Accurate mass in agrochemical analysis

Poster 232

Correctly expressing mass accuracy and mining accurate mass data for hard-to-find compounds

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Abstract

Accurate mass instruments are becoming more common in agricultural chemical analysis. The accuracy of the results is typically expressed in ppm. Understanding the true, practical meaning of ppm is critical to interpreting the data. Most accurate mass instrumentation actually produces data with accuracy that is more practically measured in mDa rather than ppm especially at low mass-to-charge ratios (smaller compounds). This poster presents examples of data showing that an error of 10 ppm, though seeming quite far off, can actually be quite good data at some mass values while on the other hand an error of 1 ppm on its own may not be good enough at other masses. It also demonstrates how the use of isotope pattern information and the accurate mass MS/MS fragmentation pattern of a compound supplement information found in the accurate mass value alone and can help to confirm or to deny the assignment of a chemical formula to an accurate mass value. The isotope pattern found in the dosing solution and a compound's fragmentation pattern can also be used to find transformation products related to the starting material, pulling the proverbial "needle" in the matrix haystack for compounds which may not ionize well in the mass spectrometer source.

Methods

- Data were collected using a Sciex TripleTOF® LC-MS/MS system with a Shimadzu Prominence HPLC and Analyst® TF software and a Berthold FlowStar model LB513 radiochemical detector and FlowStar software. The data were processed using PeakView® software, version 1.2.
- The data presented are from an OECD 501 (metabolism in rotational crops) study and an OECD 316 (photolysis) study. Any identifying information in the data has been removed for reasons of client confidentiality.
- The LC-MS/MS data were acquired using IDA (information-dependent acquisition) methods which automatically generate product ion spectra for chromatographic peaks meeting specified criteria. Most of the time, using an IDA method avoids the need to re-inject the sample which is not always plentiful.
- In its most simple implementation, IDA automatically acquires MS/MS spectra on the most abundant "n" ions in each spectrum where the number "n" is specified by the user.
- The next level of sophistication, dynamic background subtraction, instructs the IDA engine to not acquire data on signals from ions whose signal intensities are not increasing. This eliminates MS/MS collection on the downslope sides of chromatographic peaks and from relatively constant background ions. Analyst TF software has an additional level of selection. Real-time mass defect filtering moves ions with mass defects matching those of the test material and its predictable transformation products to the top of the list for automated product ion spectra collection, even if they're not the most abundant ions in the spectrum.

Data

The most common way to express mass accuracy is in ppm:

$$\text{mass error (ppm)} = \frac{(\text{experimental } {}^{m}/_{z} - \text{ calculated } {}^{m}/_{z})}{\text{calculated } {}^{m}/_{z}} \times 1{,}000{,}000$$

$$\frac{(100.0010 - 100.0000)}{100.0000} \times 1{,}000{,}000 = 10 \text{ ppm}$$

Another way to express mass accuracy is in mDa:

$$\text{mass error (mDa)} = (\text{experimental } {}^{m}/_{z} - \text{ calculated } {}^{m}/_{z}) \times 1{,}000$$

$$(100.0010 - 100.0000) \times 1{,}000 = 1 \text{ mDa}$$

In the past, mass spectrometrists who worked on magnetic sector instruments tended to use this way to express accuracy for low mass ions. The ppm error value is relative to the calculated mass (by definition!), *i.e.*, the calculation above shows that a 1 mDa error is a 10 ppm error at m/z 100 but the same 1 mDa error at m/z 800 results in a lower (at least lower looking) error when expressed in ppm

$$\frac{(799.9990 - 800.0000)}{800.0000} \times 1{,}000{,}000 = -1.25 \text{ ppm}$$

Based on the fact that the ppm method of reporting mass accuracy is m/z-dependent and the mass reproducibility of many mass spectrometers actually seems to be more constant in mDa, it may be better to express mass accuracy using mDa than ppm especially at lower m/z values. Errors expressed in mDa "appear" to be better at low mass. Furthermore the expression of mass error in mDa would make it constant or consistent across the mass range. This, however, is not the current industry standard.

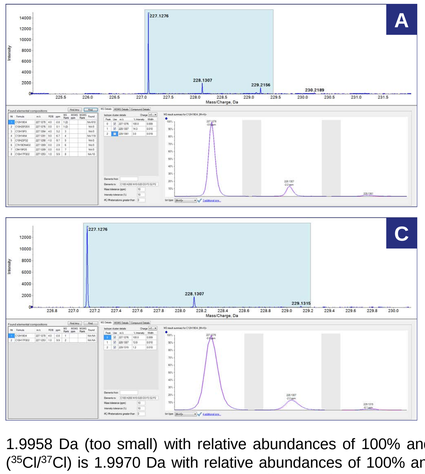

Figure 1: Mass accuracy at m/z ca. 225. Panel A shows an isotope pattern where what appears to be an A+2 isotope (229.2156) is more intense than one would expect for a $C_wH_xN_yO_z$ formula, though it might (based on height only) possibly have sulfurs. Eight formulae are now possible within ± 10 ppm using the information in only the first two isotopes. Panel B shows the tiny (unlabeled) ion at 229.1361 Da that is 2.0085 Da higher in mass than the monoisotopic peak. If this is the A+2 peak, then the formula cannot contain S or Cl, as their presence would require a higher abundance and the mass spacing would also be off. The mass difference between the sulfur isotopes ($^{32}S/^{34}S$) is 1.9958 Da (too small) with relative abundances of 100% and 4.5%. The mass difference between the chlorine isotopes ($^{35}Cl/^{37}Cl$) is 1.9970 Da with relative abundances of 100% and 32% (too high). Panel C shows a background subtracted spectrum a technique which can sometimes sort out relative abundance issues where there is a high background. Now we can use the third isotope spacing and abundance to cut the options to two, the second of which is far enough off in mass to be ignored.

"Problems" Turned into "Opportunities"

Frequently, for very small molecules, there is a high background, when this is the case it can be helpful to add a 2nd label and completely throw away the isotopic envelope information that was illustrated in Figure 1 (which may not be significant at very low mass anyway). This problem turned opportunity is illustrated in Figure 2.

In compounds which have a ^{14}C-labeled phenyl portion of the molecule, it is rare to find 100% of the molecules have six ^{14}C atoms included. It is much more common to find a distribution with molecules having 0, 1, 2, 3, 4, 5, and 6 ^{14}C atoms included. Taking advantage of the resulting artificial isotope pattern is shown in Figure 3.

Finally, when looking for compounds which don't ionize terribly well, it can be helpful to look for characteristic fragments or neutral losses (Figure 4).

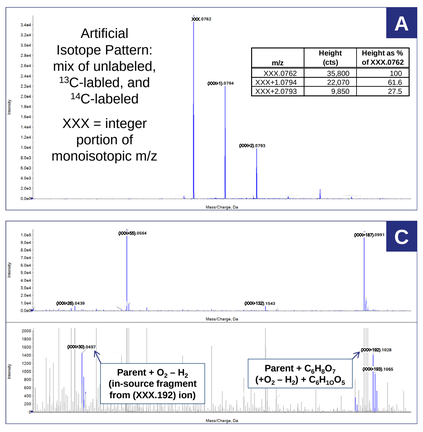

Figure 2: Low mass artificial isotope patterns. Panel A shows an isotope pattern created by mixing unlabeled test material with both its ^{13}C-Labeled and ^{14}C-Labeled analogs. The top trace in Panel B shows the TIC (almost impossible to find anything in) and the bottom trace shows the radiochromatogram. The middle trace is a mass pattern-filtered chromatogram based on the isotope pattern in Panel A. Panel C shows a mass spectrum from the chromatographic peak at 15.6 minutes. The peaks in blue (to draw attention) are those showing the compound-related ions with the same artificial isotope pattern.

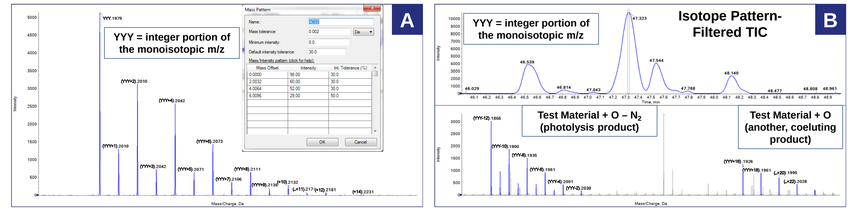

Figure 3 – Isotope pattern from a supplier's labeled test material. This data is from a hydrolysis study. Panel A shows the MS spectrum of a ^{14}C-phenyl labeled compound showing it has 0, 1, 2, 3, etc. ^{14}C atoms included. The upper trace of Panel B shows a zoomed-in portion of the filtered chromatogram with that isotope pattern applied. The lower trace is the spectrum showing two compound-related products. The filter, once more, helps pull the useful information from the dross.

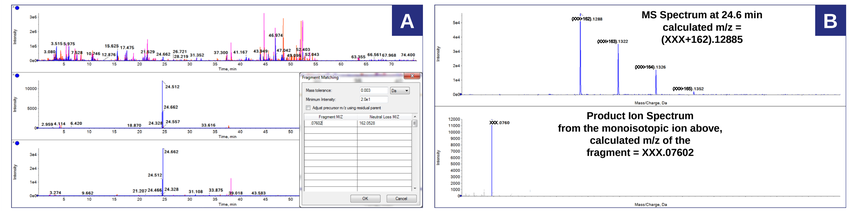

Figure 4 – Product Ion and Neutral Loss filtered data. This data is from the same plant metabolism study shown in Figure 2. The top trace in Panel A shows the overlaid MS/ MS TIC chromatograms from a data-dependent experiment. The middle trace shows those TICs filtered for spectra containing the m/z of the protonated test material. The bottom trace shows them filtered for a neutral loss of 162.0528 Da which corresponds to the loss of a hexose (*e.g.*, glucose). The top trace of panel B shows the MS spectrum at 24.5 minutes and the bottom panel the MS/MS spectrum. These indicate a metabolite that is a hexose adduct to the test material. This data mining technique found another metabolite with much lower level ionization efficiency at 15.5 minutes in the lowest trace of panel A (matching a peak in the radiochromatogram).

Conclusions

- Using the isotope pattern information as well as the accurate mass can be very important in paring down the potential formulae matching an ion suspected of being related to a test material
- Use of the isotope pattern present in the stock material or creating your own artificial one by intentionally adding ^{13}C or some other isotopic label to the mix can help find the compound-related "needles" in the "haystack" of the MS background ions
- Using specific fragments and/or neutral losses can help find or confirm a proposed structure
- Without the advanced filtering techniques, the chemist must either correctly predict the transformation products, use sample/control comparisons which may be difficult in environmental matrices, or attempt to concentrate the sample without losing the products to ensure adequate S/N to find them
- It is sometimes important to take advantage of all the information available in the data
- Expressing mass error is mDa rather than ppm is consistent across all masses and therefore less prone to misinterpretation

Future work/Software Improvement Suggestions

- Current software packages allow the user to set minima and maxima for elements in a formula. A suggestion to the software teams would be to allow a specification of multiples for certain elements in a formula, *e.g.*, in compounds containing trifluoromethyl groups, it would be helpful to be able to specify that the number of fluorine atoms in the formula must be a multiple of three.
- Current software requires the user to calculate the isotope ratio using external software or by "eyeballing" it. It would be more convenient to be able to select a spectrum or a portion thereof and specify that the isotope pattern found there be used.

Acknowledgments

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