Bethany Hood – Accurate mass of transition metal complexes  
9th March 2021

This is work left over, embarrassingly, from November 2020, with huge delays because of Covid issues.

# Method

I ran the samples as 400-fold dilutions in acetonitrile, prepared by addition of 40μL to 760μL acetonitrile, repeated. The samples were run on a Shimadzu IT-ToF, using a Prominence/Nexera UPLC as a sample introduction system, but with no chromatography. The LC pump was set up to deliver acetonitrile at 300μL.min-1 for 2 min following each 10μL injection. The column was replaced by a length of tubing. The LC system did collect UV/visible spectra (200-700nm) but I have not reported on these. The mass spec was set up to collect both positive and negative mode electrospray spectra from *m/z* 200-2000 with a maximum ion accumulation time of 20msec and an automatic sensitivity control target of 70% optimum base peak intensity. The instrument also collected automatic (data-dependent) MS2 spectra of the most abundant ions from *m/z* 50 upwards, at 50% collision energy, 50% collision gas, and an isolation width of *m/z* 3.0. The instrument ignored each precursor for 4 sec after two successive spectra had been collected.

Spray chamber conditions were 250°C curved desorbation line, 300°C heat block, 1.5 L.min-1 nebuliser gas, and drying gas “on”. The instrument was calibrated immediately before analysis, using sodium trifluoroacetate clusters according to the manufacturer’s instructions.

I present only the data that seem immediately relevant; please let me know if you need further MS2, or data in the other polarity.

# Accurate mass of isotope clusters

Last time I produced accurate mass data for your group, there was some discussion of the different masses estimated by different software products. The tool I use is Shimadzu’s accurate mass calculator. Here I describe briefly the problem of accurate mass in isotope clusters, and Shimadzu’s approach to solve it; online software tools may use different approaches, so their results may differ.

The problem is that only a handful of truly extreme mass spectrometers can actually separate all possible isotopologues. For example, Mo has numerous isotopes, of which these are the lightest:

|  |  |  |
| --- | --- | --- |
| nominal mass | % of total | accurate mass |
| 92 | 14.84% | 91.90681 |
| 94 | 9.25 | 93.90509 |
| 95 | 15.92 | 94.90584 |
| 96 | 16.68 | 95.90468 |

Note that in a hypothetical product Mo2, there are two ways to make the isotope peak at 188, but they have slightly different masses

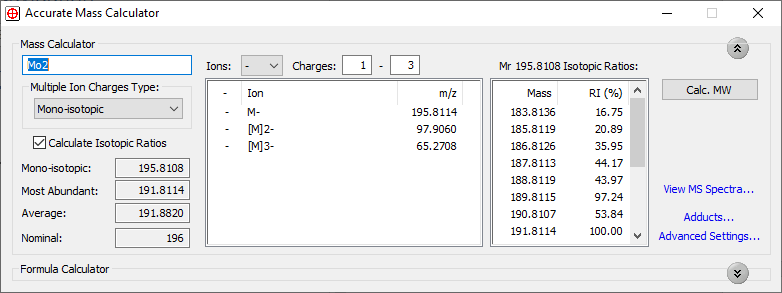
|  |  |
| --- | --- |
| 92Mo + 96Mo | 91.90681 + 95.90468 = 187.81149 |
| 94Mo + 94Mo | 93.90509 + 93.90509 = 187.81018 |

These differ by only a mDa, which is the sort of mass difference that is enough to affect the process of identification using accurate mass, but not enough to allow us to distinguish two peaks. Instead we will see a single isotope peak at some sort of hybrid mass value.

It's possible to estimate the measured mass of this hybrid peak by calculating a weighted average of the two genuine accurate masses, weighted by the abundance of each isotopologue (which can be calculated from the natural abundances of the isotope peaks). For interest, there are two basic ways to do the whole process. One can either find the abundances of all possible combinations of isotopes using the polynomial method, and then group those isotopologues that cannot be resolved. Or one can start with the known spectrum of each element recorded at an appropriate precision for the instrument, and convolute the spectra; if the original spectra are kept as centroids, the final result can be convoluted with the typical resolution of the instrument. This second method is far, far more efficient than the polynomial method, and is almost certainly what the Shimadzu tool is doing.

The Shimadzu tool that I use gives me results in the following form:

A



C

B

Here I’m using the tool in +/- ion mode, not allowing it to add adducts. First it finds (A) the neutral masses of all isotopologues of the specified formula. These are the convoluted/weighted average masses of indistinguishable isotopologues. Then, for negative ions, it adds electrons (mass 0.00055 per electron), and divides by the charge, to find the measured values (B). When the software plots predicted spectra, these are calculated as in (B), but for all isotope peaks. The mono-isotopic mass relates to the isotopologue containing only the most abundant isotope of each element, in this case 98Mo.

Note that we can estimate the Mo2 isotope peak at 188 by finding the abundance of each isotopologue

|  |  |
| --- | --- |
| 92Mo + 96Mo |  |
| 94Mo + 94Mo |  |

We can then find the weighted average mass

You will notice that this agrees closely with Shimadzu’s result.

# Results

## BRH2-195 (1/400th)

The full spectrum in negative mode was



Zoomed



This is the mass you expected, but the stated formula including counter ions, C52H92N6Mo6O20 doesn’t match. I assume the anion is C20H20N2Mo6O20(2-), which has the following simulation, which matches the data closely:



Singly-charged cluster