

Effect of Resolution and Mass Accuracy on Empirical Formula Confirmation and Identification of Unknowns

Technical Overview

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Introduction

When synthetic chemists generate new compounds, they need to quickly confirm that the compounds they created are the compounds they intended to create. Multiple compounds with different empirical formulas can share a common nominal molecular weight. Both very good mass resolution and mass accuracy are required in order to positively distinguish between compounds with the same nominal mass and confirm compound identity.

Traditionally, sufficient mass resolution and mass accuracy were only available in expensive, difficultto-use instruments such as double-focusing, magnetic sector mass spectrometers. Recent improvements in time-of-flight (TOF) technology have significantly increased TOF mass resolution and mass accuracy. The Agilent LC/MSD TOF mass spectrometer benefits from these improvements. Its outstanding mass resolution and mass accuracy, combined with the powerful and easyto-use atmospheric pressure ionization sources, make it a good solution for empirical formula confirmation of small molecule organic compounds.

This overview discusses some of the theory and concepts behind mass resolution and mass accuracy and how they affect the ability to unambiguously confirm empirical formulas of small molecule organic compounds or identify unknowns.



The Effect of Mass Resolution on Empirical Formula Confirmation and Identification of Unknowns

Mass resolution has a large influence on whether a mass spectrometer can unambiguously determine elemental composition for confirmation of empirical formulas or identification of unknowns. The better the mass resolution, the less likely it is that a mass peak of interest will be merged with an interfering ion from the sample or background.

This section explains some of the basic concepts and terminology related to mass resolution, and demonstrates how the mass of the analyte affects the mass resolution required.

Mass resolution and resolving power

Mass resolution (R) is the degree of separation (ΔM) between two adjacent ions in the mass spectrum that can be distinguished at a given mass (M). The ability to resolve singly charged ions one mass unit (1 u)* apart is called "unit" mass resolution. A typical quadrupole mass spectrometer has a resolution from a few tenths of a mass unit to one mass unit (e.g. 0.25–1 u). A typical TOF mass analyzer can resolve mass differences of a few hundredths of a mass unit to a few tenths of a mass unit (e.g. 0.025–0.25 u).

Resolving power (RP) is the nominal mass (M) to be measured divided by the difference in the masses to be identified (Δ M).

$$RP = M/\Delta M$$

Thus resolution and resolving power have an inverse relationship. In practice, the terms resolution and resolving power are used somewhat interchangeably and it is common to see references to a resolution of 10,000, although that would actually be a very bad mass resolution but a good resolving power.



Figure 1. The high resolving power of a TOF mass analyzer helps to reduce the chances of having the mass peak of interest merged with an interfering ion from the sample or the background

Resolving power is mass dependent. For a given degree of separation (Δ M) that can be distinguished, resolving power is lower at lower masses and higher at higher masses. For example:

At 100 u, RP =
$$\frac{100}{0.05}$$
 = 2,000
At 1000 u, RP = $\frac{1000}{0.05}$ = 20,000

Nominal mass

Nominal mass is the mass of a given empirical formula using the integer mass numbers of the most abundant isotope of each element (C=12, H=1, O=16, N=14, Cl=35). Purine ($C_5H_4N_4$) and acetophenone (C_8H_8O) have the same nominal mass of 120 u. On a typical quadrupole mass analyzer, under unit mass resolution conditions, the purine and acetophenone ions are indistinguishable.

^{*}For simplicity, this overview discussed masses in terms of unified atomic mass units (u). This implies singly charged ions. For multiply charged ions, the same principles and formulas apply, but the correct "units" would actually be the mass-to-charge ratio (m/z).



Figure 2. Structures of a) Purine and b) Acetophenone

Average mass

Average mass is the mass of a given empirical formula calculated using the atomic weights of each element in the compound (e.g. C=12.011, H=1.0080, O=15.9994, N=14.0067, Cl=35.453). The atomic weight is a weighted average of the naturally occurring stable isotopes of an element. Natural variations in the isotopic ratios of some elements limit the precision of some atomic weights. The average mass for purine is 120.11 u. The average mass for acetophenone is 120.15 u. Typically, the average mass is reported as the molecular weight in reference books such as the Merck Index.

Monoisotopic mass

Monoisotopic mass is the mass of a given empirical formula calculated using the exact mass of the most abundant isotope of each element (C=12.000000, H=1.007825, O=15.994915, N=14.003074, Cl = 34.968851). The monoisotopic mass of purine is 120.0436 u. The monoisotopic mass for acetophenone is 120.0575 u. Monoisotopic mass is sometimes referred to as exact mass.

Resolving compounds with identical nominal masses

Although purine and acetophenone have identical nominal mass (120), they have different empirical formulas (purine, $C_5H_4N_4$ and acetophenone, C_8H_8O). Their exact mass difference is 0.0139 u. In order to resolve purine and acetophenone, a mass spectrometer would need a resolving power of:

$$\mathrm{RP} = \frac{120}{(120.0575 - 120.0436)} = 8633$$

If a mass spectrometer does not have sufficient power to resolve this difference, it cannot positively confirm, without additional information, which of the two molecules is present.

Design for resolving power

The Agilent LC/MSD TOF includes a number of design elements designed to enhance its resolving power. These include:

- A "beam shaper" and related ion optics that reduce variations in ion position and energy before they enter the mass analyzer
- One-dimensional "harp" grids oriented in the direction of ion travel in both the pulser and ion mirror (reflectron)
- A mechanical design that automatically creates proper alignment (parallelism)

Mass Accuracy, Accurate Mass, and Limitations on Empirical Formula Confirmation and Identification of Unknowns

As demonstrated previously, high resolution is necessary to positively confirm identities and to identify unknowns. High resolution alone, however, is not sufficient. A mass spectrometer must also have a high mass accuracy.

Mass accuracy

Mass accuracy is the difference between the theoretical (calculated) mass of a compound and the mass measured by the mass spectrometer (see Figure 3). This is also called mass error.

Mass accuracy/error can be expressed in either parts-per-million (ppm) or milli-mass units (mmu). Parts-per-million is not an absolute measurement; it varies according to the mass-to-charge ratio. For example, at 200 u, a 1 milli-mass unit (0.001 u) mass error is 5 ppm. At 600 u, the same 1 millimass unit error is 1.7 ppm. The errors are mass



Figure 3. Mass accuracy is independent of resolving power, but the LC/MSD TOF exhibits outstanding resolving power and mass accuracy

dependent and get larger at the low masses or smaller at the high masses. The relationship of milli-mass unit (mmu) mass error to parts-permillion (ppm) mass error at a given mass is shown in Table 2.

Table 2: Relationship of milli-mass units (mmu) to parts-per-million (ppm) at a given mass-to-charge ratio

	ppm						
	100 u	200 u	300 u	400 u	500 u	600 u	1000 u
1 mmu	10.0	5.0	3.3	2.5	2.0	1.7	1.0
2 mmu	20.0	10.0	6.7	5.0	4.0	3.3	2.0
3 mmu	30.0	15.0	10.0	7.5	6.0	5.0	3.0
4 mmu	40.0	20.0	13.3	10.0	8.0	6.7	4.0
5 mmu	50.0	25.0	16.7	12.5	10.0	8.3	5.0
6 mmu	60.0	30.0	20.0	15.0	12.0	10.0	6.0
7 mmu	70.0	35.0	23.3	17.5	14.0	11.7	7.0
8 mmu	80.0	40.0	26.7	20.0	16.0	13.3,	8.0
9 mmu	90.0	45.0	30.0	22.5	18.0	15.0	9.0
10 mmu	100.0	50.0	33.3	25.0	20.0	16.7	10.0

Accurate mass measurements

Accurate mass generally refers to a mass measurement that is accurate enough to unambiguously distinguish two compounds with identical nominal masses. For 200 u, a mass error of 5 ppm or less is required to make an accurate mass measurement (\pm 0.001 u). For 1000 u, a mass error of 1 ppm would be required to achieve the same accurate mass measurement.

The example compounds, purine and acetophenone, differ in mass by 0.0139 u. At a nominal mass of 120 u, this difference translates to 116 ppm. At 120 u, the Agilent LC/MSD TOF has a mass accuracy of approximately 4 ppm, so it can easily distinguish between the two compounds.

Other contributions to confirmation and identification

For a given mass accuracy, as molecular mass increases, the number of possible elemental compositions also increases. For a totally unknown compound above about 600 u, the number of possible elemental compositions which fit the accurate mass data is too large to definitively assign an elemental composition. However, other information such as starting materials, the isotope distribution, the number of possible nitrogens and the number of unsaturated bonds in a compound may help to limit some of the elemental composition possibilities to those that make chemical sense. In the case of a target compound, the expected empirical formula is known and can be compared against the measured accurate mass data to confirm identity.

Figure 4. A second nebulizer and automated calibrant delivery system continuously introduce reference mass compound at a very low level into the LC/MSD TOF for continuous mass correction

Design for mass accuracy

One of the ways to improve the mass accuracy of TOF mass spectrometers is to periodically introduce a known "reference mass" compound into the MS. This allows mass correction based on any difference between the known exact mass of the reference mass compound and the mass measured by the instrument. One difficulty is that traditional TOF mass spectrometers tend to have very narrow dynamic ranges; as little as one order of magnitude. Thus, a reference mass compound must be introduced at a very specific (high) concentration. It can persist in the system and act as a contaminant, interfering with later measurements.

The Agilent LC/MSD TOF features an analog-todigital (ADC) acquisition system. The ADC acquisition system provides several orders of magnitude of dynamic range. Combined with an automated calibrant delivery system and a second nebulizer, this allows the continuous introduction of a reference mass compound at a concentration low enough that it is unlikely to interfere with analyses. The LC/MSD TOF gets the benefit of continuous mass correction without the drawbacks.



Mechanical and electrical changes caused by temperature changes can also have a negative effect on mass accuracy. The LC/MSD TOF features a flight tube made from a special, ultralow-thermalexpansion alloy that minimized flight path changes due to temperature changes. It also features mechanical and electronic temperature compensation in the flight tube and electronics.

Summary

Mass resolution and mass accuracy are both critical aspects of MS performance. With sufficient mass resolution and mass accuracy, a mass spectrometer can positively confirm elemental composition or identify unknows. Even if mass resolution and mass accuracy are not sufficient for positive identification, accurate mass measurements can

reduce the number of likely candidates enough that positive identification can be made based on a combination of accurate mass measurements and other information.

The Agilent LC/MSD TOF design includes unique design features that enhance both its mass resolution and mass accuracy. It offers the power of accurate mass measurements with the ease-of-use of much simpler, less powerful instruments.

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