Über die Entwicklung und Anwendung einer Referenzspektrenbibliothek zur Identifizierung von forensisch-relevanten Substanzen mittels MS/MS

Herbert Oberacher, Marion Pavlic, Kathrin Libiseller, Birthe Schubert

Abstract

The potential of a tandem mass spectral library established on a quadrupole–quadrupole–time-of-flight (QqTOF) instrument for the identification of therapeutic and illicit drugs was evaluated. Due to the mass accuracy, the stability of calibration and the reproducibility of fragmentation, the QqTOF mass spectrometer should represent an appropriate platform for the establishment of a tandem-mass spectral library. 402 substances were used as reference samples to build up the spectral library. For each reference compound, product ion spectra were acquired at ten different collision energy values between 5 eV and 50 eV. For the identification of unknown compounds, a library search algorithm was developed. The mass spectral library search approach was successfully applied to the characterization of forensic casework samples.

1. Introduction

Electrospray ionization (ESI) represents a soft ionization technique. Usually, only molecular ions are formed. Collision-induced dissociation (CID) can be used to obtain structural-related information of analytes. The diagnostic fragment ions are either formed in the ion transfer region (in-source CID) or more selectively in the collision cell of an instrument dedicated for tandem MS (MS/MS). Both techniques have been used to establish mass spectral libraries. To increase the tolerance of libraries towards the variability of instrumental parameters the application of a set of different collision energies for the collection of reference spectra was proposed [1,2]. Established mass spectral libraries are dedicated to the identification of drugs, pesticides or explosives [3]. More or less all of them were built up on quadrupole, triple quadrupole or ion trap mass spectrometers.

Our aim was to establish a tandem mass spectral reference library on an ESI-QqTOF mass spectrometer [4]. Due to the accuracy of fragment ion mass measurement, the use ESI-QqTOF-MS/MS combined with an appropriate mass spectral library search algorithm should represent a powerful tool for the identification of unknown compounds. To prove this theory, a database containing 402 drugs and xenobiotics of forensic interest was established. For each reference sample, product ion spectra were collected at ten different CE values between 5 eV and 50 eV. Due to the lack of any kind of database feature within the oper-

ating software of the mass spectrometer used, a library search algorithm was developed. The screening methodology was successfully applied to the characterization of forensic casework samples. Active agents and extenders were unequivocally identified within a number of seized drug and autopsy samples [4].

2. Experimental

2.1. Chemicals

Acetonitrile (HPLC gradient-grade), acetic acid (p.a.) and water (HPLC gradient-grade) were obtained from Fluka (Buchs, Switzerland). The drug standards of the laboratory's collection were used at the highest available purity.

2.2. ESI-QqTOF-MS and -MS/MS

ESI-MS was performed on a QSTAR XL mass spectrometer (Applied Biosystems). Mass calibration and optimization of instrumental parameters were performed in the positive ion mode by infusion of a mixture of 1.0 mg/l caffeine and 1.0 mg/l reserpine dissolved in 0.05% aqueous acetic acid solution containing 50% acetonitrile (v/v) at a flow rate of 2.0 µl/min. The spray voltage was typically in the range of 4.0 kV. Gas flows of 1-3 arbitrary units (nebulizer gas) and 40 arbitrary units (turbo gas) were employed. The temperature of turbo gas was adjusted to 200 °C. The accumulation time was set to 1.0 sec and 4 time bins were summed up. Mass spectra were collected in the range between 50 u and 700 u. For MS/MS, the Q1 resolution was set to unit resolution. The collision gas (N_2) flow was set to 5 arbitrary units. CE values in the range between 5 eV and 50 eV were applied to generate product ion mass spectra. Total ion chromatograms and mass spectra were recorded using the Analyst QS software (1.0, service pack 8, Applied Biosystems).

2.3. Mass spectral library of drugs

For the collection of product ion mass spectra characteristic for a certain drug, a solution of the respective drug in 0.05% aqueous acetic acid solution containing 50% acetonitrile (v/v) was prepared and directly infused into the mass spectrometer at a flow rate of 2.0 µl/min. Depending on the ionization efficiency of the compound, solutions with concentrations in the range of 0.10 to 10.0 mg/l were applied. Product ion mass spectra were measured at 10 different CE values, starting with 5 eV in steps of 5 eV up to 50 eV. At each CE level, mass spectra collected over a period of at least 1.0 min were averaged.

2.4. Computer-aided data interpretation

All calculations were performed on a personal computer under Windows XP operating system. Measured MS/MS spectra were exported from the Analyst QS software (Applied Biosystems) as txt-files. A detailed description of the applied library search strategy can be found elsewhere [4]. Automated library search was performed with a program written in ActivePerl 5.6.1 (Active State Corporation, Vancouver, BC, Canada).

2.5. Characterization of real world drug samples

100 ng - 1.0 mg of each sample were dissolved in 1.0 ml acetonitrile. Aliquots (250 µl) of the stock solutions were mixed with an equal volume of 0.1% aqueous acetic acid solution and analyzed via direct infusion into the mass spectrometer at a flow rate of 2.0 µl/min. Mass spectra collected over a period of at least 1.0 min were averaged. Product ion mass spectra were acquired at least at three different CE values. Results were checked by general unknown screening for common drugs with GC-MS.

3. Results and discussion

The created database and the developed search algorithm were applied to the analysis of multiple routine forensic casework samples [4]. All samples were characterized by GC-MS as well as by direct infusion MS and subsequent MS/MS experiments. The use of direct infusion MS offers the clear advantage that time and effort required for sample preparation steps such as extraction and chromatography can be saved. Typically, the characterization of a single sample including the collection of spectra and the execution of multiple library search runs was accomplished within a few minutes. The major part of the total analysis time can be used for MS/MS experiments. In the absence of a chromatographic separation step, however, matrix effects may become a concern. Thus, the applicability of the described approach to the analysis of complex biological samples like tissues, blood, urine and faeces is constricted.

The full scan mass spectrum obtained from a seized illicit drug sample is shown as typical example in Figure 1a. All species having relative signal intensities larger than 1.0% were selected as precursor ions for subsequent MS/MS-experiments. The obtained mass spectrometric information identified successfully amphetamine (Figure 1b), 3,4-methylenedioxy-N-methylamphetamine (MDMA, Figure 1c) and caffeine (Figure 1d) which were part of our database. Four more species (marked with asterisks in Figure 1a) were identified as fragment ions produced by in-source CID from the three compounds described above. The product ion spectrum obtained from the precursor ion with a monoisotopic mass of 254.1919 u (Figure 1e) matched almost equally well to the three structurally related compounds amphetamine, methamphetamine and mefenorex. The monoisotopic mass, however, did not fit to one of these species. It was likely that

the unknown compound was a derivative of amphetamine of which no reference spectrum had been stored in the mass spectral library. Among the number of impurities that might be found in (illicit) amphetamine preparations, N,N-di(beta-phenylisopropyl)amine was the only molecule that has a monoisotopic mass (254.1903 u) that comes close to the mass of the unknown. This example clearly suggests that the presented library search approach can be successfully used even for the identification of derivatized compounds or metabolites.

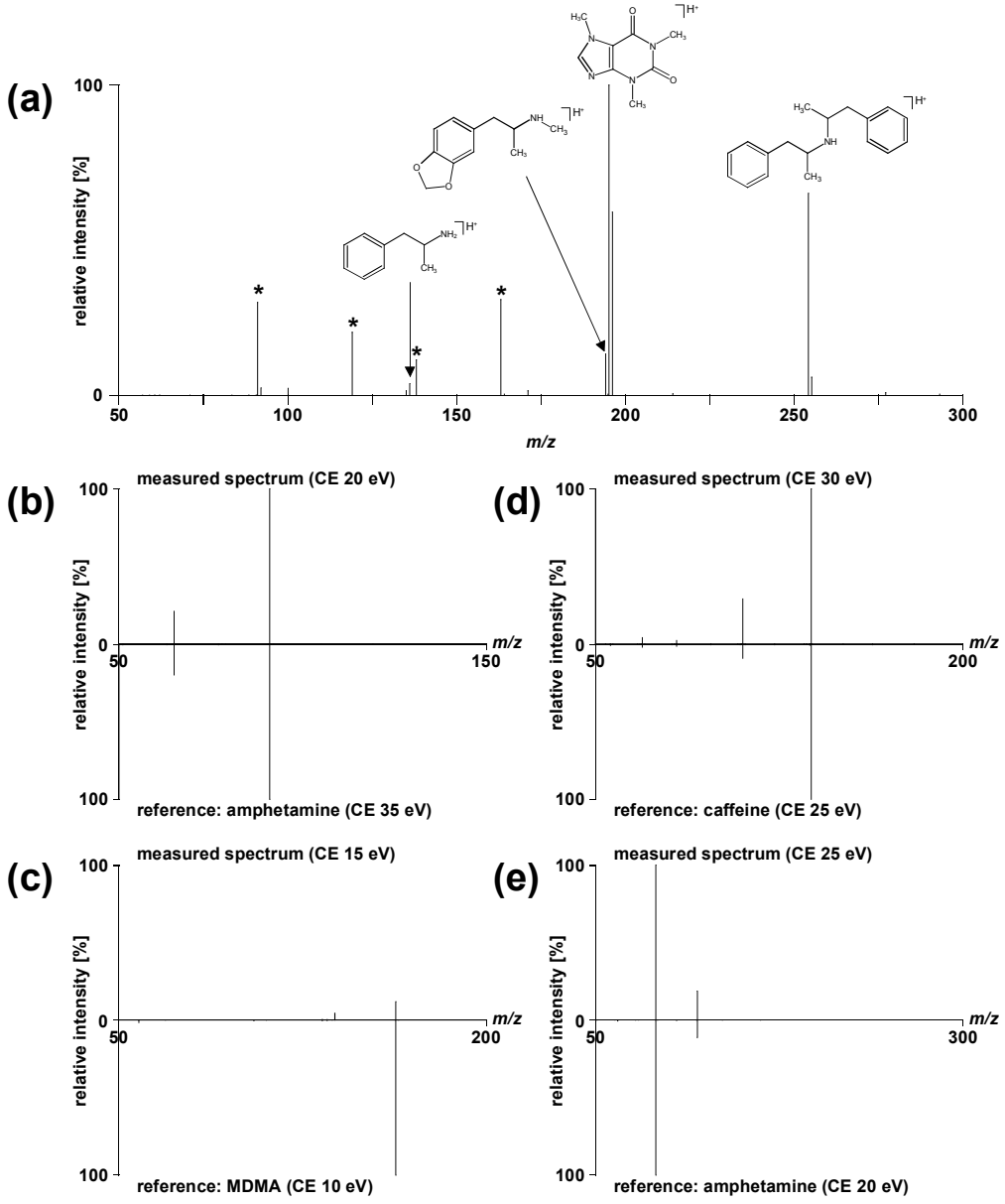

Fig. 1: Characterization of a seized drug sample via ESI-QqTOF-MS and -MS/MS experiments.

4. Conclusions

A tandem mass spectral library which has been established on a QqTOF instrument shows potential to become a valuable tool for the identification of therapeutic and illicit drugs in forensic toxicology.

5. Acknowledgements

The authors wish to thank Applied Biosystems for the generous provision of the mass spectrometer and the associated equipment.

6. References

[1] Weinmann W, Stoertzel M, Vogt S, Svoboda M, Schreiber A (2001) Tuning compounds for electrospray ionization/in-source collision-induced dissociation and mass spectra library searching. J Mass Spectrom 36: 1013-1023

[2] Jansen R, Lachatre G, Marquet P (2005) LC-MS/MS systematic toxicological analysis: comparison of MS/MS spectra obtained with different instruments and settings. Clin Biochem 38: 362-372

[3] Milman BL (2005) Identification of chemical compounds: modern qualitative analysis. Trends Anal Chem 24: 493-508

[4] Pavlic M, Libiseller K, Oberacher H (2006) Combined use of ESI-QqTOF-MS and ESI-QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. Anal Bioanal Chem 386: 69-82

Dr. Herbert Oberacher
Institut für Gerichtliche Medizin
Medizinische Universität Innsbruck
Müllerstrasse 44
6020 Innsbruck
Österreich
E-Mail: herbert.oberacher@i-med.ac.at