerland), water/2-propanol (50/50, v/v) (LC-MS grade) from Riedel-de Haën (Seelze, Germany) and (±)-methadone hydrochloride from Hoechst AG (Frankfurt a.M., Germany). 2,3-Diacetyl-6-sulfato-beta-cyclodextrin was purchased from REGIS Technologies (Morton Grove, IL, USA). The seized samples were provided by the Bundeskriminalamt Wiesbaden, Germany except of the methadone containing blood sample which was provided by the Institute of Legal Medicine of the University Heidelberg, Germany. Deionised water from a Milli-Q system was used to prepare the samples.

2.2 Instrumentation

The CE-system used for all experiments was a Beckman-Coulter P/ACE 5000 system coupled via a coaxial sheath liquid sprayer interface (Agilent Technologies Palo Alto, CA, USA) to an HCT plus ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). All samples were introduced into the CE by hydrodynamic injection (3.45 kPa for 5 s in screening runs and for 10 s in impurity profiling runs). An untreated fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA) with an inner diameter of 50 µm, an outer diameter of 360 µm and a total length of 82 cm was used. For conditioning, a new capillary was flushed without connection to the MS for 20 minutes with NaOH (0.1 mol/L), for 5 minutes with water, for 10 minutes with HCl (0.1 mol/L) and for 20 minutes with buffer solution. Between runs the capillary was rinsed with running buffer for 2 minutes. For CE system control the software Beckman P/ACE Station 1.2 was used. Electrospray ionisation (ESI) was performed at

4500 V. Dry nitrogen gas was delivered at 4 l/min at 250°C. The pressure of nebulising nitrogen gas was set at 4 psi. The sheath liquid was isopropanol/water (50/50, v/v) supplied at a flow rate of 3 µl/min by a syringe pump (Cole-Parmer, Vernon Hill, IL, USA). ESI-MS spectra were obtained in the positive ion mode with a scan speed of 26000 m/z per second in the mass range 60-350 m/z with a target mass of 220 m/z. MS^n experiments were performed by isolation and subsequent fragmentation. Software esquireControl™ (version 5.2, Bruker Daltonics) was used for data acquisition and postprocessing software DataAnalysis (version 3.2, Bruker Daltonics) was used for data processing. For data interpretation the software ProfileAnalysis (version 1.0, Bruker Daltonics) was used.

2.3 Separation conditions

To achieve chiral separation of the model analytes (amphetamine, methamphetamine, ephedrine, N-methylephedrine, norephedrine, synephrine, methadone, tetamisole and MDMA), a running buffer consisting of 1 mol/l formic acid containing 1 mmol/l 2,3-diacetyl-6-sulfato-beta-cyclodextrin and 10 mmol/l 2-hydroxypropyl-beta-cyclodextrin was used. The separation was achieved at 20°C using a high voltage of +25 kV.

2.4 Sample preparation

The stock solutions of the reference substances (amphetamine, methamphetamine, ephedrine, norephedrine, synephrine, methadone, tetramisole and MDMA) consisted of a 5 mmol/L solution in water. For the reference substance N-methylephedrine the stock solution consisted of a 5 mmol/l solution in methanol. For the CE-ESI-MS measurements the stock solutions were diluted 1:200 in 1 mol/l formic acid. The sample solutions were prepared by dissolving approximately 20 mg of powdered sample in 1 ml of water. For the CE-ESI-MS measurements the samples were diluted with 1 mol/l formic acid in water.

3. Results and discussion

3.1 Chiral CE-ESI-MS procedure

A chiral separation of the nine model analytes was achieved within 30 minutes. The corresponding electropherogram is shown in Figure 2. For the development of a chiral CE-ESI-MS method it is not possible to simply use an already existing chiral CE analysis method with UV detection [1] and replace the detector, because the employed cyclodextrins are non-volatile and thus will contaminate the mass spectrometer. Furthermore, they suppress the ion formation in the electrospray, whereby the sensitivity of the mass spectrometer will be reduced.

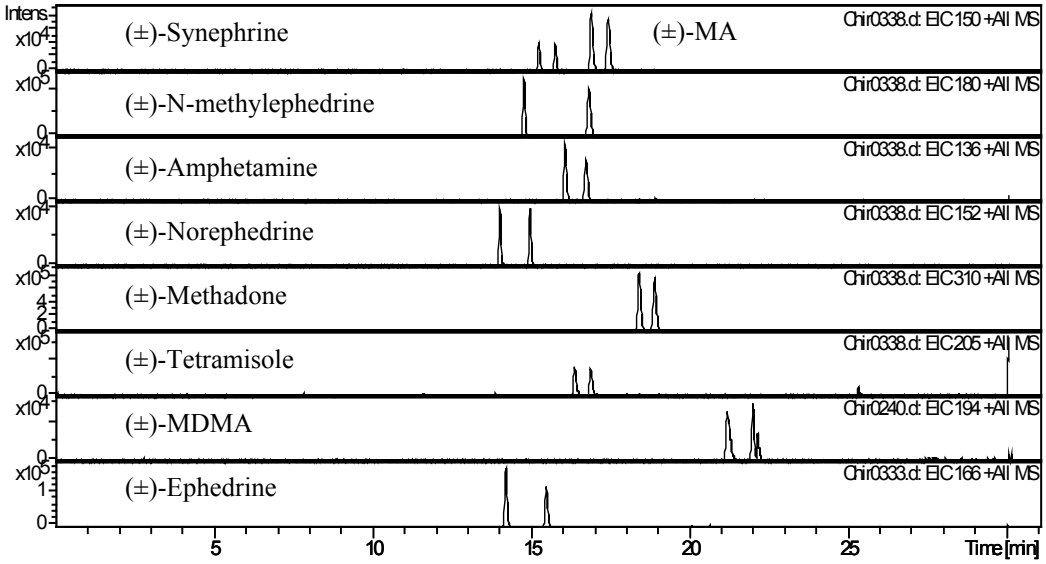


Figure 2: Electropherogram of the chiral separation of the nine model analytes

To avoid this problem for the CE-ESI-MS method only a low concentration of a mixture of selected derivatized cyclodextrins (2,3-diacetyl-6-sulfato-beta-cyclodextrin (HDAS-β-CD) and 2-hydroxypropyl-beta-cyclodextrin (HP- β-CD) was used as chiral selector. Reducing the concentration of the chiral selector has resulted in lower enantiomeric resolutions, but has the advantage of providing unequivocal identification by the mass spectrometer as well as a higher sensitivity than UV detection. This effect is illustrated taking tetramisole as an example. The enantiomeric resolution for (±)-tetramisole by using the method described in [1] (capillary electrophoresis with a PDA detector) is 6.4 compared to 1.7 for the method employing mass spectrometric detection, however, the 3σ limit of detection is 5,7 µmol/l (1,16 mg/l) for PDA detection and 400 nmol/l (81,7 µg/l) for mass spectrometric detection. The detection limits were calculated using three times the standard deviation of the baseline signal and the peak height at an analyte concentration of 125 µmol/l for UV detection and 12,5 µmol/l for mass spectrometric detection. The resolution R of the enantiomeric pair was calculated using Equations 1 and 2 and the corresponding electropherograms are shown in Figures 3 and 4.

$$R = 2\frac{\left|t_{R_2} - t_{R_1}\right|}{w_2 + w_1} \qquad (1)$$

t_{R_1} : retention time of Enantiomer 1; t_{R_2} : retention time of Enantiomer 2

w_1 : peak width for Enantiomer 1; w_2 : peak width for Enantiomer 2

$$w = 1.699427 w_{0.5} \qquad (2)$$

$w_{0.5}$: peak width at half height

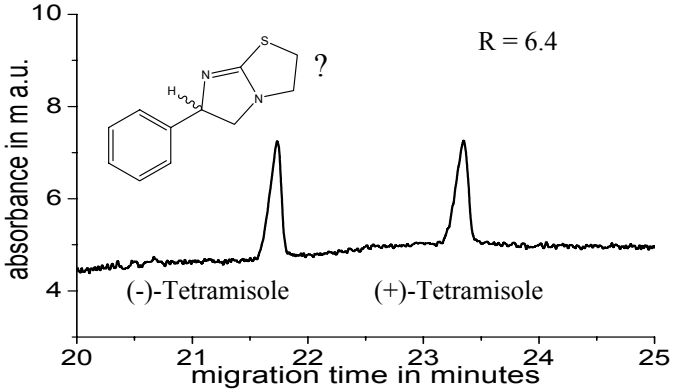


Figure 3: Electropherogram CE-DAD (running buffer: 2.5% (w/w) sulfated β-CD [1])

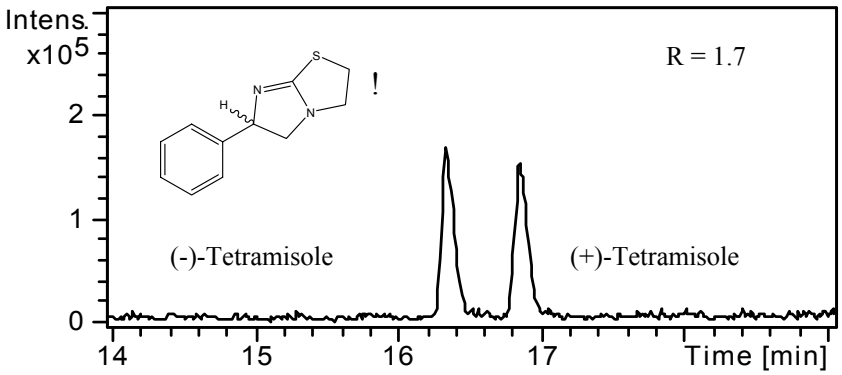


Figure 4: Electropherogram CE-ESI-MS (running buffer: 0.25% (w/w) (1 mmol/l) HDAS-β-CD and 1.5% (w/w) (10 mmol/l) HP-β-CD)

3.2 Forensic applications

Several samples of forensic interest were investigated using the described CE-ESI-MS method. One example of the indispensability of the mass spectrometric detection is the analysis of an illicit methamphetamine sample. The electropherogram of the chiral CE analysis of this sample with UV detection [1] is shown in Figure 5.

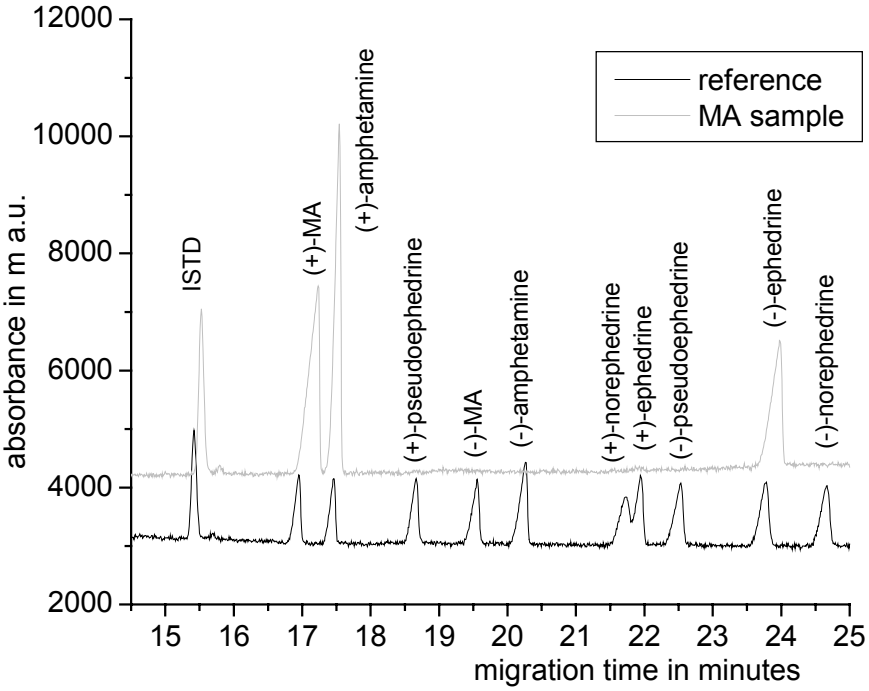


Figure 5: Chiral CE analysis of an illicit MA sample using CE-UVD (possible misinterpretation of procaine as (+)-amphetamine)

According to the results represented in Figure 5 the sample contains (+)-methamphetamine, (-)-ephedrine and apparently (+)-amphetamine. However, the results obtained by CE-ESI-MS prove unequivocally the absence of (+)-amphetamine in the methamphetamine sample. The detected peak which was misinterpreted as (+)-amphetamine is due to the local anaesthetic procaine, which was verified by MS/MS experiments. The extracted ion electropherograms (EIE´s) of the CE-ESI-MS measurement and the recorded MS/MS-spectrum of procaine are shown in Figures 6 and 7.

Another application of the enantioselective CE-ESI-MS method developed is the investigation of a methadone containing blood sample extract spiked with methadone-d9. This extract was provided by the Institute of Legal Medicine of the University Heidelberg, Germany in order to investigate whether this sample contains racemic methadone or levomethadone (e.g. Polamidone®). The enantioselective CE-ESI-MS analysis shows that the sample contains racemic methadone. The corresponding electropherogram (EIE) is shown in Figure 8.

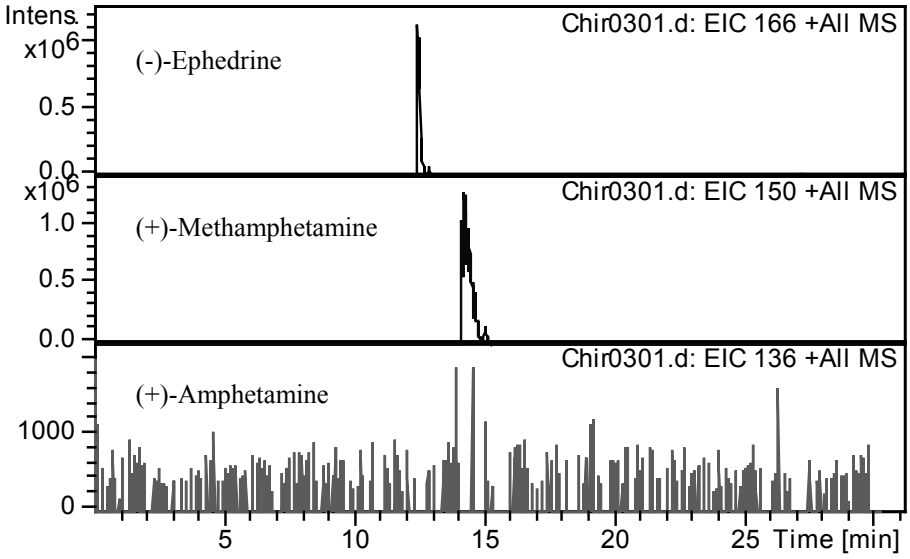


Figure 6: Chiral CE analysis of an illicit MA sample using CE-ESI-MS

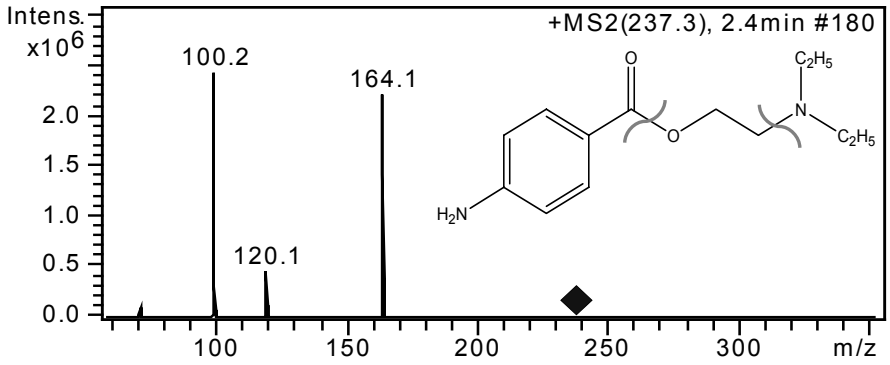


Figure 7: MS/MS-spectrum of procaine (Identification)

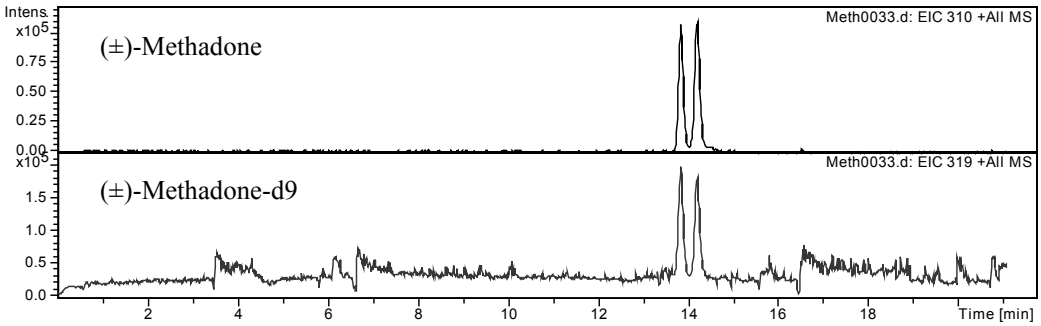


Figure 8: CE-ESI-MS analysis of a blood sample extract spiked with methadone-d9

It can clearly be seen that the unlabelled methadone and the added isotopically labelled methadone-d9 comigrate. By using the enantioselective CE-method with UV detection this comigration takes also place. Consequently, the isotopically labelled methadone can only be distinguished from the unlabelled compound by employing mass spectrometric detection.

Another forensic application presented here is the chiral identification of an unusual cutting agent in a cocaine sample. This cutting agent is levamisole, the levo enantiomer of tetramisole, which is used in human and veterinary medicine as anthelminticum against worm infestations [11], while the treatment with the racemic tetramisole is restricted to veterinary medicine. Figure 9 shows the electropherogram of the sample and of the tetramisole reference material. The identification of levamisole was confirmed by spiking. Figure 10 shows the MS/MS-spectrum of levamisole.

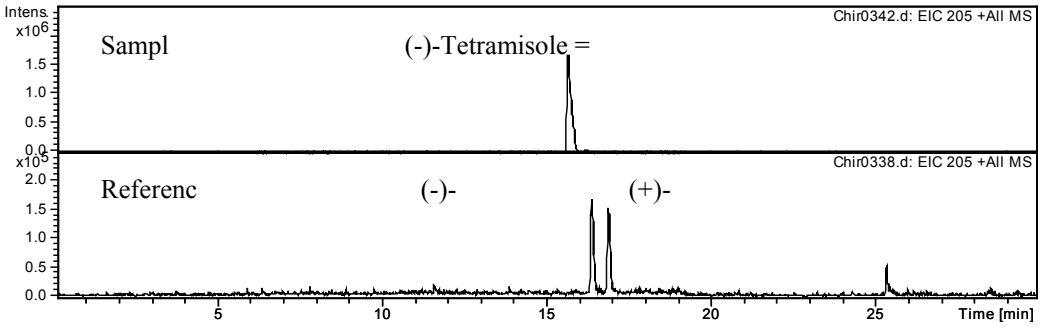


Figure 9: Chiral CE-ESI-MS analysis of an illicit cocaine sample containing levamisole as cutting agent

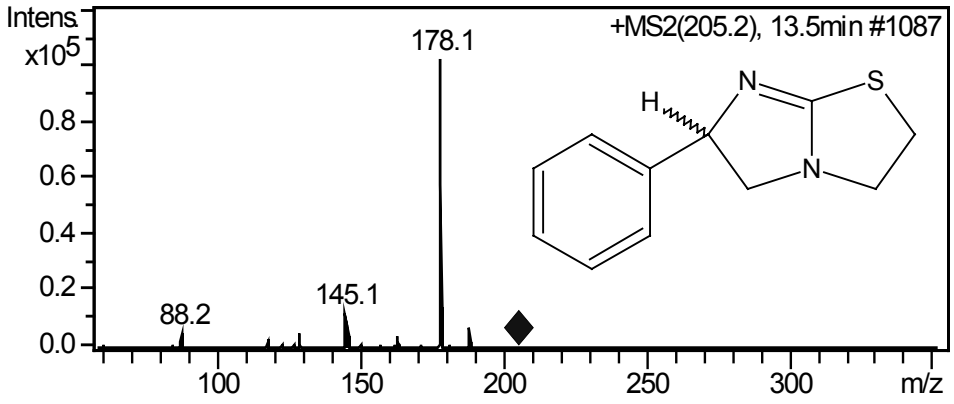


Figure 10: MS/MS spectrum of levamisole

With the CE-ESI-MS method described in this work it is also possible to discriminate between different batches of illicit methamphetamine samples by comparing the peak pattern of their chiral trace impurities. Consequently, the chiral impurities were analysed in sample solutions with a high concentration of

the active substance (stock solutions were diluted 1:4 in 1 mol/l formic acid) at an increased injection time (10 seconds). Figure 11 shows the CE-ESI-MS measurement of a methamphetamine street sample. Peak areas of selected chiral impurities (for example ephedrine and N-methylephedrine) were used for the chiral impurity profiling of the seized methamphetamine samples. Therefore the obtained data were evaluated with the Bruker software ProfileAnalysis, which uses multivariate statistical methods. The exploratory pattern recognition technique implemented in ProfileAnalysis corresponds to a principle component analysis (PCA). The visualization of the data is shown in Figure 12. On the basis of the chemometric results it was possible to discriminate between two different batches of illicit methamphetamine samples.

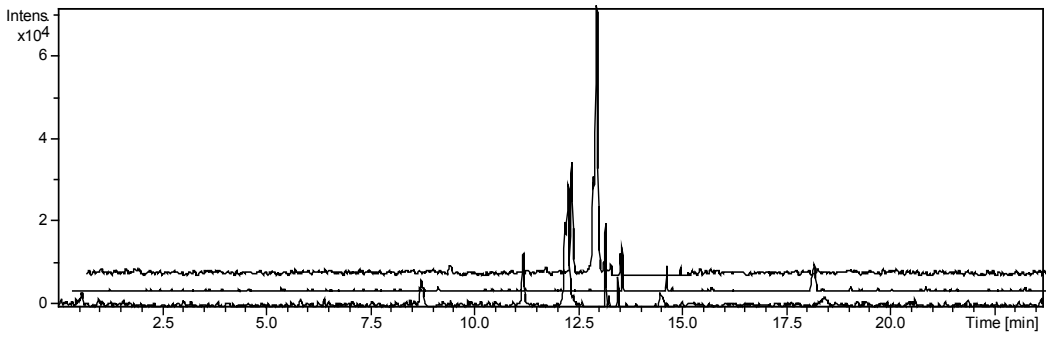


Figure 11: Chiral impurities of an illicit MA sample analysed by CE-ESI-MS

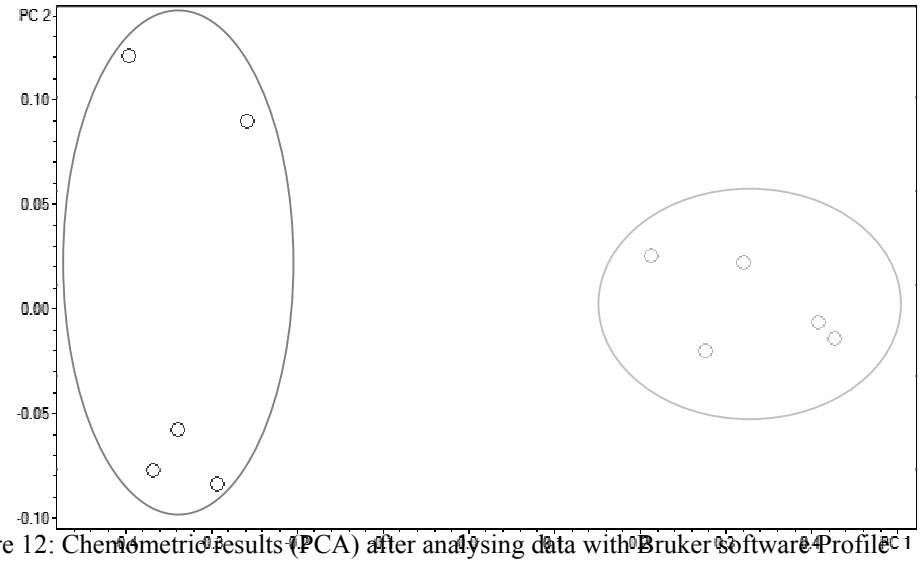


Figure 12: Chemometric results (PCA) after analysing data with Bruker software ProfileAnalysis

4. Conclusions

In this work, the potential of capillary electrophoresis coupled to mass spectrometry for the enantioselective identification of several drugs and adulterants was investigated. The developed CE-ESI-MS method allows the chiral identification of a wide range of drugs and adulterants including amphetamine, methamphetamine, ephedrine, norephedrine, N-methylephedrine, synephrine, methadone, tetramisole and MDMA within 30 minutes. Enantiomeric resolution was achieved using a running buffer containing hydroxypropyl-beta-cyclodextrin and 2,3-diacetyl-6-sulfato-beta-cyclodextrin as chiral selectors at low concentrations to avoid contamination of the mass spectrometer and to minimise ion suppression. Several forensic applications including enantioselective determination of isotopically labelled and unlabelled methadone in a blood sample show the importance of the selectivity of the mass spectrometer. The definite identification was achieved by recording MS-MS spectra in the positive polarity detection mode. With the developed method it was also possible to discriminate between different batches of illicit methamphetamine samples by comparing their chiral impurities.

5. Literature

[1] Dieckmann S, Pütz M, Dahlenburg R (2006) Chiral profiling of illicit methamphetamine samples by capillary electrophoresis. Tagungsband XIV. GTFCh-Symposium Praxis der forensischen Toxikologie, 14th-16th April 2005 in Mosbach (Baden), Germany: 225-235. Editor(s): Pragst F, Aderjan R. Publisher: Verlag Dr. Dieter Helm, Heppenheim, Germany

[2] Fanali S (2000) Enantioselective determination by capillary electrophoresis with cyclodextrins as chiral selectors. Journal of Chromatography A 875: 89-122

[3] Heo YJ, Whang YS, In MK, Lee KJ (2000) Determination of enantiomeric amphetamines as metabolites of illicit amphetamine and selegiline in urine by capillary electrophoresis using modified beta-cyclodextrin. Journal of Chromatography B 741: 221-230

[4] Iio R, Chinaka S, Takayama N, Hayakawa K (2005) Simultaneous Chiral Analysis of Methamphetamine and its Metabolites by Capillary Electrophoresis/Mass Spectrometry with Direct Injection of Urine. Journal of Health Science 51: 693-701

[5] Iio R, Chinaka S, Takayama N, Hayakawa K (2005) Simultaneous Chiral Analysis of Methamphetamine and Related Compounds by Capillary Electrophoresis/Mass Spectrometry Using Anionic Cyclodextrins. Analytical sciences 21: 15-19

[6] Iwata YT, Garcia A, Kanamori T, Inoue H, Kishi T, Lurie IS (2002) The use of a highly sulfated cyclodextrin for the simultaneous chiral separation of amphetamine type stimulants by capillary electrophoresis. Electrophoresis 23: 1328-1334

[7] Liau AS, Liu JT, Lin LC, Chiu YC, Shu YR, Tsai CC, Lin CH (2003) Optimization of a simple method for the chiral separation of methamphetamine and related compounds in clandestine tablets and urine samples by beta-cyclodextrin modified capillary electrophoresis: a complementary method to GC-MS. Forensic Science International 134: 17-24

[8] Lurie IS, Klein RFX, Dal Cason TA, LeBelle MJ, Brenneisen R, Weinberger RE (1994) Chiral resolution of Cationic Drugs of Forensic Interest by Capillary Electrophoresis with Mixtures of Neutral and Anionic Cyclodextrins. Analytical Chemistry 66: 4019-4026

[9] Lurie IS, Odeneal II NG, McKibben TD, Casale JF (1998) Effects of various anionic chiral selectors on the capillary electrophoresis separation of chiral phenethylamines and achiral neutral impurities present in illicit methamphetamine. Electrophoresis 19: 2918-2925

[10] Rätsch C (1998) Enzyklopädie der psychoaktiven Pflanzen (Botanik, Ethnopharmakologie und Anwendung). AT Verlag 228-230

[11] Valentino AMM, Fuentecilla K, (2005) Levamisole: An Analytical Profile. Microgram Journal 3: 134-137

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