High-Resolution, Accurate-Mass Orbitrap Mass Spectrometry – Definitions, Opportunities, and Advantages

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Introduction

Resolution and resolving power are terms used in mass spectrometry, various optical spectroscopy techniques such as microscopy, digital image acquisition, and processing tools.

A particular (high) mass resolution (e.g. 100,000) is necessary to separate or resolve two peaks that have a certain small mass difference of e.g. 0.01 u. High mass resolution is necessary to separate signals of one compound from those of another and ensure that ions of only one kind contribute to a particular measurement. The measurement may be an accurate-mass determination or a highly specific quantification measurement.

High mass resolution is particularly important for all types of experiments involving complex mixtures, such as samples generated from a matrix (e.g. biological, environmental, food), since these contain a significant number of background (matrix) ions in addition to the possible analytes of interest. In such cases, high mass resolution makes the difference between detecting analyte molecules at low concentration and not detecting them due to the masking effect of isobaric matrix interferences (see Figure 1).

Definitions

In mass spectrometry, four terms—**mass resolving power, mass resolution, mass accuracy, and mass precision**—are used to characterize the performance of high-resolution, accurate-mass (HRAM) mass spectrometers. This Technical Note considers and applies the latest IUPAC recommendations regarding the above terms.[1] The IUPAC recommendations published in 2013 clear up the controversial usage of mass resolution and mass resolving power, as well as the usage of the term mass resolving power with or without a unit. The 2013 paper by Murray et al., and the papers referenced therein, help to explain the (historically-grown) controversy and define the state of current understanding and definition of terms.[1]

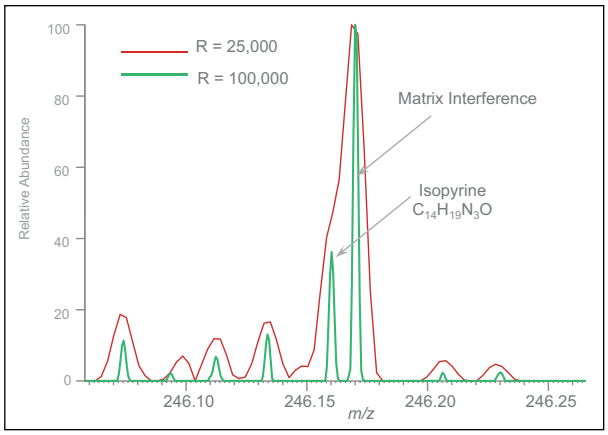

Figure 1. Mass resolution of R = 25,000 at *m/z* 200 masks the pesticide isopyrine due to a non-resolved matrix interference (red); whereas, resolution setting of R = 100,000 at *m/z* 200 resolves the analyte molecule from the matrix background.

The glossary of the 2013 IUPAC recommendation explains the term mass resolving power as a "measure of the ability of a mass spectrometer to provide a specified value of mass resolution", while mass resolution is defined as the observed *m/z* value divided by the smallest difference (Δ) *m/z* for two ions that can be separated: (*m/z*) / Δ (*m/z*).[1] The *m/z* value at which the measurement was reported should be reported; the definition for (Δ) *m/z* - measured at which peak height - should be reported; for details we recommend referring to the referenced paper.

Mass resolution is typically a large number that describes the ability to distinguish between ions differing in the mass/charge (*m/z*) value by a small increment. The mass resolution is characterized by giving the peak width, measured in mass units, expressed as a function of mass, for at least two points on the peak. Specifically, this is defined at fifty percent of the maximum peak height (FWHM) in Orbitrap technology.

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In an example of a peak at *m/z* 200.0000, with a peak width of 0.002 FWHM, the mass resolution is R = m/Δm = 100,000. As a consequence, two peaks of equal height at *m/z* 200.0000 and 200.002 cannot be baseline resolved. Only two peaks of equal height at *m/z* 200.0000 and 200.004 can be baseline resolved, if the mass spectrometer delivers a peak width of 0.002 u (FWHM) at these given *m/z* values.

Taking the above into account, consider the isotope pattern of the tetrapeptide MRFA, which—as it contains the amino acid methionine—contains the element sulphur. Sulphur naturally (and predominately) occurs with two isotopes ^{32}S and ^{34}S in high and low abundance, respectively. For the (A+2) peak[2] of the protonated, singly charged MRFA signal at *m/z* 526, (predominantly) two signals with a mass difference of about 0.011 u are expected. Two peaks of a mass difference of 0.011 u at *m/z* 526 must be resolved to distinguish between the MRFA isotopologue with ^{34}S (and ^{12}C isotopes only, *m/z* 526.2608) and the MRFA isotopologue ^{32}S (and $^{13}C_2$ replacing two of the ^{12}C isotopes, *m/z* 526.2717). To resolve these two main signals within the (A+2) peak of the MRFA peptide, each of these signals must have a FWHM peak width of approximately 0.011/2. Note that we neglect the existence of less-abundant signals within this (A+2) peak occurring between theses two main ones (^{34}S and ^{32}S). Nevertheless, mass resolution must be 526 / (0.011/2), i.e. approximately 100,000, to see the signals containing ^{34}S and ^{32}S clearly separated, though, still not baseline resolved.

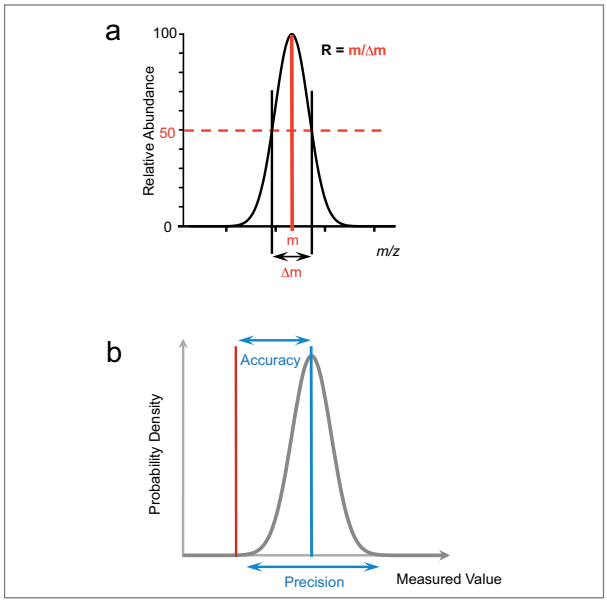

Figure 2. (a) Mass resolution R = m/Δm at FWHM, (b) accuracy and precision of mass determination.

Mass accuracy is the closeness of the agreement between the result of a measurement and a true value (exact mass).

Mass precision is the closeness of agreement between independent mass measurement results.

Orbitrap Mass Spectrometry

In Orbitrap mass spectrometry, the mass resolution is inversely proportional to the square root of the *m/z* value and proportional to the acquisition time. Refer to the literature for more detailed information.[3-5] The angular frequencies ω_z of ion movements along the central electrode acquired in the Orbitrap device are determined by the following equation in which *k* is an instrumental constant:

$$\omega_z = \sqrt{\frac{k}{m/z}}$$

A specific frequency ω_z is indicative of a specific *m/z* value (assuming fixed charge state). The abundance of a given ion is reflected by the amplitude of the given frequency ω_z of this ion. The information in the Orbitrap device with many different *m/z* values moving along the central electrode contains therefore both, the *m/z* information by different frequencies ω_z and the intensity information by the amplitude of the individual frequency ω_z. This is called the transient signal.

A mathematical operation called Fourier transformation (FT) translates these frequencies into *m/z* values and their amplitudes into intensities. The longer the transient signal is recorded, the higher is the resolution of the mass spectrum obtained.

The FT of a transient signal yields a highly-resolved, accurate-mass Orbitrap mass spectrum.

The different resolution achievements of Orbitrap instruments compared to commercially available time-of-flight mass spectrometers are shown in Figure 3.

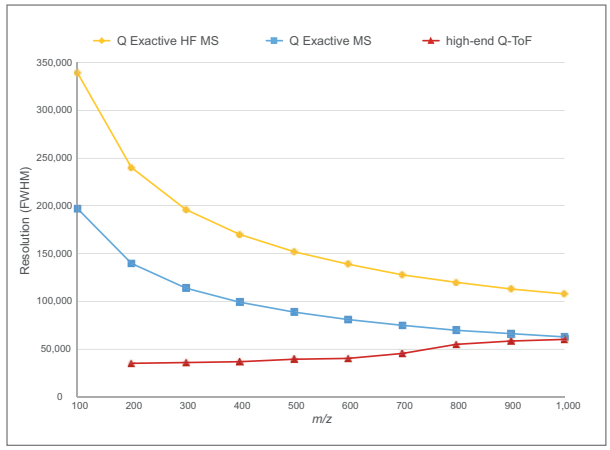

Figure 3. Resolution vs. *m/z* for small molecule mass range.

Discussion

The main purpose of mass resolution is depicted in Figure 4—the ability to distinguish between ions differing in the mass/charge ratio by a small mass increment. Here, monoisotopic masses of two pesticides quite close in their molecular ion masses are simulated *in silico* at different mass resolutions of R = 25,000, R = 35,000 and R = 65,000 (FWHM).

- dimethoate ($C_5H_{12}NO_3PS_2$), *m/z* 230.00690 as $(M+H)^+$ and
- dicryl ($C_{10}H_9Cl_2NO$), *m/z* 230.01340 as $(M+H)^+$

The basis of the simulation is that dicryl shows about 50% intensity/abundance compared to dimethoate.

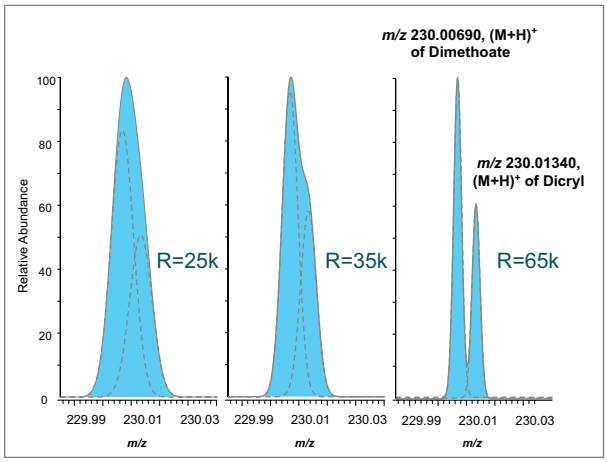

Figure 4. Two compounds with their respective monoisotopic masses at *m/z* 230 simulated at various mass resolution settings.

The individual mass spectrometric peaks are depicted in dashed, grey lines, while the resulting mass spectrometric peak observed is displayed by the blue area. It is obvious from the resulting (blue) peak area that neither a mass resolution of 25,000 nor 35,000 are sufficient to distinguish between and resolve the two compounds. In the case of R = 65,000, both ions are separated and only then is the mass of each compound determined accurately.

Accurate Mass and Elemental Composition

The most striking feature of high mass resolution, or the ability to distinguish small mass increments (i.e. mass resolving power, see above) along with high mass accuracy, is the ability to directly determine the identity of the elemental composition of an ion signal based on the *m/z* value determined from the mass spectrum. First, the accurate determination of the monoisotopic mass (A) peak[2] restricts the pool of possible elemental composition combinations significantly. Second, high mass resolution in combination with accurate-mass measurements enable the user to directly depict fine structures, which further eliminates possible elemental compositions.

The example shown in Figure 5 illustrates the isotope pattern and accurate-mass measurement of a singly charged compound with the (A) peak (monoisotopic mass) at *m/z* 202.04336. The inset displays and interprets the fine structure of the (A+1) peak of the pattern.

Table 1 shows the elements in use and their particulate numbers to calculate the possible elemental compositions for *m/z* 202.04336. Searching for the identity of a suited sum formula matching the isotope pattern given in Figure 5 retrieves 35 elemental compositions in a ±20 ppm mass tolerance window. For details on this and on the number of elemental composition proposals applying smaller tolerance windows, refer to Table 2.

Table 1. Elements in use to retrieve the number of possible elemental compositions of the ion at *m/z* 202.04335.

Isotope	Minimum	Maximum
^{14}N	0	5
^{16}O	0	10
^{12}C	3	20
^{1}H	1	10
^{31}P	0	5
^{32}S	0	5
^{35}Cl	0	3
^{19}F	0	3
^{79}Br	0	3

Table 2. Numbers of elemental composition proposals in given mass tolerance windows considering the fine structure of the entire isotope pattern.

Mass Tolerance Window	± 20 ppm	± 10 ppm	± 5 ppm
Monoisotopic	35	18	9
At least 1 N, 1 S, no Cl, no Br	6	2	1

Exploring the fine structure of the isotope pattern (illustrated in Figure 5 and displayed for the (A+1) peak) unambiguously reveals that the correct sum formula contains sulphur (S) and nitrogen (N). The absence of chlorine (Cl) and bromine (Br) is clearly demonstrated by the low abundance of the (A+2) peak.

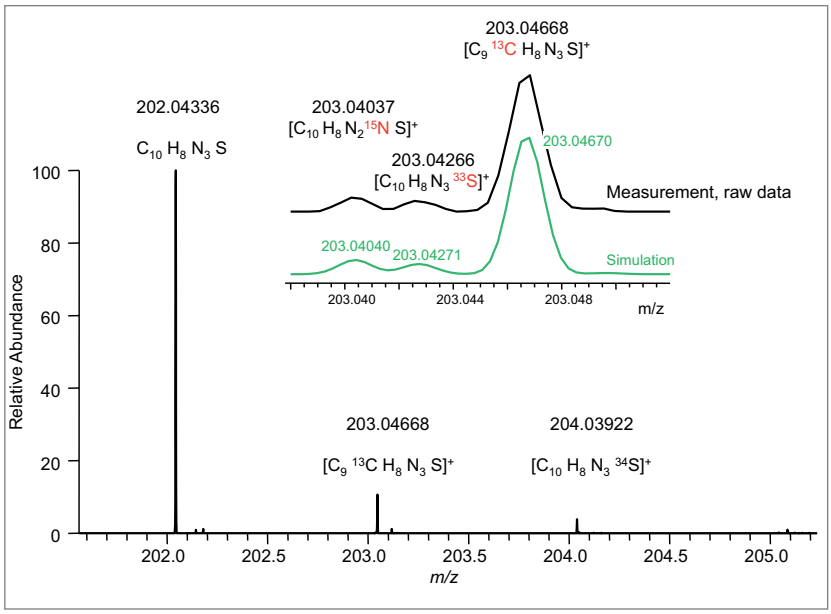

Figure 5. Thiabendazole at *m/z* 202.04334 with(A), (A+1), and (A+2) peaks (zoom on the (A+1) peak in the inset) measured and simulated data with resolution setting of 140,000 at *m/z* 200.

If the mass tolerance window is set to ±5 ppm and the findings about the isotope pattern fine structure are considered, the list of possible elemental compositions is limited to only one result, which is $C_{10}H_8N_3S$.

With the information considered above in mind, a database search (such as on ChemSpider), easily reveals the identity of the compound, which is the commonly used fungicide thiabendazole, also named tiabendazole or 2-(1,3-thiazol-4-yl)-1H-benzimidazole.

Quantification

Usually, extracted ion chromatograms (XIC) of ions of interest are created by extracting a theoretical *m/z* value with an MS-analyzer-suited mass tolerance window from the data set. Considering the high mass accuracy of Orbitrap-based instrumentation, a suited tolerance window for XIC extraction is ±2 to ±5 ppm. For less accurate MS analyzer types, the tolerance window should be set wider; as a consequence, more chemical noise is extracted as well and is visible in the XIC. A wider mass tolerance window and more chemical noise can lead to overlapping peaks and incorrect data interpretation, such as false positives. The more narrow the mass tolerance window can be set, the less chemical noise is expected to appear in the XICs of selected *m/z* values.

Mass precision is one main prerequisite to achieve proper XIC traces to be utilized for quantification. Precise quantification is only possible if the selected *m/z* value can be extracted from all scans in the raw data with the same narrow mass tolerance window. Figure 6 shows an XIC of the compound carbaryl. The individual measurements of the eluting LC peak are displayed. Carbaryl shows a signal at *m/z* 202.0863 and is extracted here with a window of ±3 ppm. The mass precision across the LC peak ensures an interference-free extraction of the signal, and only because the scan-to-scan precision is high (all data points are within 1 ppm), an undistorted peak shape is extracted, enabling interpretation of the correct quantitative value.

Dependency of Mass Accuracy, Mass Precision, and Resolution

In order to achieve the most precise mass determination, two different aspects must be considered.

First, the mass spectrometer itself needs to deliver precise, reproducible data. Precise mass determination is easiest to measure in a (neat) standard sample. This is the most common type of sample utilized for mass calibration to ensure not only the best mass precision but also mass accuracy.

Secondly, one must consider the mass analyzer's ability to resolve the ions of interest from all possible interferences originating from both matrix ions as well as other chemical background in complex samples. If the mass resolution of the mass spectrometer is sufficient to separate ions of interest from the chemical background, the molecular ions can be measured with high precision and accuracy in a wide range of complex samples in different matrices.

Sufficient mass precision can be achieved only if the system provides enough mass resolution and thereby the ability to distinguish and separate the peak of interest from adjacent, irrelevant signals. For acquisitions done with insufficient mass resolution, the compound of interest can be hidden in the background. This is illustrated in Figure 7 for the protonated ion of the pesticide iprovalicarb at *m/z* 321.21713 with the elemental composition of $C_{18}H_{28}N_2O_3$ of the $(M+H)^+$ species.

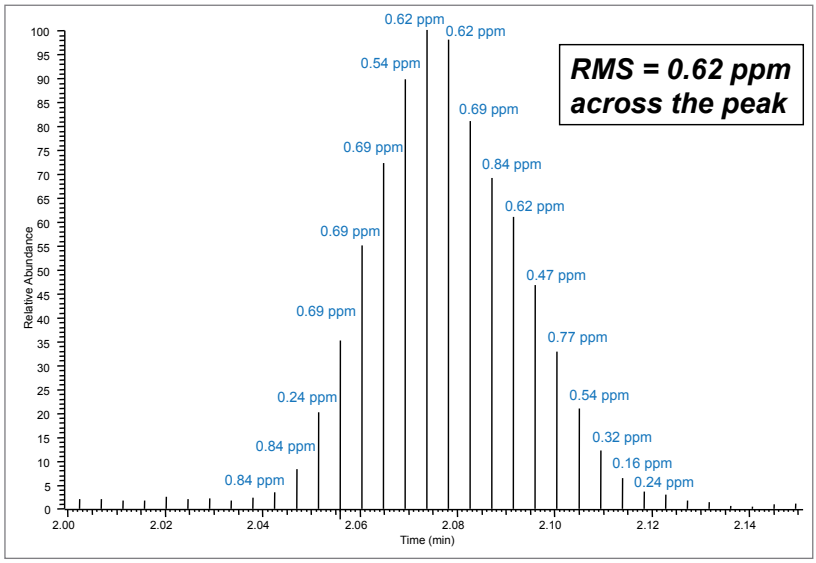

Figure 6. Precision of mass determination displayed for the XIC of *m/z* 202.0863 ±3 ppm, the $(M+H)^+$ ion of carbaryl.

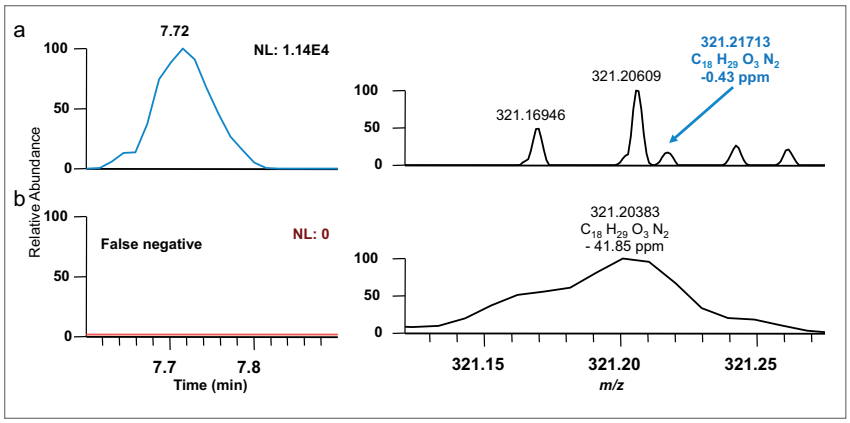

Figure 7. XIC of *m/z* 321.21713 (protonated ion of iprovalicarb) with (a) narrow (±5 ppm) and (b) wider (±150 ppm) extraction window due to resolution chosen; (a) acquired with 70,000 mass resolution at *m/z* 200, (b) acquired with 10,000 mass resolution at *m/z* 200.

Concluding Remarks

Mass spectrometry has undergone a paradigm shift in the last decade. Today, accurate mass data with unambiguous mass assignments are mandatory for MS and, increasingly, for MS/MS mass spectra. This paradigm shift was initiated by the introduction of the user-friendly ion trap / Fourier Transform ion cyclotron resonance hybrid mass spectrometer, the Thermo Scientific™ LTQ™ FT MS, in 2003, and later strengthened by the ion trap / Orbitrap hybrid mass spectrometer, the Thermo Scientific™ LTQ™ Orbitrap™ MS, and its successors starting in 2005. With Exactive series instrumentation, introduced in 2008, various benchtop Orbitrap MS systems have become available. An overview of the Orbitrap mass analyzer portfolio is illustrated in Figure 8. With Orbitrap technology, true high mass resolution and mass accuracy as described above are delivered by one compact and easy-to-use instrument that can deliver sub-ppb sensitivity and sub-ppm mass accuracy reliably and routinely.

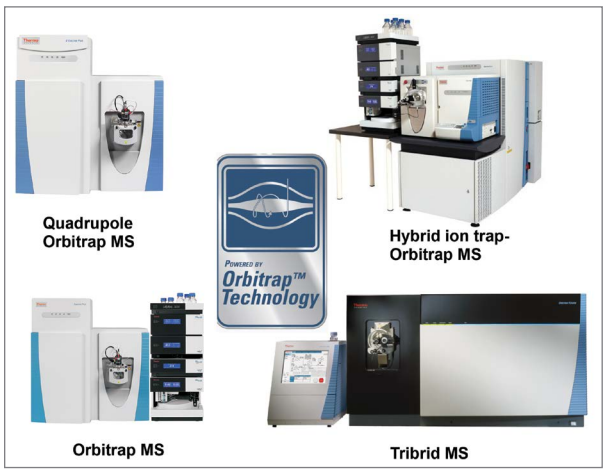

Figure 8. Portfolio of Orbitrap-based mass spectrometers.

Reference

1. Murray, K.K.; Boyd, R.K.; Eberlin, M.N.; Langley, G.J.; Li, L.; Naito, Y. *Pure Appl. Chem.*, **2013**, *85*, 1515-1609. http://dx.doi.org/10.1351/PAC-REC-06-04-06" 10.1351/PAC-REC-06-04-06
2. McLafferty, F.W.; Frantisek, T. Interpretation of Mass Spectra, 4th ed.; University Science Books; Mill Valley, CA, **1993**. ISBN-13: 978-0935702255
3. Makarov, A. Electrostatic axially harmonic orbital trapping: A high-performance technique of mass analysis, *Anal. Chem.*, **2000**, *72* (6), 1156–62. doi:10.1021/ac991131p
4. Makarov, A.; Denisov, E.; Kholomeev, A.; Balschun, W.; Lange, O.; Strupat, K.; Horning, S. Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer, *Anal. Chem.*, **2006**, *78* (7), 2113–20. doi:10.1021/ac0518811
5. Makarov, A.; Scigelova, M. Coupling liquid chromatography to Orbitrap mass spectrometry, *J. Chromatography* A **2010** 1217 (25): 3938–3945. doi:10.1016/j.chroma.2010.02.022

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