AUGUST 26, 2014

Last quarter I shared my thoughts on challenges and solution to potency determination in compounds that have low purity levels.

Following are my observations/learnings for minimizing probability/rate of degradation of HPLC solutions of metabolites that are prone to hydrolysis/degradation in acidic aqueous solutions.

Solution stability of hydrolysable metabolites such as acyl-glucuronides, sulfates, and phosphates:
Typically, the major hydrolysis degradation pathway in aqueous solution is:
Metabolite → API + free acid ; often catalyzed by strong acid or strong base.

Without going into too much details of kinetics, the major factors that determine the rate of degradation in acid modified HPLC solutions are concentration of metabolite [metabolite] and concentration of strong acid [strong acid] that catalyzes the hydrolysis.

Rate of hydrolysis in aqueous solutions = [metabolite]^{x} [strong acid]^{y} [weak acid]^{z}
- where ***x***, ***y*** and ***z*** exponents depend on many factors and vary for each metabolite and type of acid.

In non-aqueous solutions, trans-esterification can be the major degradation pathway.

In order to increase the stability of metabolite solutions, first, the concentration of strong acid has to be minimized. One can do that by using weak acids such as acetic acid instead of strong acids (TFA) as HPLC solution modifiers – the chromatography can sometimes be different but will often be reproducible. As you can imagine, the stronger acids ensure that all counter ions are identical, whereas weaker acids may (in some instances) not be able to displace strongly bound counterions .

Second, lower the concentration of the metabolite in solution; i.e. prepare dilute solutions.
At higher concentration of metabolites (in weak acid solutions), the rate of degradation is slowest initially, but as it degrades and additional strong free acid is formed to catalyze the hydrolysis, and the rate of degradation increases rapidly.

Another factor that plays an important role in determining the stability of the metabolite is its level of purity. If the purity level is low and it already contains strong free acid, the rate of hydrolysis/degradation of metabolite will be significantly higher than that of more pure metabolites. So, given a choice, my recommendation would be to work with metabolites that have highest purity levels.

In summary, when working with metabolites prone to hydrolysis my recommendation would be as follows:
1) Try to use the highest purity metabolites that are available
2) Use acetic acid modified HPLC solutions
3) Prepare and use dilute solutions – avoid preparing and storing concentrated solutions

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,
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chemtos