HOWTO for Identification of an Unknown Using Spectrometric Data

Identification of an unknown is a central aspect of chemistry. Given the number of tools available, the process can be daunting, as it involves a series of hypotheses and tests to winnow the literally millions of possibilities down to one structure, consistent with the data. We provide here a general approach that should be useful for your challenge in CH 362. There will be additional steps described that you do not have to take, but bear these in mind for future use when you have to deal with a real unknown. We'll present these as a series of questions and suggestions for how to answer them.

1. Is this a pure compound? (What is the evidence?) If not, what do you know about impurities—solvent? Water? Grease? You should identify artifacts from known imputities in each spectrum before trying to assign a structural interpretation. (Generally, CH362 unknowns are pure other than NMR solvents and maybe a trace of water.)

2. What is the molecular formula? We obtain this from the mass spectrum in a logical manner:
    a. Identify the parent ion and any heavy-isotope peaks: a small (M+1) for ^{13}C, significant (M+2) peaks for Cl or Br.
    b. Do you know of any structural component? All CH 362 unknowns are disubstituted aromatics and therefore must have C_6H_4 as part of the molecular formula. Subtracting that mass from the parent ion gives the mass of what's left. As you identify other pieces, you can subtract those as well until you have nothing left to explain.
    c. Can you observe major fragmentation? Loss of substituents from the parent ion can give clues to their identity based on mass. Halogens are typical (and the fragments no longer have the heavy isotope peak), but carboxylic acids, esters, aldehydes and alkyl groups often show characteristic mass losses.
    d. Does the spectrum show fragments typical of particular kinds of functional groups?

3. We do not do this for the CH 362 unknowns, but using IR at this point to identify functional groups would be a normal part of the process.

4. Examine the ^1H NMR and identify the number of chemically distinct positions. If the aromatic group is para substituted, there will be symmetry, otherwise there should be 4 different chemical shifts for multiplets (possibly overlapped), plus any protons in the substituents.

5. You should (to the extent possible) analyze the coupling patterns in the 1-D ^1H NMR and measure the coupling constants. Overlapping signals may make this impossible, but there are cases where a single multiplet can provide most of the coupling information in a system. It helps to make a prediction for what typical coupling constants will produce for each signal in both a meta and an ortho disubstituted compound. And remember that triplets may separate into doublets of doublets if the two coupling constants differ (as they often do). The best approach:
    a. Identify the peaks in the multiplet on the peaklist printout. Use the absolute frequency in Hz.
    b. Whatever the pattern, there should be two pairs of outer peaks in the multiplet separated by the same frequency difference in Hz. This is the smallest coupling constant.
    c. If that outer pattern is a doublet, find all possible pairs of peaks separated by that frequency difference. (If it is a triplet, you look for all possible 1:2:1 triplets with that separation.)
    d. You should now be able to find regular groupings of these pairs (or triplets) separated by regular frequency differences.

Repeat for all identifiable multiplets; there should be some consistency in J values (± 0.1 Hz)

allowing you to identify which protons couple where (and how). In rare cases you will know the substitution pattern and compound identity at this point.

6. The COSY spectrum now helps you confirm these coupling relationships. Identify (on the bases of off-diagonal peaks) which protons (identified by the chemical shift of the center of the multiplet) couple to which others. Make a table listing each of the 4 aromatic chemical shifts, and which other protons it couples to. (Include chemical shifts of substituents for completeness.)

7. The HSQC spectrum now tells you which carbons are attached to each proton. Add that information to your table. There will be 2 carbons (probably weak signals) that do not show up; give these separate lines in your table. Also include carbons from any substituents.

8. The HMBC spectrum is the most complex, but that means it holds the most useful information. Approach it as such:

a. Identify any one-bond coupling. This is typically identified by seeing cross peaks that may not correspond to a ^{1}H chemical shift; they are symmetrically placed about the ^{1}H shift and usually 80-100 Hz on either side (up to 0.25 ppm). You might find these for only a few carbons, and often not at all.

b. For each carbon line, identify which protons show correlations. List these in a new column in your table. These can be 2-, 3-, or 4-bond couplings but most are 3-bond.

c. If you have a carbon in a substituent, it is easiest to trace the connectivity from there into the ring. The strongest correlations for this carbon will be to any proton ortho to the substituent—the 3-bond coupling. If there is only one, in all likelihood your substituents are ortho to each other. If there are two, then most likely it is a meta disubstituted compound.

d. Do your best to trace out all the correlations from carbon to protons. You should end up with an unambiguous assignment of all carbon and proton chemical shifts where the 2-D correlations and J values all fit.

9. Last: make a prediction for what your chemical shifts should be based on additivity tables (Lab Manual, pp. 44-45, Appendices B and C). The carbon shifts should agree to within 1-2 ppm (and they should wildly disagree with the other isomer). Proton shifts are highly solvent dependent, so the absolute values may differ a bit, but the chemical shift order in the spectrum should fit. If you have a meta isomer with no carbons in the substituent, this set of predicted shifts is the ONLY way of completing the assignment and removing the ambiguities.

10. Those of you lucky enough to have fluorine as a substituent have additional information: the ^{19}F spectrum and additional coupling visible in the ^{1}H and ^{13}C spectrum. Just as for protons and carbons, the magnitude of the coupling to fluorine decreases as the number of intervening bonds increases. In the 13C spectrum particularly, you can usually “step around” the ring on the basis of the decreasing J values for ^{19}F-coupled doublets. The ^{1}H-^{19}F J values from the ^{19}F spectrum should match otherwise unexplained J values in the ^{1}H spectrum.