

# Mass Resolution and Resolving Power

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The Thermo Scientific Exactive LC-MS has the speed, accuracy and precision to routinely give the most confident analysis of both simple and complex samples.

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## Resolution and Resolving Power

The terms high (mass) resolution and resolving power are often used interchangeably. High resolution is necessary to separate peaks of one mass 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. High resolution is particularly important for all types of experiments involving complex mixtures, such as samples generated from a matrix (e.g. biological, environmental), since these will contain a significant number of background ions. In such cases high resolving power will make, for example, the difference between, detecting analytes at low concentration or not detecting these analytes due to the masking effect of isobaric matrix interferences (see Figure 1).

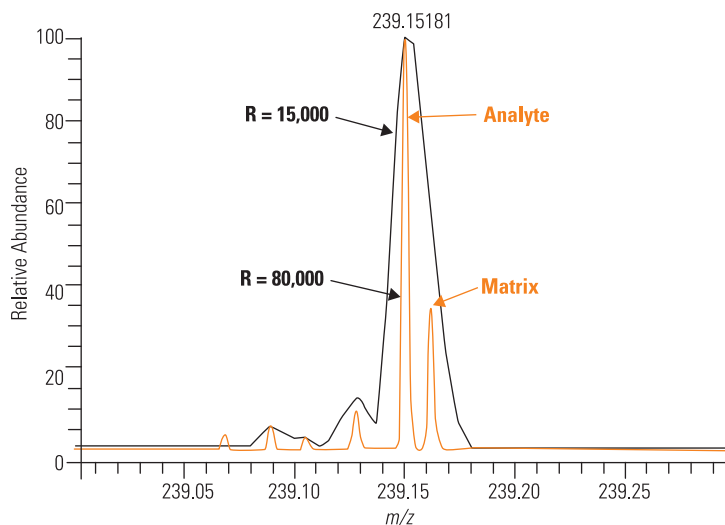


Figure 1: Analysis of the MH<sup>+</sup> peak of Pirimicarb at 15,000 and 80,000 resolution.

# Resolution and Resolving Power

Because two terms, resolution and resolving power, are used to characterize mass spectrometers, it is useful to look at their definitions. IUPAC defines:

## Resolving Power in Mass Spectrometry

The ability to distinguish between ions differing in the quotient mass/charge by a small increment. It may be 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 at fifty percent and at five percent of the maximum peak height.

## Resolution in Mass Spectrometry ( $m/\Delta m$ )

- (10% valley definition): Let two peaks of equal height in a mass spectrum at masses  $m$  and  $m - \Delta m$  be separated by a valley which at its lowest point is just 10 per cent of the height of either peak. For similar peaks at a mass exceeding  $m$ , let the height of the valley at its lowest point be more (by any amount) than ten per cent of either peak height.
- (peak width definition): For a single peak made up of singly charged ions at mass  $m$  in a mass spectrum, the resolution may be expressed as  $m/\Delta m$  where  $\Delta m$  is the width of the peak at a height which is a specified fraction of the maximum peak height. It is recommended that one of three values 50%, 5% or 0.5% should always be used. For an isolated symmetrical peak recorded with a system which is linear in the range between 5% and 10% levels of the peak, the 5% peak width definition is technically equivalent to the 10% valley definition. A common standard is the definition of resolution based upon  $\Delta m$  defined as the Full Width of the peak at Half its Maximum height, sometimes abbreviated 'FWHM'. This acronym should preferably be defined the first time it is used.



The terms resolution and resolving power are basically interchangeable. In fact, both resolution and resolving power are defined by  $R = (m/\Delta m)$ . The difference is, however, how  $\Delta m$  is defined. Resolution of magnetic sector instruments is normally given according to the 10% valley definition, which defines  $\Delta m$  by the mass difference between two resolved peaks, with a 10% valley between them. All other definitions for resolution are measured on a single peak. Since there are several ways to do this one should always look at the actually used definition before comparing numerical values. For example, the 10% valley definition, where  $\Delta m$  is based on a real peak separation, is equivalent to a single peak measurement where  $\Delta m$  is defined by the peak width at 5% peak height, as can be seen in Figure 2.

The most commonly used method to measure the resolution of Quadrupole MS, FT-ICR MS, Orbitrap MS and ToF MS follows the Full Width at Half Maximum (FWHM) definition, which uses the width of a peak at 50% of its height as a measure for  $\Delta m$  (see Figure 2).

The numerical value arrived at using the FWHM definition is always larger than the value one would determine using the “width at 5% peak height” (10% valley) definition. In fact, the numerical value of the “FWHM resolution” necessary to achieve mass separation with a 10% valley is approximately twice the value according to the “10% valley definition”. This means that a resolution of 20,000 (FWHM) is equivalent to a resolution of 10,000 (10% valley). An instrument which has a resolution of 10,000 (FWHM) will separate ions at  $m/z$  500.0 from ions at  $m/z$  500.1 (and not 1000.0 from 1000.1).

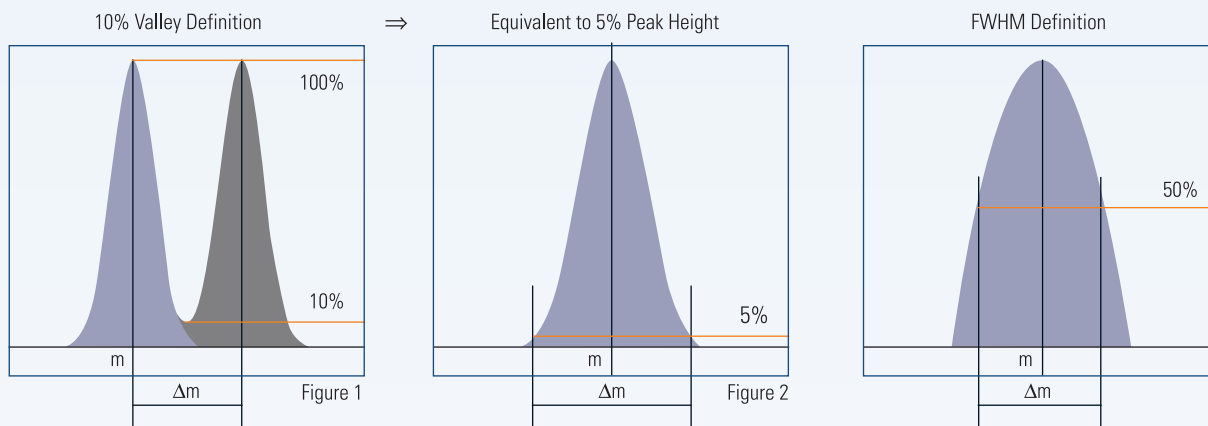


Figure 2: Examples for different resolution definitions.

In Figure 3, where resolution values are given according to the FWHM definition, we show the separation of CO from N<sub>2</sub> at different resolution settings. At an instrumental resolution of 2,300 (FWHM) the individual CO and N<sub>2</sub> peaks (green and blue) merge to give the signal outlined by the thick black line (left panel).

The instrumental resolution has to be increased to about 5,000 (FWHM) in order to separate and accurately measure the mass of each peak (right panel).

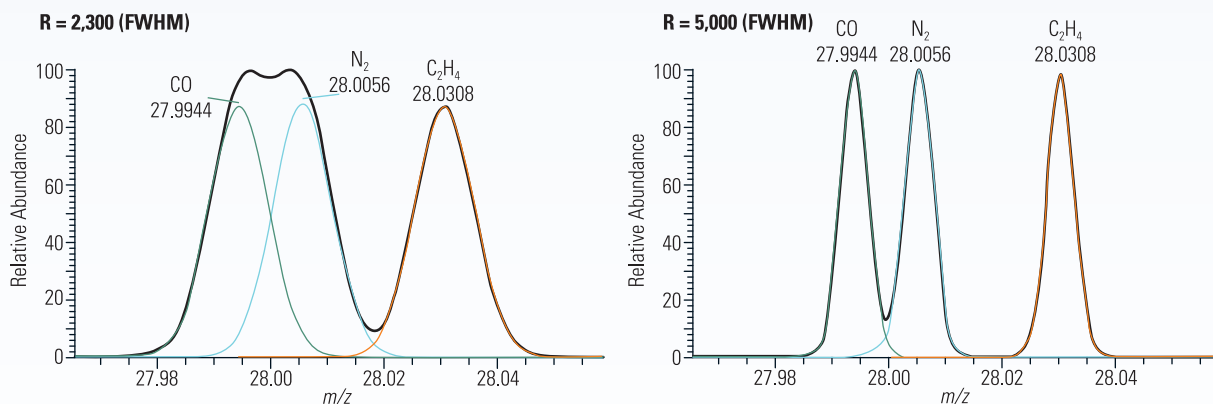


Figure 3: Separation of isobaric peaks at different resolution settings.

## Instrument Types and Mass Resolution

In all following examples, “resolution” and “resolving power” are used synonymously and numerical values follow the FWHM definition. In the case of quadrupole ion traps (e.g. Thermo Scientific LTQ) and transmission quadrupoles (e.g. Thermo Scientific TSQ Quantum) the instrumental resolution increases linearly with mass, i.e., if the instrument is set to resolve masses 100 and 101 with unit mass resolution then  $R = 100/0.5 = 200$ . At mass 1000 however the resolution needed to separate it from mass 1001 will be  $R = 1000/0.5 = 2000$ . This resolution setting is called “unit mass resolution”. ToF and double-focusing mass spectrometers (e.g. Thermo Scientific DFS High Resolution Magnetic Sector GC/MS) operate at constant resolving power.

This means that the set resolution will be the same for the entire mass range. FT-ICR and Orbitrap mass spectrometers operate at varying resolving power. In the case of FT-ICR MS the resolution decreases linearly with mass and for Orbitrap MS the resolution decreases with square root of mass:

### Orbitrap MS, for example:

$R = 100,000$  at  $m/z$  400 becomes  $R = 50,000$  at  $m/z$  1600

### FT-ICR MS:

$R = 100,000$  at  $m/z$  400 becomes  $R = 25,000$  at  $m/z$  1600

## Why is Mass Resolution Important

High resolution mass spectrometry is generally used to either, measure accurate masses (and thereby enable the determination of elemental compositions) or to enable highly specific quantification. In either case the mass centroid of a peak profile is measured. For a quantification experiment unambiguous results can only be obtained if sufficient resolving power is used to separate the target analyte from any possible interference. If the resolving power of the instrument is insufficient, it will result in false positive or in false negative signal responses. Figure 4 shows the mass chromatogram of Butyl Phthalate and Ethinyl Estradiol at resolution of  $R = 10,000$  (top chromatogram) and at  $R = 100,000$  (bottom chromatogram).

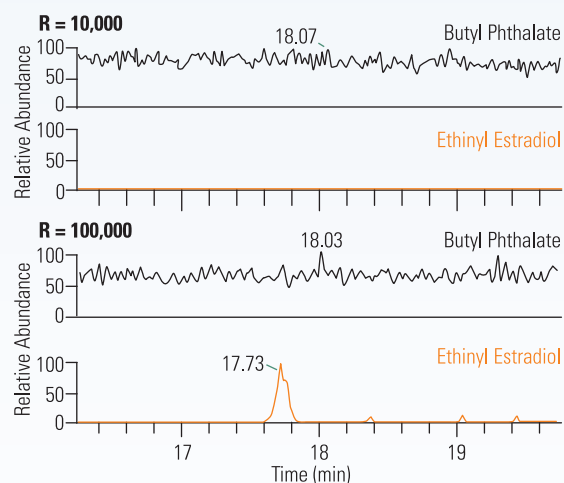


Figure 4: False negative hormone detection at low resolution (top) and correct detection at high resolution (bottom).

Another example of the benefit of high resolution mass spectrometry can be seen for the analysis of the two isobaric pesticides Thiamethoxam and Parathion which differ in mass by  $\sim 13.5$  mu (Figure 5). Clearly at a resolving power of 50,000 (FWHM) these two isobaric pesticides are resolved and each of their masses can be accurately determined. At lower resolution settings however (top panel) the mass assignment of the Thiamethoxam peak is shifted towards the centroid of the unresolved peak doublet which leads to a mass error for the Thiamethoxam of more than 40 ppm.

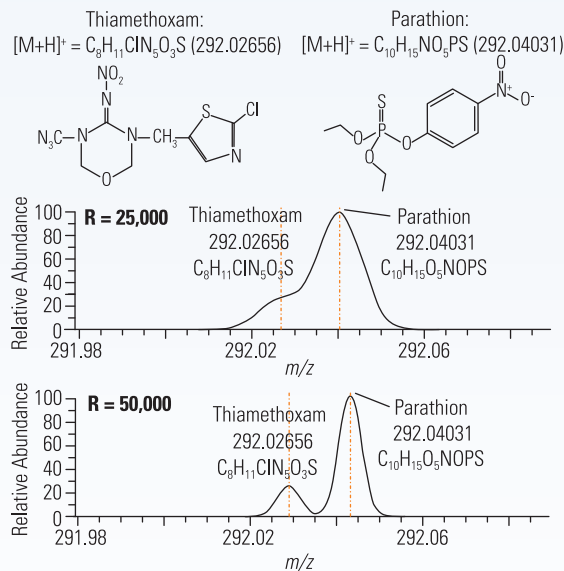


Figure 5: Correct (bottom) and incorrect (top) mass measurement of the pesticides at different resolution settings.

## Accurate Mass and Elemental Composition

Accurate mass measurement is used to determine the elemental composition of an analyte and thereby to confirm the identification of target compounds or support the identification of unknowns by providing possible elemental compositions. In addition to using the mass of the  $^{12}C$  isotope, additional confirmation of elemental composition can be provided by use of information about isotopic masses and intensities if the resolution is high enough to ensure separation of possible interferences as shown in Figure 6 for the isotopic peak at  $m/z$  405. This information significantly reduces the number of elemental composition proposals and helps to identify a single candidate with the highest confidence.

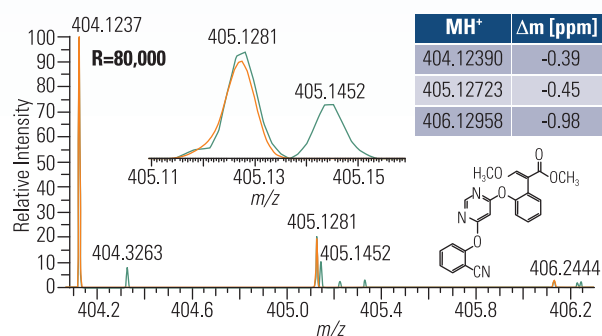


Figure 6: Use of the isotopic information for confirmation of elemental composition.

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