APRIL 29, 2014

Last quarter I shared my thoughts on how stability of a compound is also related to its physical state (crystalline vs amorphous).

Following are my observations relating to potency determination when impurities are present.

Even though potency determination of pure compounds in absence of impurities is relatively simple, determination of potency of Reference standard compounds when impurities are present can be a significant challenge – the potential error increases significantly with increasing fraction of impurities. The impurities can be organic compounds or inorganic salts/ions. The UV absorbance (profile and extinction coefficient) of organic impurities is often not identical to that of the parent compound. The MW of the impurities is also often different from that of the parent compound. Ideally, one needs an analytical technique that has (intensity) response for all components matching its relative weight, along with another distinct resolvable response (such as peak retention time) for the parent compound and impurities within it. But alas, no such analytical technique or detection technique exists!

HPLC UV purity is an approximation for the purity analysis of compounds that have UV absorbance. The UV response for the parent compound is often different from that of the impurities that are present, and is not proportional to the weight fraction of the impurities. Nonetheless, when the level of impurities are low (<1%), UV HPLC is the best available and widely used analytical technique for determination of purity of a (non-volatile) compound and needs only a tiny amount (< 1 mg) of compound (without a need for accurate weight). But, when purity of a compound is low, the HPLC UV purity values are not close to the weight fraction purity values, and introduce significant error in potency determination.

Almost all other analytical techniques used for determination of weight fraction of impurities require accurate weighing. As a result, a larger amount of substance has to be weighed / analyzed for accurate measurements. These include techniques such as Karl Fischer titrations for water content and Elemental analysis.

Proton NMR is an analytical technique that has the potential to provide accurate values of potency. If appropriate acquisition parameters are used, each peak in proton NMR spectrum represents mole% response from each of the proton containing groups present in the solution analyzed. If the identity of each of the proton containing components is known, the NMR mole% response (obtained without accurate weighing of sample) can be converted to relative weight% measurement. Unfortunately, we often do not know the identity of each of the impurities that might be present. An alternative is to accurately weigh known amount of compound and add an accurately weighed amount of an internal reference standard whose NMR peaks do not overlap with that of the compound or the impurities present within it. With proper acquisition parameters, resonance line shape, phasing, and baseline correction, such proton NMR with internal reference standard can be used to accurately determine the weight fraction purity of a compound, without having knowledge of the identity of impurities. Some refer to this method as “Quantitative proton NMR” and it accurately determines potency of the major compound, even if the purity of the compound is low. It also accounts for inorganic impurities, residual solvents, water, and other invisible impurities that might be present in the compound. So, as long as sufficient amount of compound is available for accurate weighing, “Quantitative proton NMR” is likely to be the best option for potency determination, especially whenever purity level is low. We do have this analytical service available in case you need it.

Next quarter, I plan to discuss and share some pointers on preparation of solutions of weakly stable compounds such as metabolites.

Additional reference standards that we have in-stock are listed on our website catalog. If you are looking for a particular compound we have not listed, please contact us - we can often quickly procure or synthesize those that we do not have in-stock using advanced intermediates that we might have.

Our standard practice is to place a one year recertification date in a comprehensive Certificate of Analysis (CoA) that includes all supporting analytical data (HPLC, LCMS, NMR) freshly obtained from the batch/container that is shipped. This does entail a cost overhead for each shipment, but provides guarantee and assurance that the high purity values of the compound listed in the CoA are accurate and enables compliance with regulatory requirements.

Please do let me know if I can answer any questions or if we can be of assistance.

Best Regards,
Khalid

Khalid Thakur