**Actual Instrumentation**

*August 2017*

See also [http://www.uni-bielefeld.de/chemie/analytik/ms](http://www.uni-bielefeld.de/chemie/analytik/ms) for additional information

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1. MALDI TOF Mass spectrometry on the Ultraflex

experimental section:

Samples were mixed on a polished steel target with 1 µL of matrix solution (either 50 mg/mL 2,5-DHB in 50% ACN, 0,1% TFA or saturated DCTB in methanol). MALDI TOF mass spectra were recorded with a Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) operated in reflectron mode (For very large analytes, e.g. proteins: linear mode). Ionisation was achieved using a LTB nitrogen laser MNL 200 (337 nm beam wavelength, 50 Hz repetition rate). Maximum resolution achieved with this instrument is approx. 20,000.

The Instrument Default Calibration was used for calibrating the mass axis.

or

The mass axis was externally calibrated with (PEG or red phosphorous¹) as calibration standard on the same target.

or

The mass axis was internally calibrated with ... as internal calibration standard.

The spectra are recorded with FlexControl Software 3.0 (Bruker Daltonik, Bremen, Germany) by accumulation and averaging of ... single laser shots. FlexAnalysis 3.4 (Bruker Daltonik, Bremen, Germany) was used for processing the spectra.

specify (example):

MS (MALDI TOF, positive ions, Matrix, Nr.of summed Spectra)

\[ m/z = \text{Mass}_1 \left[M_1+H\right]^+, \text{Mass}_1 \left[M_1+Na\right]^+, \text{Mass}_1 \left[M_1+K\right]^+, \text{Mass}_2 \left[M_2+H\right]^+, \ldots \]

MS (MALDI TOF, negative ions, Matrix, Nr.of summed Spectra)

\[ m/z = \text{Mass}_1 \left[M_1-H\right]^{-}, \text{Mass}_1 \left[M_1+Cl\right]^{-}, \text{Mass}_2 \left[M_2-H\right]^{-}, \ldots \]

For soft ionisation techniques relative (!) intensities in the spectra should not be cited because they are not reproducible in substance mixtures and on different MS instruments. If the isotopic cluster of a compound shall be cited as a proof for the elemental composition of a special compound, relative ion abundances have to be given as values or (better) the cluster may be depicted in comparison with the calculated isotopic distribution. example (or use example depicted in the Esquire 3000 section):

abbreviations:

Mass: \( m/z \) of the pseudomolecular ion; \( M \): number of the compound or its formula (C\(_{x}\)H\(_{y}\)O\(_{z}\))

Experimental section:

Nano-ESI mass spectra were recorded using an Esquire 3000 ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a standard nano-ESI source. Samples were introduced by static nano-ESI using in-house pulled glass emitters. Nitrogen served both as the nebulizer gas and the dry gas. Nitrogen was generated by a Bruker nitrogen generator NGM 11. Helium served as cooling gas for the ion trap and collision gas for MS^n experiments.

The spectra shown here are recorded with the Bruker Daltonik esquireNT 5.2 esquireControl software by the accumulation and averaging of several single spectra (as given in print outs). DataAnalysis™ software 3.4 was used for processing the spectra.

Specify (examples):

**MS** (ESI, positive ions)

\[m/z = \text{Mass}_1 [\text{M}_1 + H]^+, \text{Mass}_1 [\text{M}_1 + \text{Na}]^+, \text{Mass}_1 [\text{M}_1 + \text{K}]^+, \text{Mass}_2 [\text{M}_2 + H]^+, \ldots\]

**MS** (ESI, negative ions)

\[m/z = \text{Mass}_1 [\text{M}_1 - H], \text{Mass}_1 [\text{M}_1 + \text{Cl}], \text{Mass}_2 [\text{M}_2 - H], \ldots\]

**MS/MS** (ESI, P1 (m/z XXX), positive ions)

\[m/z (\%) = \text{P}_1 [\text{precursor}]^+, \text{D}_1 (\text{int}) [\text{daughter 1}]^+, \text{D}_2 (\text{int}) [\text{daughter 2}]^+, \ldots\]

**MS^3** (ESI, P1 (m/z XXX) → P2 (m/z YYY), positive ions)

\[m/z (\%) = \text{P}_2 [\text{precursor}]^+, \text{D}_1 (\text{int}) [\text{daughter 1}]^+, \text{D}_2 (\text{int}) [\text{daughter 2}]^+, \ldots\]

For soft ionisation techniques relative (!) intensities in the spectra should not be cited (exception: MS^n spectra) because they are not reproducible in substance mixtures and on different MS instruments. If the isotopic cluster of a compound shall be cited as a proof for the elemental composition of a special compound, relative ion abundances have to be given as values or (better) the cluster may be depicted in comparison with the calculated isotopic distribution.

Example:

\[[2M + H]^+ = 2218.6\]

\[\text{C}_{136}\text{H}_{216}\text{N}_8\text{O}_{16} + H^+\]

Abbreviations:

Mass: \(m/z\) of the pseudomolecular ion

M: number of the compound or it’s formula (C\(_x\)H\(_y\)O\(_z\))

P: \(m/z\) of the precursor ion

D: \(m/z\) of the daughter ion
3. Accurate Mass ESI mass spectrometry on the Agilent 6220

**Experimental section:**

ESI mass spectra are recorded using an Agilent 6220 time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) in extended dynamic range mode equipped with a Dual-ESI source, operating with a spray voltage of 2.5 kV. Nitrogen served both as the nebulizer gas and the dry gas. Nitrogen was generated by a nitrogen generator NGM 11. Samples are introduced with a 1200 HPLC system consisting of an autosampler, degasser, binary pump, column oven and diode array detector (Agilent Technologies, Santa Clara, CA, USA) using a C18 Hypersil Gold column (length: 50 mm, diameter: 2.1 mm, particle size: 1.9 µm) with a short gradient (in 4 min from 0% B to 98% B, back to 0% B in 0.2 min, total run time 7.5 min) at a flow rate of 250 µL/min and column oven temperature of 40°C. HPLC solvent A consists of 94.9% water, 5% acetonitrile and 0.1% formic acid, solvent B of 5% water, 94.9% acetonitrile and 0.1% formic acid. The mass axis was externally calibrated with ESI-L Tuning Mix (Agilent Technologies, Santa Clara, CA, USA) as calibration standard.

The mass spectra shown here are recorded in both profile and centroid mode with the MassHunter Workstation Acquisition B.04.00 software (Agilent Technologies, Santa Clara, CA, USA). MassHunter Qualitative Analysis B.07.00 software (Agilent Technologies, Santa Clara, CA, USA) was used for processing and averaging of several single spectra (details given in print outs).

**Specify (examples):**

**MS** (ESI, positive ions)

\[m/z = \text{Mass}_{1}\ [\text{M}+\text{H}]^{+}, \text{Mass}_{1}\ [\text{M}+\text{Na}]^{+}, \text{Mass}_{1}\ [\text{M}+\text{K}]^{+}, \text{Mass}_{2}\ [\text{M}+\text{H}]^{+}, \ldots\]

**MS** (ESI, negative ions)

\[m/z = \text{Mass}_{1}\ [\text{M}-\text{H}]^{−}, \text{Mass}_{1}\ [\text{M}+\text{Cl}]^{−}, \text{Mass}_{2}\ [\text{M}-\text{H}]^{−}, \ldots\]

For soft ionisation techniques relative (!) intensities in the spectra should not be cited (exception: **MS** spectra) because they are not reproducible in substance mixtures and on different MS instruments. If the isotopic cluster of a compound shall be cited as a proof for the elemental composition of a special compound, relative ion abundances have to be given as values or (better) the cluster may be depicted in comparison with the calculated isotopic distribution.

**Example:**

![Mass spectrum example](image)

\[\text{[2M + H]}^{+} = 2218.6\]

\[\text{C}_{136}\text{H}_{216}\text{N}_{8}\text{O}_{16} + \text{H}^{+}\]

**Abbreviations:**

Mass: \(m/z\) of the pseudomolecular ion

M: number of the compound or its formula (C\(_x\)H\(_y\)O\(_z\))
4. EI Mass spectrometry on the Autospec X

experimental section:

EI mass spectra were recorded using an Autospec X magnetic sector mass spectrometer with EBE geometry (Vacuum Generators, Manchester, UK) equipped with a standard EI source. Samples were introduced by push rod in aluminium crucibles if not otherwise noted. Ions were accelerated by 8 kV in EI mode.

The spectra shown here are recorded and processed with the OPUS software (V3.6, Micromass 1998) by the accumulation and averaging of several single spectra.

Exact mass measurements were performed in the high resolution mode with PFK as internal standard. Centroided spectra were used to determine the mass accuracy.

specify (examples):

\[ \text{MS (EI, 70eV)} \]
\[ m/z \% = P \text{ (int)} [\text{Parent}]^*, F_1 \text{ (int)} [\text{Fragment 1}]^*, F_2 \text{ (int)} [\text{Fragment 2}]^*, ... \]

abbreviations:

\( M_x \): number of the compound or its formula \((C_{x}H_{y}O_{z})\)
\( P_i \): \(m/z\) of the parent ion
\( F_i \): \(m/z\) of the fragment ion
5. ESI and MALDI spectra on the Synapt G2Si (Q-IMS-TOF MS)

experimental section:

MALDI or nano-ESI experiments are performed using a Q-IMS-TOF mass spectrometer Synapt G2Si (Waters, Manchester, UK) in resolution mode, interfaced to a MALDI or nano-ESI ion source. Nitrogen serves both as the nebulizer gas and the dry gas for nano-ESI. Nitrogen is generated by a nitrogen generator NGM 11. Helium 5.0 is used as buffer gas in the IMS entry cell, nitrogen 5.0 is used for IMS separations. Argon serves as collision gas for MS/MS experiments. 1,3-dicyanobenzene is used as electron transfer reagent in ETD experiments. The MALDI source is equipped with a 2.5 kHz solid state laser at a wavelength of 355 nm. MALDI samples are mixed on a polished steel target with 1 µL of matrix solution (either 50 mg/mL 2,5-DHB in 50% ACN, 0.1% TFA or saturated DCTB in methanol or 10 mg/mL CHCA in 50% ACN, 50% ethanol with 0.1% TFA).

ESI samples are introduced by static nano-ESI using in-house pulled glass emitters.

The Instrument Default Calibration was used for calibrating the mass axis.

or

The mass axis was externally calibrated with (PEG or red phosphorous) as calibration standard on the same target. (use for MALDI experiments!)

or

The mass axis was externally calibrated with fragment ions of GluFib as calibration standard. (use for nano-ESI experiments!)

or

The mass axis was internally calibrated with the doubly protonated GluFib ion as internal calibration standard. (use for nano-LC/ nano-ESI experiments!)

Scan accumulation and data processing was performed with MassLynx 4.1 (Waters, Manchester, UK) on a PC Workstation. The spectra shown here were generated by the accumulation and averaging of several single spectra (as given in print outs). Determination of exact masses were performed using centroided data. Drift spectra were visualized and selected with DriftScope v2.8.

specify (examples):

MS (ESI, positive ions)
\[ m/z = \text{Mass}_1 [M_1+H]^+, \text{Mass}_1 [M_1+Na]^+, \text{Mass}_1 [M_1+K]^+, \text{Mass}_2 [M_2+H]^+, \ldots \]

MS (ESI, negative ions)
\[ m/z = \text{Mass}_1 [M_1-H], \text{Mass}_1 [M_1+Cl], \text{Mass}_2 [M_2-H], \ldots \]

MS/MS (ESI, P, (m/z XXX), positive ions)
\[ m/z (\%) = P_1 [\text{precursor}]^+, D_1 \text{ (int)} [\text{daughter } 1]^+, D_2 \text{ (int)} [\text{daughter } 2]^+, \ldots \]

For soft ionisation techniques relative (!) intensities in the spectra should not be cited (exception: MS^2 spectra) because they are not reproducible in substance mixtures and on different MS instruments. If the isotopic cluster of a compound shall be cited as a proof for the elemental composition of a special compound, relative ion abundances have to be given as values or (better) the cluster may be depicted in comparison with the calculated isotopic distribution. (Compare example shown with Esquire 3000)

For details on nano-LC/ nano-MS experiments (proteomics) refer to the operator.

6. HPLC/TLC ESI mass spectrometry on the ZQ2000

Experimental section:

ESI mass spectra are recorded using a ZQ2000 single quadrupole mass spectrometer (Waters, Manchester, UK) equipped with an ESI source, operating with a spray voltage of 3.5 kV. Nitrogen served both as the nebulizer gas and the dry gas and is generated by a nitrogen generator NGM 11. The mass axis was externally calibrated with ESI-L Tuning Mix (Agilent Technologies, Santa Clara, CA, USA) as calibration standard.

HPLC: Samples are introduced with an AllianceHT 2795 HPLC system consisting of an autosampler, degasser, binary pump and 996 photodiode array detector (Waters, Manchester, UK) using a C8 Symmetry column (length: 100 mm, diameter: 2.1 mm, particle size: 3.5 µm) with a gradient (in 10 min from 5% B to 95% B, back to 5% B in 0.2 min, total run time 25 min) at a flow rate of 400 µL/min. HPLC solvent A consists of water and 0.1% formic acid, solvent B of acetonitrile and 0.1% formic acid. UV spectra are recorded in the range from 190 nm to 800 nm.

TLC: Samples are introduced using an in-house built TLC interface, operated with a Hitachi L-6000 LC pump (isopropanol, flow rate 400 µL/min)

Scan accumulation and data processing was performed with MassLynx 4.1 (Waters, Manchester, UK) on a PC Workstation. The spectra shown here were generated by the accumulation and averaging of several single spectra (as given in print outs).

Specify (examples):

**MS (ESI, positive ions)**

\[ m/z = \text{Mass}_1 [M_1 + H]^+, \text{Mass}_1 [M_1 + Na]^+, \text{Mass}_1 [M_1 + K]^+, \text{Mass}_2 [M_2 + H]^+, \ldots \]

**MS (ESI, negative ions)**

\[ m/z = \text{Mass}_1 [M_1 - H]^-, \text{Mass}_1 [M_1 + Cl]^-, \text{Mass}_2 [M_2 - H]^-, \ldots \]

For soft ionisation techniques relative (!) intensities in the spectra should not be cited (exception: MS^n spectra) because they are not reproducible in substance mixtures and on different MS instruments. If the isotopic cluster of a compound shall be cited as a proof for the elemental composition of a special compound, relative ion abundances have to be given as values or (better) the cluster may be depicted in comparison with the calculated isotopic distribution.

Example:

\[ [2M + H]^+ = 2218.6 \]

\[ C_{136}H_{216}N_8O_{16} + H^+ \]

**Abbreviations:**

Mass_x: \( m/z \) of the pseudomolecular ion  
\( M_x \): number of the compound or it’s formula (C_{x}H_{y}O_{z})

1 Luftmann, H Analytical and Bioanalytical Chemistry 2004, 378, 964-968  
2 https://www.uni-muenster.de/imperia/md/content/organisch_chemisches_institut2/massenspektrometrie/dgms-hl.pdf
7. GC/MS on the Varian Saturn II

experimental section:

EI/CI GC/MS spectra were recorded using an Varian Saturn II GS/MS mass spectrometer equipped with an ion trap used with EI (70 eV) or CI with internal ionization.

The spectra shown here are recorded and processed with the Varian Saturn II Software Version 5.2 by the accumulation and averaging of several single spectra for each GC peak using background subtraction. The GC used a 25 m silica capillary column HP 5 MS (0.2 mm ID, 0.33 µm film) ¹.

specify (examples):

MS (EI, 70eV)

\[ m/z \text{ (%) } = P \text{ (int) [Parent]}^+, F_1 \text{ (int) [Fragment 1]}^+, F_2 \text{ (int) [Fragment 2]}^+, \ldots \]

abbreviations:

Mᵢ: number of the compound or it’s formula (CₓHᵧOᵣ)
Pᵢ: m/z of the parent ion
Fᵢ: m/z of the fragment ion

¹ Please ask a GCMS operator for the actual column your samples have been separated with!