

Internal Standards

There are many tradeoffs in picking internal standards, which see use in quantifying absolute NMR yields, GLC analyses, and partition coefficient determinations, among other things. Some general considerations are noted below.

1. Avoid Volatile Internal Standards. An ideal internal standard is crystalline with a low vapor pressure. Volatile internal standards, which may evaporate, resulting in quantitative errors, should be avoided (there are almost always good alternatives). A standard solution of a volatile internal standard in a solvent will sometimes give reliable data. However, a volatile solvent further complicates the matter.

Water is considered a reasonably non-volatile solvent for standard solutions. If you need to prepare a standard solution of an internal standard in an organic solvent, avoid something like dichloromethane and favor something with a vapor pressure greater than or equal to water, such as toluene, methylcyclohexane, or tetrachloroethane.

2. Significant Digit Problem. If your written experimental procedure indicates that you only used 2 mg of an internal standard, any yields calculated are not accurate enough for publication (only one significant digit!).

If your experiment requires that you weigh out an internal standard, make sure you use a good balance so that three significant digits can be obtained (for example, 13.2 mg).

If your experiment allows the use of a standard solution, follow the guidelines in (1) and make sure that the standard is weighed out to at least three significant digits, or delivered by volume to three significant digits (a weighed syringe is also an option).

3. Response Factor Problem. This is exemplified for the determination of absolute yields by NMR, although analogous considerations apply to UV/vis, HPLC, and GC measurements.

Consider the internal standard candidate 1,3,5-tris(*t*-butyl)benzene (MW = 246). The most conspicuous singlet in this compound is due to 27 protons. Suppose you want to determine the yield of an organometallic complex, MW = 600, that you expect to form in about 50% yield, by integrating the Cp protons vs. a standard. How much standard should be used?

You should always perform a trial calculation of this type before executing the experiment. With many instrumental techniques (particularly in the old days), the calculated yield will not be very accurate if the area of the standard is 100 and the area of the peak in the compound you are trying to quantify is 1-2. Try to avoid extracting quantitative data from peaks of very different areas. Accuracy is maximized when peaks are of similar areas.

Return to the above example and consider a run with 30 mg (0.050 mmol) of a cyclopentadienyl complex. We expect 0.025 mmol of product, or "0.125 mmol of Cp protons" to integrate. How much of the 1,3,5-tris(*t*-butyl)benzene internal standard should be used? We would want 0.125 mmol of *t*-butyl protons, which is (\div 27) 0.0046 mmol of standard – which is in turn 1.14 mg of standard – far too small an amount by criterion 2 above.

One might consider using the integral of the aromatic protons of 1,3,5-tris(*t*-butyl)benzene. This would give much better results, but the analyte will often have interfering aromatic protons.

So the lesson is: pick NMR internal standard with a higher molecular weight (ideally crystalline, air stable, etc.) and a unique peak due of just a few protons, not a multitude of protons.

4. Recommended (and Forbidden) Internal Standards. One good starting example for an internal standard is Ph_3SiCH_3 (exercise: calculate how many mg would be optimum for use in reaction in 3). There was once a large supply of this easily synthesized compound in the group. There are many other good silicon containing standards, but tin is more reactive and should be avoided.

Another possibility is 1,2,4,5-tetrachlorobenzene (the aromatic protons shift downfield out of the range of most benzenoid groups), but this should not be used when electron transfer mechanisms are possible. There are many inexpensive polychlorinated aliphatic molecules.

Methylnaphthalene would be a reasonable internal standard for many purposes, but a bromomethylnaphthalene (as one isomer) would be even better. For NMR determinations, avoid *t*-butyl groups (as groups to be integrated).

Very bad internal standards would include ether, dichloromethane, and any similar volatile solvent.