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## Tech Tip

### Confusion Resulting from Molecular Weight and the Nominal Mass, Monoisotopic Mass, and Average Molar Mass



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David Sparkman

# Confusion Resulting from Molecular Weight and the Nominal Mass, Monoisotopic Mass, and Average Molar Mass

**Mass associated with chemical formulae is different for those working in mass spectrometry as compared to those working in other fields of chemistry and in physics.**

Very early in science training, students are introduced to units of mass on the atomic scale. These units of mass are called *atomic weights* or *atomic masses*. The students are told that the values of these atomic weights are determined relative to the assigned mass of carbon (and in some cases, the term C-12 is used); however, they are almost never told that the atomic weight of an element is the weighted average of the masses of its naturally occurring isotopes. There is never a discussion of isotopic mass of an element, a molecule, or an ion. They are rarely, if ever, told about the 1815 proposal of British chemist William Prout that the atomic weight of all elements was approximately a whole number, the *Whole Number Rule*. When the atomic weight of chlorine was determined to be  $\sim 35.5$  u, Prout and his theory was discredited. It was not until 1920 when Francis W. Aston published his list of isotopes of the various elements and showed that chlorine had two isotopes (one with a mass of  $\sim 35$  u and the other with a mass of  $\sim 37$  u and that these two isotopes existed in a 3:1) that Prout's *Whole Number Rule* was finally accepted. Some even give Aston credit for the *Whole Number Rule*.

Little attention is given to the mass of isotopes even in organic chemistry where the concept of mass spectrometry should be introduced. Most teachers of organic chemistry skip mass spectrometry in favour of NMR and IR. Oftentimes, even in graduate courses called *Spectrometric Identification of Organic Compounds*, the concept of isotopic mass is not discussed. A review of the

books used for these courses shows that a great deal of the information on mass spectrometry is incorrect, poorly presented when correct, and that the technique is relegated to determining the molecular weight and/or confirming the elemental composition of the compound.

One of the first things that has to be taught in a mass spectrometry course is the concept of **isotopic mass**. Teaching this concept requires the definition of **isotope** (atoms of the same element that have different numbers of neutrons in their nuclei). The terms **nominal mass** (the integer mass of the most abundant naturally occurring stable isotope) and **monoisotopic mass** (the exact mass of the most abundant naturally occurring stable isotope) have to be introduced along with a new definition of atomic weight (the weighted average of the exact masses of the naturally occurring stable isotope) and molecular mass (mass of a molecule based on the atomic weights of its elements) have to be discussed. It is also necessary to introduce a more precise definition of the **molecule** (a neutral group of bonded atoms that has an even number of electrons) which gives rise to the definition of an **ion** (a charged particle consisting of one or more atoms that can have an even number or an odd number of electrons) and a **radical** (a neutral particle consisting of one or more atoms that has an odd number of electrons). Elements have **nominal** and **monoisotopic** masses. The difference between an **exact mass** (calculated based on published values for the various nuclides) and an **accurate mass** (the

# “For many years, mass spectrometry referred to the nominal mass of a molecule as its molecular weight”

measured mass that is close enough to the exact mass to provide an elemental composition or a series of elemental compositions) must also be explained.

Isotopes of elements have integer and exact masses. Molecules have **molecular weights** (a.k.a. average **molecular masses**,  $M_r$ ) based on the atomic weights of their elements. They also have **nominal masses** and **monoisotopic masses** when all the atoms of their various elements are the most abundant naturally occurring isotope. Ions and radicals (encountered in mass spectrometry) do not have molecular masses because they are not molecules. The best example to illustrate all of these concepts is hydrogen bromide (HBr). A molecule of HBr has a molecular mass ( $M_r$ ) of 80.917 u or an integer molecular mass of 81 u. HBr has a nominal mass of 80 u and a monoisotopic mass of 79.9261 u. A unit resolution mass spectrum has four peaks at  $m/z$  80, 81, 82, and 83. The peak at  $m/z$  80 represents  $^1\text{H}^{79}\text{Br}^+$ , an odd-electron molecular ion; the peak at  $m/z$  81 represents  $^2\text{H}^{79}\text{Br}^+$ , an odd-electron H-2 isotope ion;  $^1\text{H}^{81}\text{Br}^+$ ; the peak at  $m/z$  82 represents an odd-electron Br-81 isotope ion; the peak at  $m/z$  83 represents an odd-electron isotopic variant of the molecular ion

where the bromine is  $^{81}\text{Br}$  and the hydrogen is  $^2\text{H}$ . The intensity of the peak at  $m/z$  81 relative to the intensity of the peak at  $m/z$  80 is <0.01% because the abundance of deuterium relative to protium is <0.01%. There are no atoms of bromine that have a mass of 80 u (the atomic weight of Br). For many years, mass spectrometry referred to the nominal mass of a molecule as its molecular weight. As mass spectrometry began to deal with hydrocarbons that had nominal masses >500 u (which meant that the ion would have >70 atoms of hydrogen), the concept of mass defect became important. Mass defect is defined as the difference between the integer mass and the **exact mass** of an **atom, molecule, ion, or radical**. With respect to hydrocarbons, the hydrogen mass defect (0.007825 u) could be significant and affect the value observed. A molecular ion containing 50 atoms of carbon and 102 atoms of hydrogen would have a nominal mass of 702 u; however, because of the hydrogen mass defect, the ion would have an exact mass of 702.7982 u, which would be observed at  $m/z$  703 in a unit resolution mass spectrometer that would not report a  $m/z$  value to a greater accuracy than the resolution. This meant that the nominal mass and the integer mass of the same ion would be different. Even though this was the case, the convention of the nominal mass being molecular weight of the compound was maintained for all integer data. Not only is the molecular weight of the compound reported as the nominal mass, but the mass spectra of the compounds are adjusted for the mass defect and the peaks appear at the value that is consistent with the ion's mass being calculated based on the number of atoms of each element being multiplied by their individual nominal masses and these products summed to obtain the ion's mass.

Other elements besides hydrogen have mass defects that cause the integer mass of bonded atoms to be different than its nominal mass. Decabromobiphenyl ether has an integer mass

of 949 u (exact mass of 949.17830 u) because of the large negative mass defect of bromine (-0.0817 u) and a nominal mass of 950 u. Had this compound been in the NIST08 Database, its EI mass spectrum would have had a molecular ion peak at  $m/z$  950, NOT  $m/z$  949, and the NIST08 Database mass spectrum of this compound would have reported its molecular weight as 950 u. Some mass spectrometers adjust the observed data for mass defect as it is acquired and stored. However, this could be a problem if a positive hydrogen mass defect (resulting in a higher than nominal value for an ion) was being applied to the acquisition of decabromobiphenyl ether, which will have a lower than nominal value because of the negative mass defect of bromine. The hydrogen mass defect would result in the subtraction of 0.949 from the observed value of 949.17830 to give 948.2293 u, which would be reported as  $m/z$  948 in a unit resolution mass spectrometer.

Confusion such as just described is rare when considering ions with multiple atoms of bromine and/or chlorine because of the distinctive **isotope** peak patterns associated with such ions; however, if multiple atoms of iodine are present, the elemental composition of the ion might not be so obvious. Distinguishing as to when to apply a hydrogen mass defect correction or a bromine/chlorine **isotope** correction should be easy for even the inexperienced operator.

When the instrument does not provide an adjustment in the data for mass defect, this adjustment has to be made before the **Nitrogen Rule** (all **molecules** that have an odd number of nitrogen atoms have an odd **nominal mass**) can be applied as the first determination as to an ion's elemental composition. This is very significant with LC/MS data because of the often high  $m/z$  values (> $m/z$  500) of peaks representing ions in the mass spectrum. In the case of electrospray (ES), be sure to be mindful of peaks representing an ion with more than a single charge.

One way to help to eliminate some of the confusion would be to try to differentiate between  $M_r$  [a molecular mass based on atomic weights, a.k.a. as the molecular weight (MW) in most areas of chemistry and physics] of a molecule and mass based on nominal or **monoisotopic** mass. It is possible that this could be accomplished by adopting a convention for the units of mass. The u (unified atomic mass unit) and the Da (dalton) are currently used synonymously. It might be better if u is the unit of mass for a nominal mass or a monoisotopic mass and Da is the unit of mass for  $M_r$ .

Regardless of what terminology is used (molecular weight, MW, average molecular mass,  $M_r$ , nominal mass, monoisotopic mass, exact mass, and accurate mass,) there is always going to be confusion. In mass spectrometry, ions in and peaks on mass spectra have  $m/z$  values. The  $m/z$  symbol should always be used as an adjective; i.e., the ion with  $m/z$  356 or the peak at  $m/z$  256. The  $m/z$  symbol should never be used as a unit; i.e., the 256  $m/z$  peak was the most intense peak in the spectrum. Ions have mass; i.e., the ion (or peak) with  $m/z$  256 represented an ion with a mass of 512 u. When the mass (a numeric value) is stated, it would always have associated units; i.e., the mass of the molecule is 256 u (indicating a nominal mass), 256.029108 u (indicating a monoisotopic mass), or 257 Da or 256.688 Da (indicating an average molar (molecular) mass,  $M_r$ ). If the symbol MW has to be used, the units could differentiate between nominal/monoisotopic mass and average mass (based on atomic weights). One important point: **collections of bonded elements that are neutral and do not have an even number of electrons DO NOT have a molecular weight (MW) or an average molar mass ( $M_r$ ).**

A mass spectrum can have nominal/monoisotopic  $m/z$  value peaks representing any monoisotopic ion (an ion where there is only a single **isotope** for any of the elements, and that isotope is the most abundant isotope of the

element). The mass of an isotopic ion cannot be said to be nominal or monoisotopic; it can be said to be integer (a whole number) or accurate (a number specified to a specified precision). The unit resolution mass spectrometer no longer really exists. Most transmission quadrupole and quadrupole ion trap mass spectrometers have a resolution of 0.3  $m/z$  units and because they take between 10 and 20 measurements per  $m/z$  unit will report a mass accuracy of  $\pm 0.05$   $m/z$  units. If the mass spectral peak represents a monoisotopic ion, this accuracy can be very informative. These same instruments have a precision such that an ion reported as  $m/z$  250.25 one time, will be reported with the same value time after time and on instrument after instrument (within  $\pm 0.05$   $m/z$  units). A reflectron time of flight (TOF) mass spectrometer with a resolving power of 20,000 ( $R = M/\Delta m$ ) will separate an ion with  $m/z$  500.000 from an ion with  $m/z$  500.025. Such an instrument can provide an accurate mass measurement for a monoisotopic ion to the nearest 0.01 millimass unit (mmu). Software, such as the **Cerno Bioscience MassWorks**, can be used to provide accurate mass information resulting in an unambiguous elemental composition, which can be included in a database spectrum.

With such accuracies for mass measurements and existing precisions for these measurements, databases of standard spectra should be built using these values rather than the standard nominal mass paradigm. Large databases such as the NIST/EPA/NIH Mass Spectral Database will have to continue to be built with nominal mass values because to recalculate all of the peaks in each spectrum would require that the elemental composition of each ion be known, and the proportion of peaks representing the members of doublets such as a methyl acylium ion ( $H_3CC\equiv O^+$ ) and a propyl ion ( $C_3H_7^+$ ), each having a nominal mass of 43 u and requiring a resolving power  $>5,000$  to separate, would also have to be known. What is now possible is to perform a conventional

nominal mass library search of a sample spectrum while confirming the monoisotopic mass of the molecule using an Exact Mass constraint against the observed accurate (measured) mass with a precision window.

***“A reflectron time of flight (TOF) mass spectrometer with a resolving power of 20,000 ( $R = M/\Delta m$ ) will separate an ion with  $m/z$  500.000 from an ion with  $m/z$  500.025”***

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He also provides general consulting services in mass spectrometry for a number of instrument manufacturers, manufacturing companies, and government agencies.

# Featured Applications



## Determining Pesticides in Dietary Supplements with QuEChERS Extraction, Cartridge SPE, and GCxGC-TOFMS

Company: Restek

Regulatory requirements are driving the development of new multiresidue pesticide methods for dietary supplements. Minimizing matrix interference is critical for data accuracy. The novel approach employed here combines QuEChERS extraction, cartridge SPE cleanup, and GCxGC-TOFMS analysis, and results in good recoveries across a range of compounds found in these complex matrices. This work shows the application of QuEChERS, cSPE, and GCxGC-TOFMS with an Rxi-5Sil MS x Rtx-200 column combination to quantify pesticides in dietary supplements. The approach used here reduces matrix interferences and improves accuracy relative to one dimensional GC-TOFMS.

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## Metabolic Profiling of Accurate Mass LC-MS/MS Data to Identify Unexpected Environmental Pollutants

Company: AB Sciex

There is growing concern that commonly used Pharmaceuticals and Personal Care Products (PPCP) are entering and contaminating the drinking water supply. The use of targeted quantitation of PPCP has been well established but there is an emerging trend to also screen for and identify unexpected environmental pollutants. The AB Sciex Triple TOF 5600 LC/MS/MS was used to profile environmental samples and unexpected pollutants, to identify and characterize the chemical composition and structure of the pollutants, and to quantify the concentration in collected water samples.

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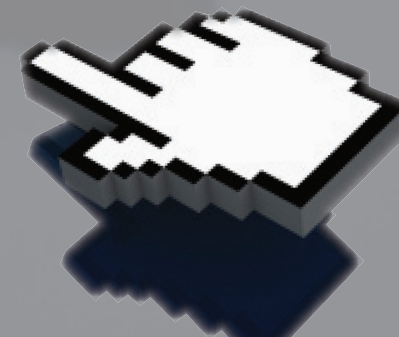
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### Detecting drugs of abuse: Enhanced identification using BenchTOF-dx and associated software

*Company: ALMSCO*

When screening urine for drugs of abuse (DOA), reliability is crucial. Detection of compounds such as marijuana, cocaine, heroin and amphetamines, as well as their metabolites, is commonly carried out by GC/MS, however the complexity of a sample such as urine can present an analytical challenge. High matrix effects and frequent co-elution can significantly compromise reliability of identification, particularly for DOA at trace level, therefore these factors need to be addressed. In this study, a urine sample was obtained for DOA analysis. Fast GC was performed with MS detection using the BenchTOF-dx.

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### Quantitative Analysis of 21 Benzodiazepine Drugs, Zolpidem and Zopiclone in Serum using UPLC/MS/MS

*Company: Waters*

Benzodiazepines are the most frequently prescribed drugs in the western world. They are indicated for a variety of disorders including: anxiety; insomnia; agitation; muscle spasms and alcohol withdrawal. They work primarily due to their interaction with the GABA A receptor. This note describes a quantitative method based on liquid/liquid extraction (LLE) and UPLC with tandem mass spectrometry (MS/MS) for the identification of 21 benzodiazepines in serum. The method's performance has been evaluated using authentic samples. Data were compared to results obtained with a validated method based on HPLC/MS/MS.

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# Products



## AB SCIEX QTRAP® 5500 LC/MS/MS System

*Manufacturer's description:* The AB SCIEX QTRAP® 5500 LC/MS/MS system has been designed to excel at metabolite identification, detection and confirmation of low-level pesticides, and protein/peptide quantitation for biomarker verification and validation. Incorporating fast eQ™ electronics, the QTRAP® 5500 system brings in a new era of performance, according to the company.

The QTRAP® 5500 system also houses a sensitive ion trap, the patented Linear Accelerator™ trap and is claimed to offer ultra-fast scan speeds, and full MS3 capabilities.

Key features of the system include:

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For more information, visit [www.absciex.com](http://www.absciex.com)

## Xevo G2 QTof Manufacturer: Waters Corp.

*Manufacturer's description:* Xevo G2 QTof is the most sensitive, exact mass, quantitative and qualitative benchtop MS/MS system according to Waters. While it maintains the integrated workflow benefits of Engineered Simplicity™ found in the company's existing Xevo QTof, it also incorporates the QuanTof™ technology of Waters' SYNAPT™ G2 system.



Reported features of the system include:

- QuanTof technology - delivers highly sensitive, exact-mass, quantitative and qualitative data UPLC/MSE - a simple, patented method of data acquisition that comprehensively catalogues complex samples in a single analysis
- An extensive range of interface capabilities to service the broadest range of applications
- Guaranteed maximum system performance and usability through our implementation of Engineered Simplicity™
- A complete system solutions, backed by high levels of support

For more information visit [www.waters.com](http://www.waters.com)

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