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## *Electrospray ionization: acquisition conditions*

Electrospray ionization product-ion spectra library of designer drugs and compounds of pharmaceutical or toxicological interest

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### **Introduction**

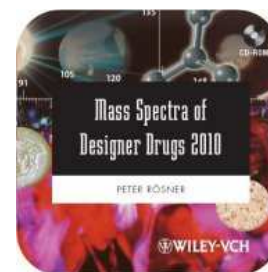
Liquid chromatography-mass spectrometry (LC-MS) has become a powerful technique with high sensitivity and specificity in forensic and clinical toxicology - . Since electrospray ionization-mass spectrometry (ESI-MS) was introduced by Yamashita and Fenn this technique and product-ion mass spectrometry (ESI-MS-MS) have been increasingly used for the analysis of compounds of forensic relevance sometimes even making a direct analysis of body fluids possible. With the introduction of wideband excitation and normalized collision energies the reproducibility of ESI spectra was significantly improved so that the construction of MS/MS spectral libraries became possible. These can be searched in the same way as electron impact (EI) libraries. The goal of perfectly matching ESI spectra from different instruments is not reached yet, but useful universal libraries may be created. This encouraged the authors to build an ESI-MS/MS library of designer drugs and compounds of pharmaceutical or toxicological interest in analogy to the electron impact mass spectral library Designer Drugs, An ESI-MS/MS library of more than 5,000 spectra of about 500 designer drugs, medicinal drugs, toxicological interesting compounds and their metabolites has been generated by using standard reversed-phase analytical columns with gradient elution or direct flow injection of solutions containing concentrations of 10 µg/mL up to 1 mg/mL of the reference compounds.

### Recording of electrospray ionization (ESI) product-ion spectra

The electrospray ionization (ESI) product-ion spectra were acquired with a LCQ DECATM and a LCQ DUOTM ion trap mass spectrometer (Thermo, San Jose). The spectra of this library were recorded by using normalized collision energies. Following additional standard parameters were used:

Source Voltage:	5.0 kV
Capillary Temp:	200 °C
Capillary voltage:	36 V
Flow Rate:	5.00 (µL/min)
Sheath gas flow rate:	80 arbitrary units
Micro Scan Count:	3
Isolation width:	1.3 (m/z)
Activation Q:	0.250
Activation time:	30 msec
Number of micro scan:	3
Max. inject time:	50 msec

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Mass range:	normal
Resolution:	Low
Dynode Voltage:	-1500 (kV)
CID gas:	Helium (~ 1 Torr)

## Promoting ESI response

Analytes suitable for analysis with ESI-MS must either exist as preformed ions in solution or charged by protonation, deprotonation, adduct formation, electrochemical oxidation or reduction. In the cases of amines and other protonable molecules methanol was used as solvent with a concentration of 0.5 % acetic acid to promote protonated molecule ion species ( $[M+H]^+$ ) and acceptable ESI responses. Organic acids were negatively charged through deprotonation in methanol as solvent containing 5  $\mu\text{L/mL}$  ammonia 25 %.

## Reference standards

Reference standards were obtained from Acros Organics and Sigma-Aldrich. Many compounds were obtained from the daily working area and from a number of colleagues others were synthesized. The authors are especially indebted to Prof. Pragst (Berlin) who generously provided us with numerous pharmaceutical reference compounds.

## Selection of ESI ions

In the case of basic analytes like amines the protonated pseudo molecular ions ( $[M+H]^+$ ) were used as precursors ions for following collision experiments and spectra acquisitions. Organic acids or other acid compounds were negatively charged ( $[M-H]$ ) via deprotonation and the deprotonated pseudo molecular ions were selected as precursor ions. Charged molecules like compounds with tertiary ammonium groups were recorded in their ionic form ( $[M]^+$ ). Some compounds are oxidized in the positive ion or reduced in negative ion mode via an electrochemical reaction. In these cases the spectra of radically molecular ions ( $M^+$ ,  $M^-$ ) species were added to the library. Spectra of sodium and potassium adduct or clusters are not part of the library.

## Assessing ESI spectra quality

The comparison of unknown and library reference spectra is the key step in the mass spectral identification of chemical compounds by automated systems. Noise can cause either false negative or false positive identifications. High levels of chemical and random noise are inherent for ESI-MS spectra and a major disadvantage of this technique, so that noise reduction and an increase of the analyte ion signal intensity become important issues.

Full scan mass spectra (MS1) are selected as the basis of all collision-induced dissociation (CID) spectra so that noise calculation and a filtering algorithm that removes poor quality spectra become necessary. All full scan mass spectra (MS1) with a total ion count below 106 inclusive their related CID spectra were not accepted. To have a quality assessment in analogy to EI spectra<sup>9</sup>, any recorded MS1 spectrum was compared to an ideal ESI-MS1 spectrum with an upper  $m/z$ -range of  $m/z = 2000$  with fragments existing only below and equal to the pseudo molecular ion species ( $[M+H]^+$ ,  $[M-H]$ ,  $[M]^+$ ). Chemical noise and clusters above the pseudo

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molecular ion inclusive its isotopic peaks represent interferences that potentially affect the fragments of the molecular ion species. An ESI-MS1 noise quality index (QIMS1) was defined on the basis of the intensity sum of fragment below and equal to the pseudo molecular ion (including  $^{13}\text{C}$  and isotopic halogen clusters) compared to the intensity sum of the total ESI spectrum up to  $m/z$  2000:

$$\text{QIMS1} = 100 \times \frac{\text{intensities} \leq \text{pseudo molecular ion mass}}{\text{intensities below mass 2000}}$$

ESI-MS1 spectra with noise quality indices below 5% were not accepted as reference spectra, so that an ESI-MS1 database with an overall average noise quality index of 38% could be generated. ESI-MS1 spectra with noise quality indices of about 30% represent visually high-quality full scan spectra with low chemical noise (Fig. 1).

In-source CID to decrease solvent adduct formation or break up dimers and other complexes was not applied.

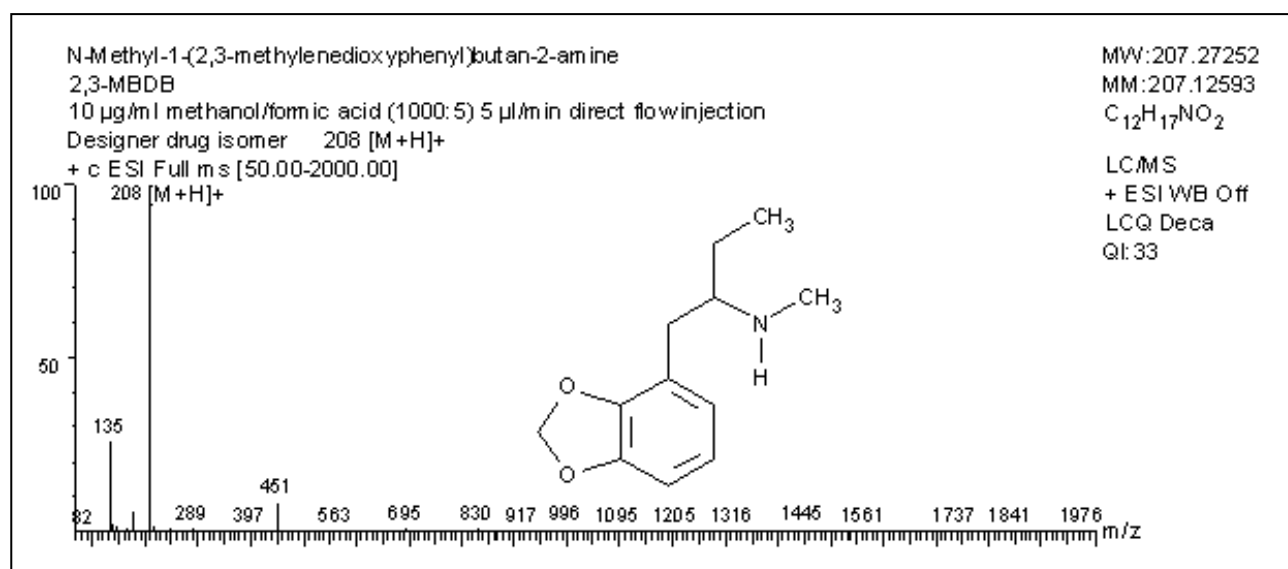


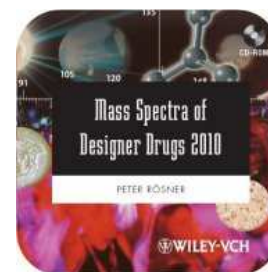
Fig. 1

For ESI-CID spectra an additional quality assessment was defined by dividing the sum of signal intensities above ten times the noise level by the total intensity sum of the spectrum. Spectra were generally added to the database only when this quotient was above 50%. In this way, an ESI-MSn-CID database with an overall noise quality index of 94 % could be generated.

$$\text{QICID} = 100 \times \frac{\text{intensities ten times higher the noise level}}{\text{intensities}}$$

As far as possible the spectra were verified by ESI mass spectra libraries and checked by mass spectral interpretations. This library contains besides MS2 spectra also higher MSn spectra up to MS6. As far as we are informed other ESI spectra collections available commercially contain MS2 spectra only. A verification of MS3-MS6 spectra of this library with foreign libraries therefore was not possible. An additionally verification index (VI) therefore could be given for ESI MS2 spectra only. The following number behind the verification index gives the number of foreign product ion

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spectra libraries where this spectrum corresponds to a reference spectrum with a similarity equal or bigger than 90%. About 14 % of all ESI-MS2 spectra could be verified in this way (NIST/EPA/NIH Mass Spectral Library 2005, nist\_msms, 5191 spectra). The low verification rate is an indicator of a high amount of mass spectra which can be found in Designer Drugs MS/MS 2010 only.

## Selection of normalized collision energies

One factor determining the successful comparison of unknown and reference spectra is their information content. The higher the number of fragments of spectra the more reliable are the library search results. The number of fragments of an ESI product ion spectrum depends on the collision energy used. The selection of normalized collision energies for library reference spectra therefore becomes critically for spectral comparisons. Especially low molecular weight ESI product ion spectra have a low information content compared to their electron impact counterparts. A strategy to generate a reliable ESI reference spectra library has to consider the need of information. Because the number of fragments increase with the collision energy a value as high as possible was selected until the precursor ion intensity dropped to a value of about 10% (Fig. 2). Spectra with this optimum of fragmentation efficiency were selected as the basis of all higher MS<sub>n</sub> product ion spectra.

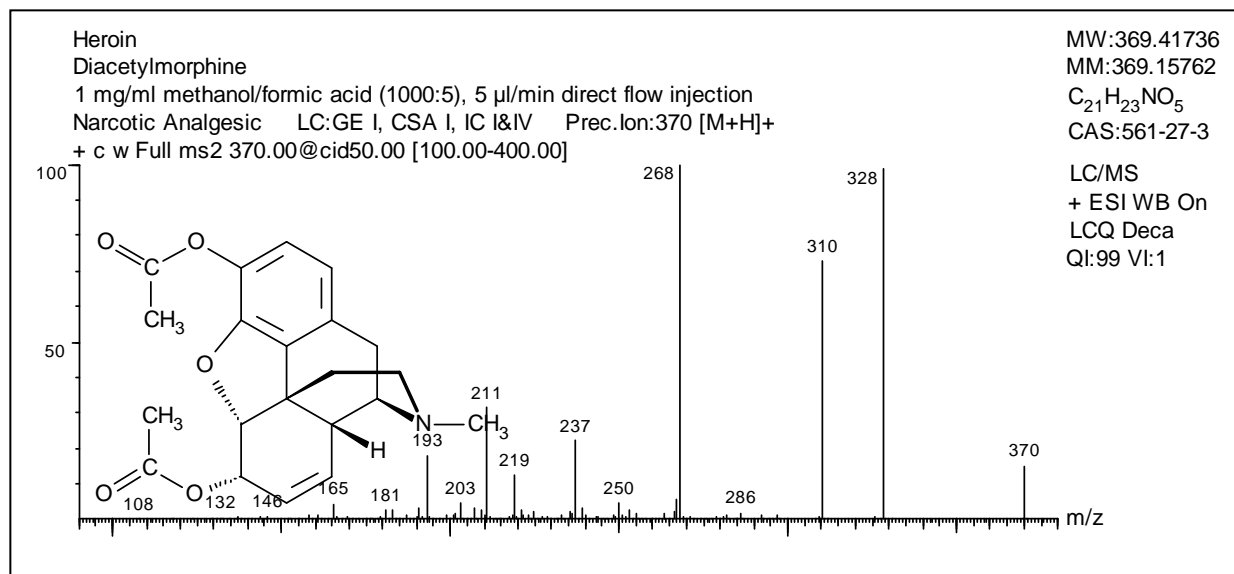


Fig. 2

Alternatively to this upper collision energy value, a lower normalized collision energy value was defined by lowering the collision energy until all fragments besides the precursor ion were below 10% (Fig. 3). All reference library CID spectra therefore are characterized by a significant high mass fragment identical with the precursor ion mass of the highest MS<sub>n</sub> experiment.

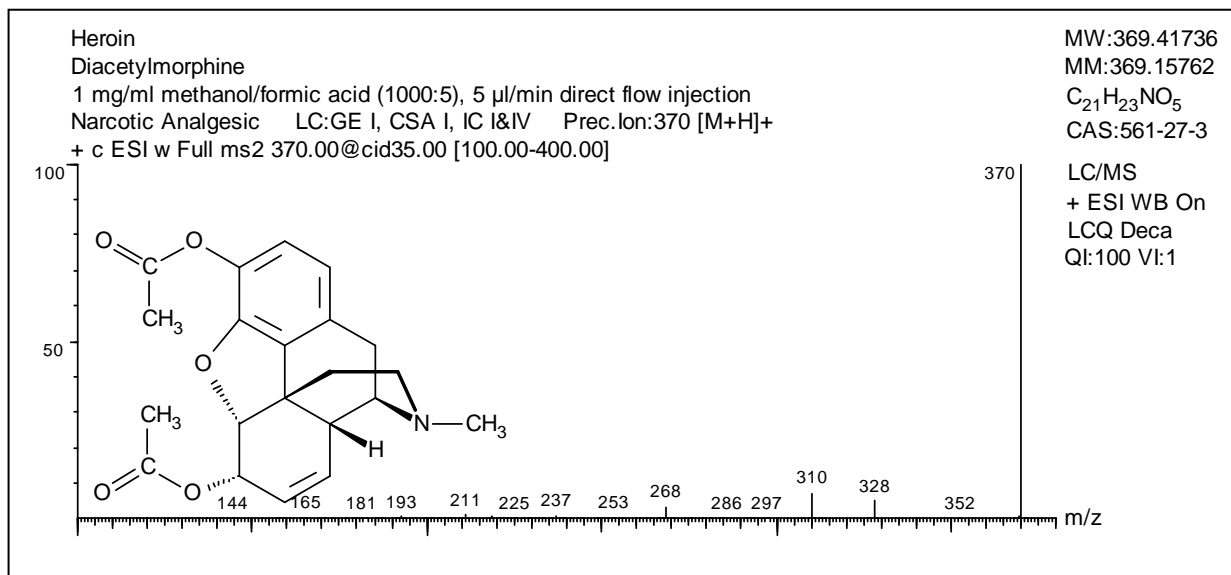
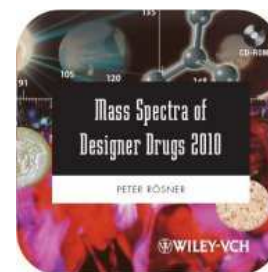


Fig. 3

CID spectra can be generated automatically by data depending acquisition modes. During data depending scans, the computer determines the mass of the most intense parent ions and subjects those ions to CID. During the automatic acquisition of data depending scans collision energies of very different values are used. To generate a reliable pool of ESI-MS<sup>n</sup> library reference spectra suited for data depending scan spectra and LC/MS-spectra of different mass spectrometer systems additional CID spectra with a stepwise distance of 5% normalized collision energies between the above defined extremes were generated.

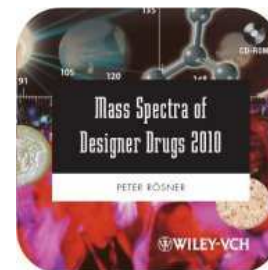
### Normalized collision energies

The collision energy needed to achieve an optimum fragmentation efficiency is proportional with the m/z value. The higher the m/z value of the precursor ion the higher the collision energy to get an effective fragmentation. Normalized collision energies vary automatically the actual collision energy according to the precursor ion m/z value<sup>7</sup>. This allows the use of a simple scale of normalized collision energy from 0 – 100 % regardless of the ion mass. Setting a value of 100% applies the maximal available collision energy of 5 eV to the precursor ion.

The use of normalized collision energies optimizes fragmentation efficiency and secures a reliable pattern of product ions, makes the MS/MS spectra remarkably reproducible and relative insensitive to instrumental settings. This MS/MS library has been built using the powerful technique of normalized collision energies.

### Wideband excitation

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During wideband excitation resonance the energy is applied over a mass range which extends to 20 amu lower than the selected ion. This ensures that both the precursor ion and any ion generated by loss of low mass neutrals (H<sub>2</sub>O, NH<sub>3</sub>) will undergo fragmentation. The absence of wideband excitation requires an additional MS/MS step to isolate the ion generated by loss of low mass neutrals. Applying more collision energy to precursor ion results in practical the same spectrum because much internal energy gets lost with the excited low mass neutrals. Wideband excitation is marked as “WB on” on the left hand column of the spectra. Spectra of this library were recorded with and without wideband excitation resonance.

## Nomenclature for CID spectra

Any CID spectrum is provided with an experiment string explaining its recording conditions. The experiment string follows the nomenclature of the Windows® based Thermo Scientific Xcalibur® software:

```
+ c ESI d w Full ms2 319.15@cid35.00 [75.00-650.00]
```

The experiment string starts with a sign + or – depicting the ionization mode. The following character describes the kind of data acquisition, centroid or profile data: c or p. ESI denotes electrospray ionization; API would denote atmospheric pressure ionization. The character d describes a precursor scan mode, a CID spectrum. The character w indicates a wideband excitation spectrum and Full denotes that a product ion mass range has been recorded. “ms2” gives the scan power (degree of consecutive fragmentation processes) and the following number indicates the m/z value of the precursor ion. After “@cid” follows the value of normalized collision energy applied for this CID process. The mass range of the measured product ion spectrum is given in squared brackets at the end of the string. The help system of the Xcalibur® software gives a comprehensive description of experiment strings so that detailed explanation is spared here.

## Data handling

The huge amount of data generated by product-ion (MS/MS) mass spectrometry can be handled adequately only with the help of computers and sophisticated software. A program has been developed under Microsoft Visual C++ 6.0 for now more than two decades. The program Chemograph Plus\*) allows to combine cross instrument spectroscopic data of different spectrometer types into a single data set and permits the production of high quality forensic reference data bases<sup>9,10</sup>. The data processing comprises standard methods like TIC baseline calculations, automatic peak identification, mass spectra underground subtraction or other mass spectral arithmetics. More sophisticated methods allow spectral quality evaluation and the digital based identification and elimination of structural, spectral or orthographic errors. Computer-aided human inspection is the most important thing in finding errors in mass values, nomenclature, and structures. Efficient error elimination functions are especially essential for larger spectral databases containing thousands of spectra. The graphical representation of the spectra is of a high quality in vectorial format and suited directly for documentation and publication. The graphical representations are placeable in all mayor text and graphic processing programs by OLE (object linking and embedding) windows metafiles or bitmaps.

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## Structural and empirical formulas

The structural formulas are uniformly drawn in their precise stereochemical representation. The elements of the empirical formulas are arranged according to Hill:

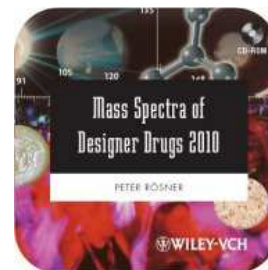
1. Number of carbon atoms
2. Number of hydrogen atoms
3. Alphabetical order of the other elements
4. Increasing number of elements

This manual has been prepared with maximal care. However, because of the extremely large data amount, errors cannot be completely excluded and no legal responsibility can be accepted for any inaccuracies of statements, data, illustrations, procedural details, or other items. Users are requested to report any error or suggestions by E-Mail to [p.roesner@t-online.de](mailto:p.roesner@t-online.de) or by Fax: 0049 431/32723. In forthcoming editions suggestions will be included and errors will be corrected.

The reproduction of any commercial or trade names etc. in this publication even if not imply specifically identified does not mean that these names have been released for general use under the legislation on trademarks and trademark protection.

We hope that this database of mass spectral data will be useful also to other analytical chemists in forensic, clinical, or university laboratory for the analysis of new designer drugs and compounds of pharmaceutical and toxicological interest.

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