COVERSTORY

Challenges in Defining and Achieving Selectivity Requirements for Phase I Stability Indicating HPLC Methods

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At all stages of clinical development, the innovator company is expected to have analytical methods in place to monitor the quality and safety of clinical trial materials. In the case of a typical small molecule developmental candidate, a key method in this context is a high pressure liquid chromatography-(HPLC) based stability indicating method (SIM). An ideal SIM should demonstrate selectivity for the active pharmaceutical ingredient (API), API process impurities, potential degradation products, and any formulation components. By later phase clinical trials (Phase III, for example), the formulation composition, API process impurity profile, and the expected degradation profile of the product under long-term storage conditions are all known. Thus, at Phase III the HPLC SIM selectivity requirements are readily defined.

The pharmaceutical scientist faces a markedly different situation when trying to rationally define the Phase I HPLC SIM selectivity requirements. At that time, the API manufacturing process and the corresponding impurity profile are only very recently established. The formulation composition is evolving, and experience with potential degradation products is very limited. In this early developmental stage, a diverse set of chromatographic data needs to be considered to appropriately define what method conditions are "stability indicating" for each developmental candidate. Current industry trends of deferring as much effort and resources as possible in early phase development until clinical proof of concept, and the concomitant effort to bring larger numbers of developmental candidates to Phase I, have further increased these challenges.

Yet any increases in throughput cannot compromise product quality and safety; rationale, effective SIM must be developed more efficiently. This article highlights these difficulties in more detail, and describes how "realistic" forced stress testing can increase the efficiency and confidence with which Phase I SIM selectivity requirements are defined and achieved. SIM development workflow is also discussed including application of Generic SIM methods, column switching, and column selectivity databases.

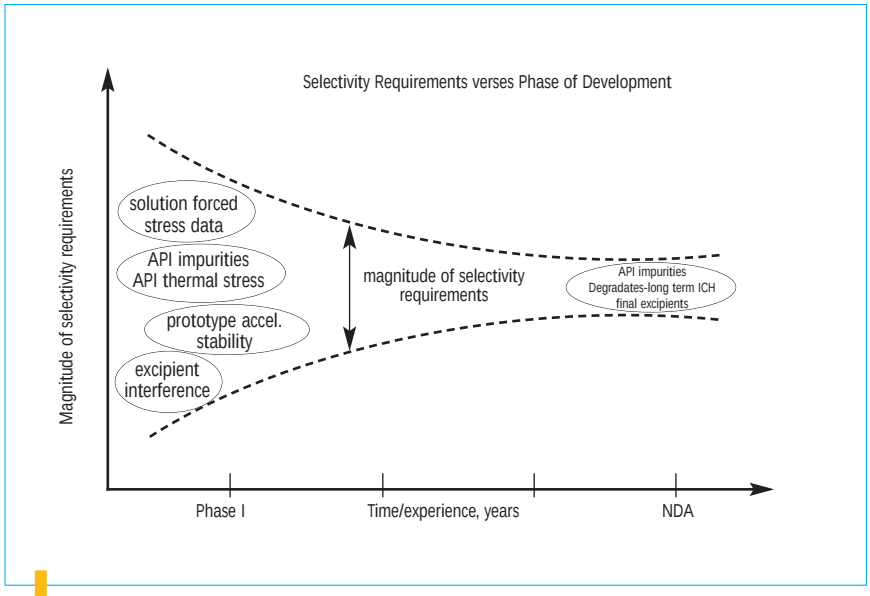

Figure 1. Distance along Y axis shows general complexity of SIM selectivity requirements, X axis shows progression of time as developmental process proceeds. Dashed lines show the SIM selectivity needs are larger in early phases and become more specific and focused as development progresses. Circles represent data sets, size of circle proportional to complexity/size of data set.

Chromatographic data sets needed for Phase I SIM development

Figure 1 attempts to depict the chromatographic "inputs" into the development of a Phase I SIM, and how the resulting selectivity requirements evolve over time. In Figure 1, the distance between the dashed lines represents the magnitude or complexity of the SIM selectivity requirements at each developmental stage, while the developmental stage (Phase I to New Drug Application) timeline is along the X-axis. The four labeled circles above the Phase I region in Figure 1 represent typical chromatographic data sets used to define the Phase I SIM selectivity needs. The data sets typically become available in the order shown in Figure 1, from top to bottom.

Forced stress testing experiments in solution are typically carried out first as any form of the drug substance can be used. Acid-, base-, and hydrogen peroxide-based stressing are common.[1] These studies are often the first view of the drug's potential chemical instabilities. Once the final crystal form of the API is available, short term accelerated thermal stress of the API (50 ~70°C) is typically carried out. This probes degradation routes such as oxidation, isomerization, epimerization, and rearrangements. When prototype formulations are made, accelerated thermal and humidity stress (open dish 40°C/75 percent RH being common) is carried out. These studies examine hydrolysis, oxidation, and excipient mediated degradation pathways. The last data set in Figure 1 refers to excipient chromatographic interferences in the final Phase I formulation, these can be very pronounced if excipients such as Tween 80, Imwitor, and miglyol are used.

Limited experience and uncertainties in the pre-Phase I interval often lead to the expansion of the selectivity requirements to encompass as much data in all four data circles as possible. This can be costly in terms of time and resources. Inevitably, in Phase I there is more complicated or broad SIM selectivity requirements compared to later stages in development. This is shown by the large distance between the dashed lines in the left hand portion of Figure 1.

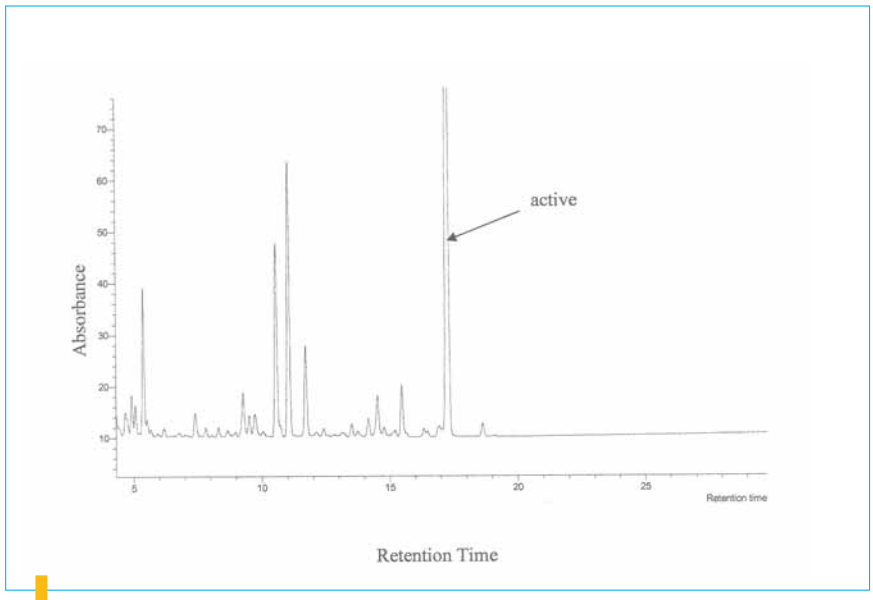

Figure 2. Chromatogram of 1 percent hydrogen peroxide stress of drug candidate; 24 hour stress in 100 percent aqueous solution, heated at 40°C. None of these degradates reflect the desired two electron oxidation products, none of these degradates appear in solid dosage forms under long-term storage conditions.

"Realistic" solution forced stress procedures for Phase I SIM

Stress testing of pharmaceuticals has recently been reviewed.[2] Stress testing is typically performed throughout product development and serves different purposes in each stage. In the context of Figure 1, in our view the stress procedures should be limited in strength so as to produce only "realistic" degradates, which might plausibly be formed in solid dosage forms on long term stability. Such realistic stress conditions, when applied to a chemically stable API, would produce no signal degradates at all. This would completely eliminate the forced stress data set circle in Figure 1. The purpose of these solution stress studies is three- fold: a) to determine if there is significant hydrolytic or oxidative sensitivity of the drug, b) if sensitivity exists, identify the one or two degradates which would first "signal" the degradation pathway, and c) quantitatively determine if there are any mass balance issues associated with the observed sensitivities.

In the case of acid and base stress, the strength of the acid and base, duration of exposure and temperature are intentionally limited. Realistic oxidative stress involves use of hydrogen peroxide in a manner to maximize two electron oxidative events such as oxidation of tertiary amines to N-oxides and oxidation of thioethers to sulfones[3] while minimizing hydroxyl radical reactivity. Azobisisobutyronitrile (AIBN) is used to create peroxy radical activity in

solution,[4] as peroxy radical mediated oxidation is the arguably most common route of oxidation of drug substances solid dosage forms.[5] These procedures when carried out as described are rather selective, and are meant to complement the two other degradate data sets described in Figure 1.

A brief outline of these procedures follows. The stress procedures require an initial solution of the API both in a methanol/water solvent system, as well as a acetonitrile/water solvent system, at the analytical concentration. All dilutions are quantitative, active area counts for all stress conditions are quantitatively compared to the initial solutions correcting for any dilutions made.

Acid and base stressing

Dilute a portion of 1.0 N HCl 10-fold into a portion of the API in the acetonitrile/water solution. The solution is mixed, then split into equal volumes and placed in flasks and cap. One sample is kept at room temperature and the other placed in a 60° C oven. The procedure is repeated except 1N NaOH is diluted 10-fold in a portion of the acetonitrile/water solution. Degradation is monitored over 1 day at a maximum, or until 10 percent of the API is degraded, whichever is achieved first.

Oxidation by hydrogen peroxide

Dilute a 1 percent hydrogen peroxide solution 10-fold into a portion of the API in the methanol/water solvent system. The sample is mixed, capped and kept at room temperature for up to 24 hours. The type of oxidation being looked for here occurs quite rapidly; 10-percent conversion of active to an N-oxide for example may occur in a few hours. If tertiary amines groups are present in the drug candidate, the pH may have to be raised to deprotonate the amine. Typically, only a few of the rapidly forming type of degradates being probed in this stress condition should be observed. Figure 2 highlights the ease with which hydrogen peroxide can be misused to generate unrealistic degradation products.

Oxidation by Peroxy Radical

Ten ml of the API in the methanol/water solvent system is pipetted into a 25 ml volumetric flask to provide ample headspace containing oxygen.[6] Solid AIBN is added to the flask to give about 5 mM AIBN. Once the AIBN has been dissolved, the flask is capped tightly and placed at 40°C. Samples are analyzed after 24 hours and up to 3 days and compared to appropriate controls containing AIBN and no drug substance. Peroxy radical is quite selective; numerous drug candidates will not show any degradation under these conditions. A significant reactivity toward peroxy radical would be ~3 percent degradation products (or greater) over 24 hours.

Photolytic Stressing

Either starting API solution is given a UV A exposure of 200 W-hr/ m2. This exposure is the minimum exposure suggested in the International Conference on Harmonization ICH Q1B but in our view is appropriate given the pre Phase I "realistic" degradates scenario being described here.

An example of these simple procedures applied to a drug candidate is shown in Figure 3. The API starting solution is shown in the lower chromatogram, with the base, acid, hydrogen peroxide, and AIBN stress chromatograms overlaid above. The acid and base stress chromatograms do not show any active loss or significant degradates peaks. Therefore there is no hydrolytic signal degradates. The same is true for the UV exposure (data not shown). The hydrogen peroxide stress shows a single major peak near 13 minutes (labeled Deg. 1 in Figure 3). The AIBN stress shows formation of a significant degradate peak near 20 minutes, labeled Deg. 2 in Figure 3. Thus for this API, the forced stress data circle in Figure 1 has only two signal degradates, and comparison of active area count loss and degradate area gained show that mass balance is excellent under the current method conditions.

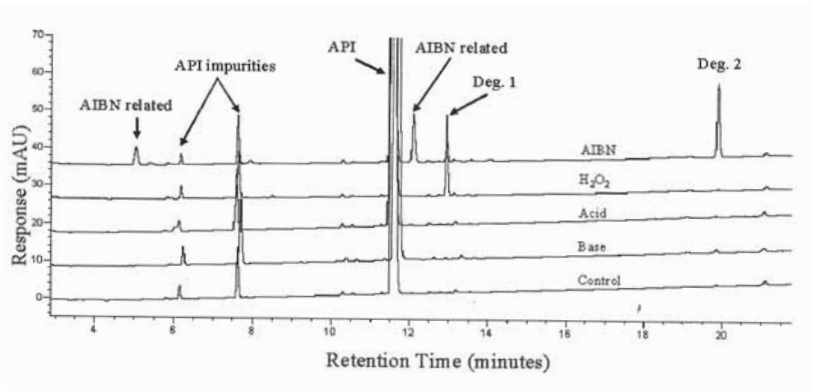

Figure 3. Lower, starting solution of drug in 50/50 methanol/water; above that are the base, acid, hydrogen peroxide, and AIBN based stresses as described in the text. Note Deg.1 formation in the 0.1 percent hydrogen peroxide in 50/50 methanol/water as a signal degradate. AIBN stress gives an additional signal degradate labeled Deg. 2 near 20 minutes.

Analytical workflows for efficient SIM development

These simple solution stress procedures and resulting data such as that shown in Figure 3 provide a degree of confidence that any significant chemical instabilities are known and can be initially monitored. This provides a solid foundation to proceed forward with SIM development. Figure 4 shows one possible general sce-

nario for Phase I SIM development workflow. The data set circles from Figure 1 are shown on the right hand side of Figure 4, while the left had side of Figure 4 shows corresponding block diagrams of workflows which assimilate each data set. There are four general features of the workflow in Figure 4: 1) generic HPLC SIM methods are applied to examine the initial forced stress solution studies, 2) the API thermal stress samples (and associated API impurity profile) are then examined; any co-elution issues are resolved by automated column switching experiments; once selectivity for signal and thermal API stress degradates is obtained, then 3) the accelerated stressed prototype formulations are examined; this insures that the HPLC method is as "stability indicating" as possible prior to the chromatographic analysis which allows for optimum decision making regarding final formulation selection, and 4) once the final formulation is selected any remaining quantitative, formulation specific resolution issues are resolved and further reductions in analysis time can be considered. A brief overview of these four aspects follows.

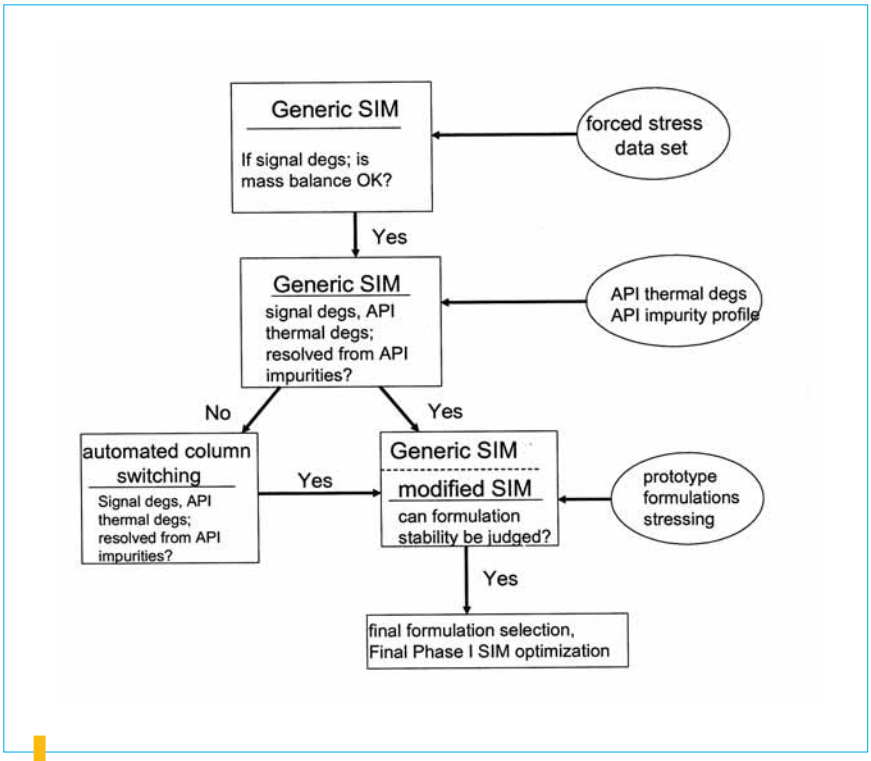

Figure 4. Analytical workflow for Phase I SIM development. Key features are use of Generic SIM to examine solution forced stress samples and requirement that signal degradates and API thermal stress degradates should be resolved from each other and API impurities prior to stability testing of stressed prototype formulations.

Generic HPLC SIM applied to forced stress samples

Generic methods harmonize analyst "starting points," provide a reasonable chance of success without any method development resources, and facilitate best practice sharing and information flow between analytical groups from different developmental areas. A typical generic method utilizes gradient elution over a wide solvent composition range, and could utilize columns with either 5 micron or 3.5 micron particle size packings. The data in Figure 3 derives from such a generic method: C18 column, 15 cm x 4.6 mm (3.5 micron particle size), with gradient elution ranging from 95/5 to 0/100 (0.1 percent phosphoric acid/acetonitrile) over 30 minutes, flow rate = 1.0 ml/min., with UV detection near 210 nm.

The selectivity requirements at this stage are only that if signal degradates form, they be resolved from the active peak. Any mass balance issues observed should be resolved and generally rationalized at this stage before proceeding.

Signal degradates, API impurities, and API thermal stress degradates

The generic method is then applied to the final form API (impurity profile), and any API thermal stress samples available. Given that the SIM being discussed is a final drug product SIM, the primary selectivity requirements are that signal degradates and any significant API thermal stress degradates cannot co-elute with each other or with API impurities. Stable API's will show no signal degradates and few if any thermal stress degradates; in these cases the selectivity requirements are often obtained with the generic method. Less stable API's have increased chances of co-elution issues at this stage. In these cases, automated column switching can be used to rapidly explore other column chemistries and mobile phases as shown in Figure 4. Snyder and Dolan[7] have recently established column databases in which columns with either very similar or very different ("orthogonal") selectivities can be chosen. If three such orthogonal columns are selected, along with two mobile phases (pH 2 and pH 7, for example), and five different samples are chromatographed (asumming 3 solution stress samples with signal degradates, the API, and thermally stressed API), the entire data set can be generated in just over 24 hours given a 30 minute gradient as in Figure 3.

Prototype formulation stability testing

The different HPLC method conditions explored by the column switching experiments will typically yield a solution to the co-elution problem, given the relatively small number of peaks being considered and the relatively large peak capacity of a typical gradient HPLC method as in Figure 3. The stressed prototype formulations can then assayed with the optimal method. While "new" degradate peaks can be observed in the prototype formulations, often the degradation products have been predicted by either the solution forced stress studies or the thermal stress of the API. Chromatographic peaks from excipients can come into play at this point and can complicate resolution and quantitation

of degradates. Given that the purpose of the SIM at this point is to judge overall stability performance, and that a number of prototype formulations may be considered, formulation specific method optimizations should be avoided if possible.

Once the final formulation is selected, final optimization of the SIM supporting the Phase I clinical materials can proceed. Any outstanding, final formulation specific resolution issues would need to be resolved at this point. For most API's with fairly simple formulations, either the original generic SIM method or the modified SIM (Figure 4) will come through the workflow in Figure 4 to provide the necessary final selectivity. This is the desired case since minimal resources are used for SIM development. For chromatographically complex liquid filled capsule formulations, there may still be significant co-elution challenges remaining for certain key degradates. The analyst should take full advantage of the column switching data set to guide method modification; software packages such as Chromsword and Drylab could be utilized to model specific elution behaviors if needed.

Summary of current state and hopes for the future

The type of HPLC SIM workflows depicted in Figure 4 currently involve gradient separations using standard HPLC hardware. Similar numbers of theoretical plates are available by using 25 cm HPLC columns with 5 micron particle size packings, or 15 cm columns with 3.5 micron packings, but the latter can effect the separation in ~35 percent less time. UPLC (Ultra high pressure liquid chromatography) systems are available which utilize columns with ~2 micron packings which can provide further improvements in plates achieved per unit of time. While UPLC capability is clearly helpful in regard to improving efficiency of SIM development, these systems have yet to prove themselves in regards to user robustness in the typical analytical laboratory setting. "Lab-on-chip" type technologies[8] remain in the future, but ideally could hold promise of generating separations with large numbers of theoretical plates per unit time. Such separations might be able to resolve all the impurities, degradates, and excipient peaks described in Figures 1 and Figures 4, thus allowing a "one method fits all" approach. Such systems would face the same robustness challenges as UPLC currently and would have to be able to generate data acceptable from a regulatory point of view. Until that time, current HPLC instrumentation and iterative SIM development approaches as described in Figure 4 will need to continue.

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