



Kick-Start the Evolution of Stability Indicating Methods with Forced Degradation

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Goals of this talk

- Place forced degradation into context with respect to method development
- Discuss some good practices
- Warn of a couple of dangerous practices
- Focus on early method development where forced degradation is most important

Disclaimer: What comes next is my opinion, and I guarantee that some smart people disagree with some of the details—judge for yourself.



Obligatory regulatory background: ICH Q1A (R2)

2.1.2. Stress Testing

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.

Stress testing is likely to be carried out on a single batch of the drug substance. It should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing), humidity (e.g., 75% RH or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing. The standard conditions for photostability testing are described in ICH Q1B.

Examining degradation products under stress conditions is useful in establishing degradation pathways and developing and validating suitable analytical procedures. However, it may not be necessary to examine specifically for certain degradation products if it has been demonstrated that they are not formed under accelerated or long term storage conditions.

Results from these studies will form an integral part of the information provided to regulatory authorities.

Gleanings from ICH Q1A(R2)

- identify the likely degradation products
- intrinsic stability of the molecule
- validate the stability indicating power of the analytical procedures used
- The nature of the stress testing will depend on the individual drug substance and the type of drug product involved
 - effect of temperatures... humidity
 - oxidation...photolysis...hydrolysis across a wide range of pH values

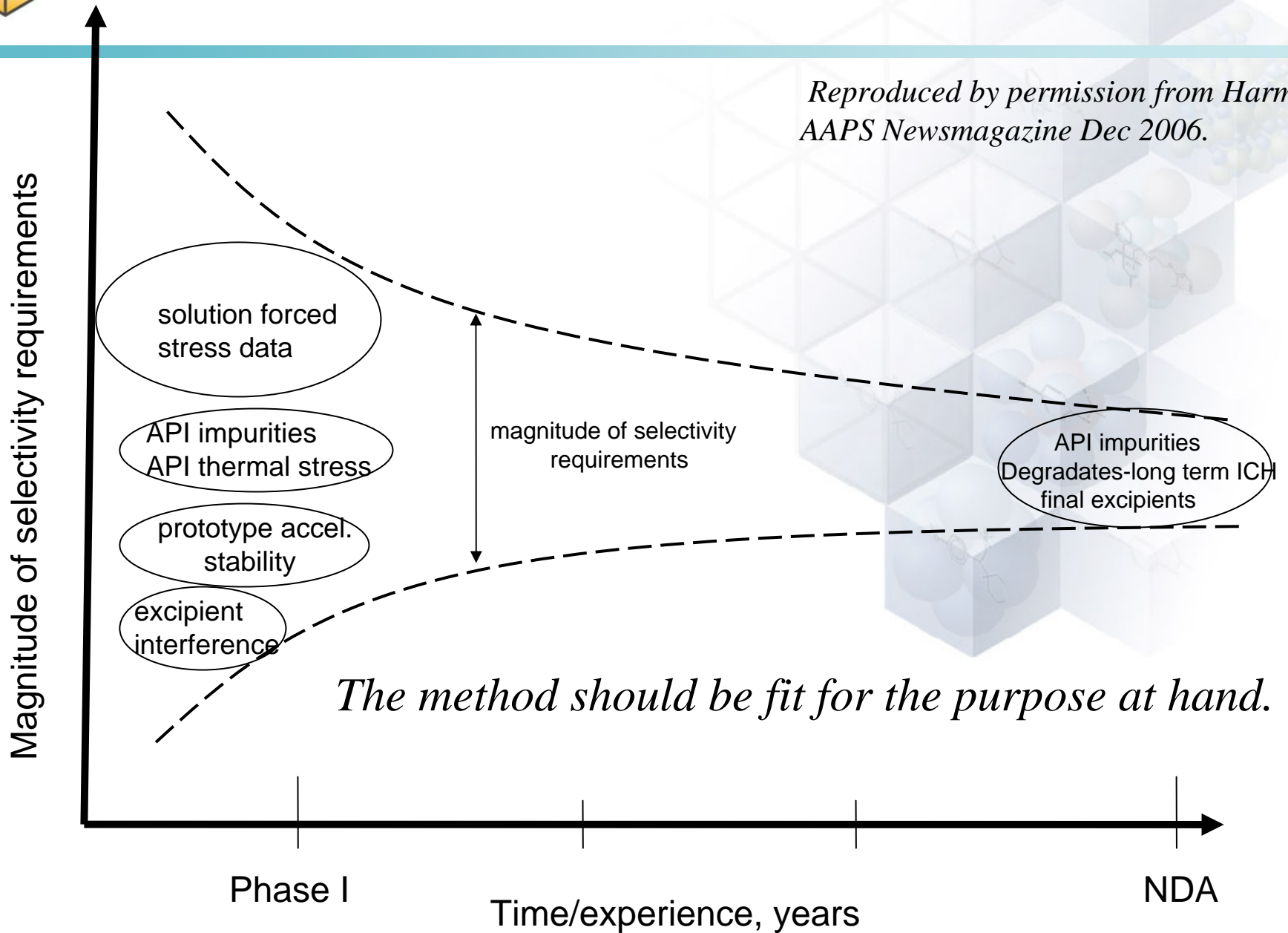


Important flavors of forced degradation that contribute to method development

- Drug Substance
 - Oxidation (H_2O_2 , AIBN, etc.)
 - Hydrolysis
 - Photostability
 - Temperature/Humidity
- Drug Product
 - Excipient compatibility
 - Temperature/Humidity
 - Photostability

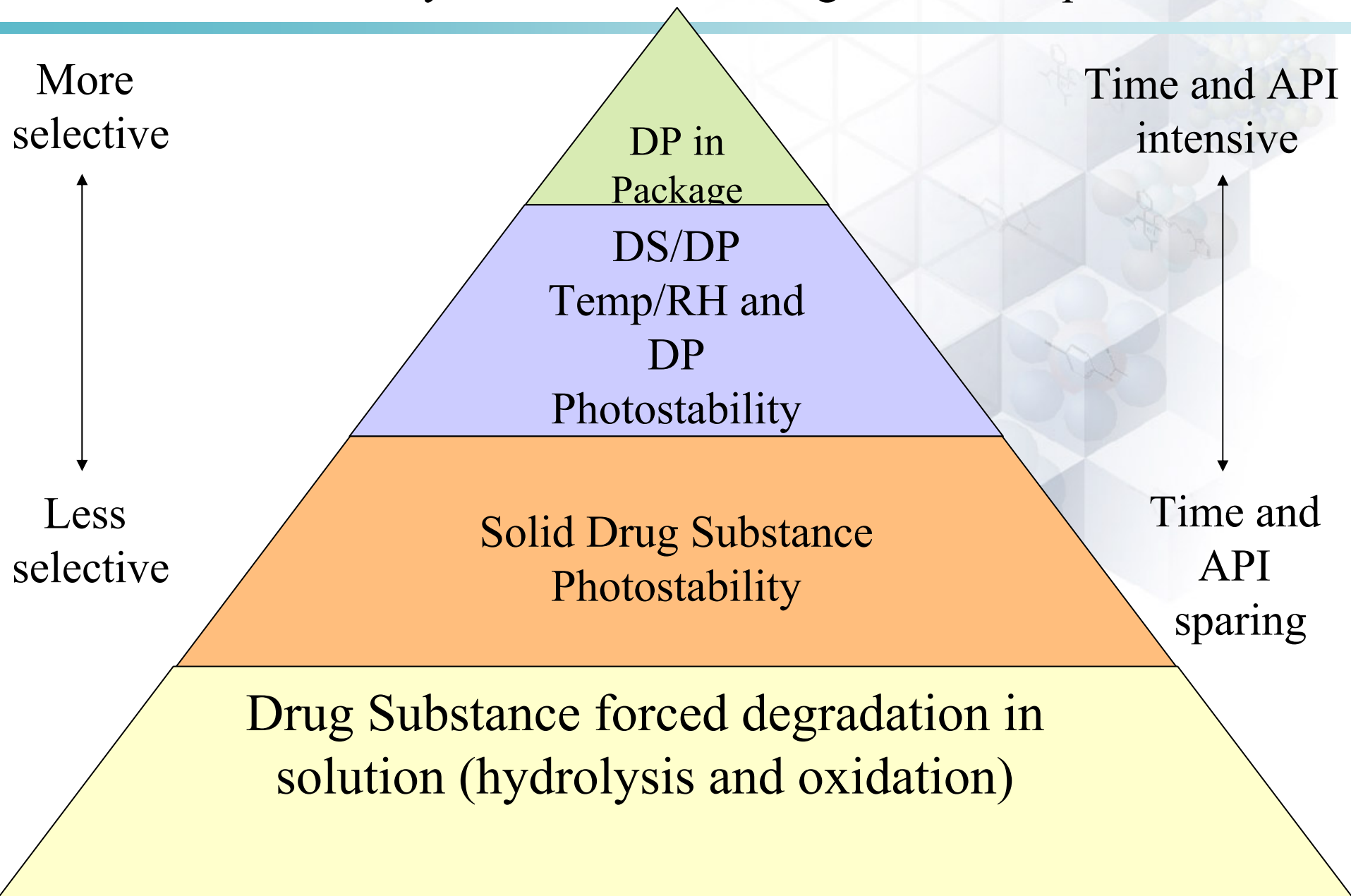


Selectivity Requirements verses Phase of Development





Some potential degradants can be ruled out based on further tiers of stability information allowing method simplification





Early method development

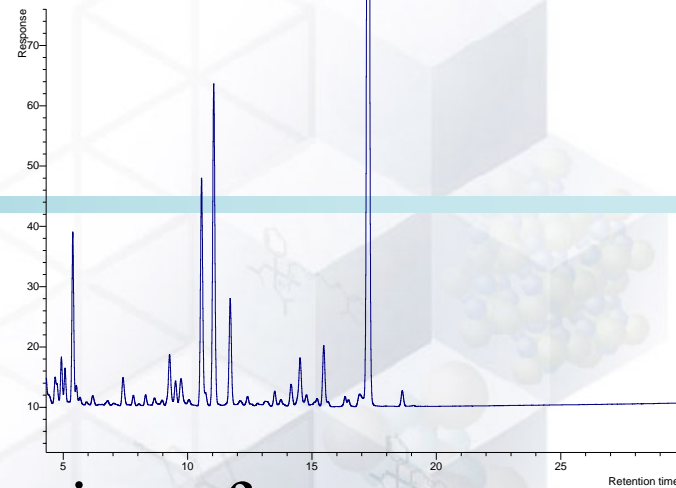
- A selective method is necessary ASAP
- Method development required prior to stability studies
- Method development parallel with formulation development
- API available but likely not abundant

Selectivity defined by drug substance forced degradation and excipient compatibility—likely many degradants to consider.



Method assumptions

- Gradient HPLC-UV
- Key separation challenge is separation of minor degradants from each other
- Variations of forced degradation methodologies produce differing degrees of degradation that range from easy to separate to migraine-inducing complexity.



Selection of degradants to be separated is the foundation of successful method development.



Key questions for early method development

- What is the intended dosage form, and what forced degradation best reflects this?
- Photostability: More representative for solid API or solution?
- Hydrolysis and Oxidation: How much is enough?
- Excipient compatibility: What excipients are relevant and *how processed*?
- What are the kinetics of degradation? Which are the *signal degradants*?



“Signal Degradants”

- Assumption: In most cases, primary degradants are much more important than secondary and tertiary degradants.
- Minor degradants seen in solution forced degradation screens are generally not the degradants that are later revealed to be important degradants in your product.
- “Signal degradants” are the major primary degradants formed in forced degradation experiments.
- Obsessing on very minor degradants causes unnecessary method development headaches.

Photostability for early method development

- Solution formulations: photostability of solutions is important and representative concentrations and compositions are desirable if possible.
- Solid formulations and API: Solution stress may significantly exceed the relevant level of stress, and lead to non-representative degradation.

“The purpose of forced degradation testing studies is to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation. This testing may involve the drug substance alone and/or in simple solutions/suspensions to validate the analytical procedures...For development and validation purposes it is appropriate to limit exposure and end the studies if extensive decomposition occurs. For photostable materials, studies may be terminated after an appropriate exposure level has been used.” from ICH Q1B



Excipient compatibility

- Compression is often important
- Representative “prototype formulations” can reveal nuances missing from binary compacts.
- Moisture is very important and can be underdone or overdone—
 - IMHO 40°C/75%RH is a good degree of humidity.
 - Wet vs Dry processing
- Excipient:Drug Ratios?
- The specifics trump the general—think about the science and adjust accordingly!



Hydrolysis: Easy does it

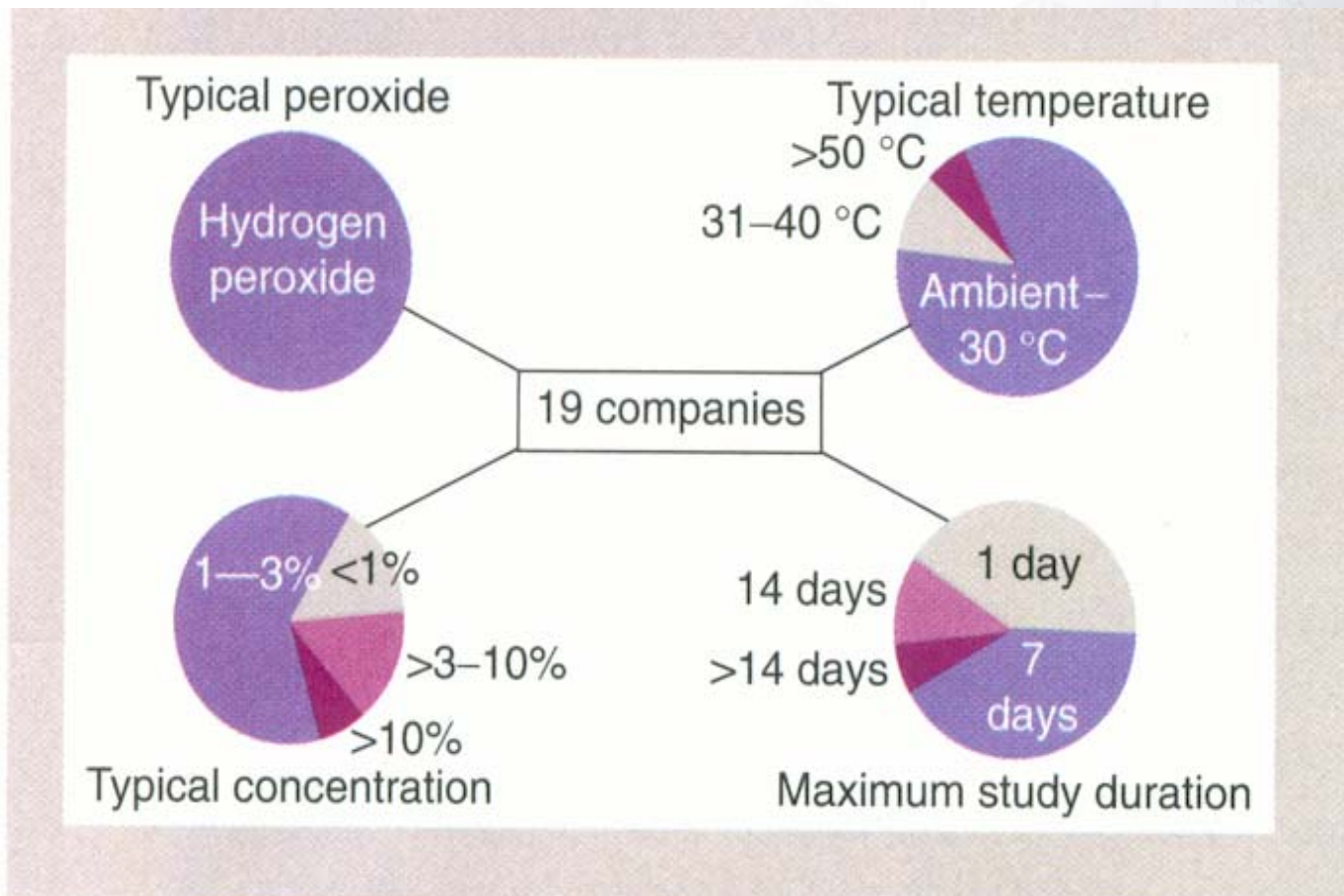
- Most acid and base catalyzed hydrolysis pathways of common drug molecules (e.g., amide/ester hydrolysis) go only slowly if at all in solid formulations.
- If a degradation mode has to be forced at high temperature in solution, is it really likely in the solid phase?
- It's very easy to force secondary and tertiary degradation in solution with acid/base catalysis.

Oxidation: understand the chemistry

- Recommended methods to force oxidation all have pitfalls and caveats—can lead to wildly unrepresentative degradation if not understood correctly.
- H_2O_2 comes with pitfalls of temperature and solvent effects
- AIBN/ACVA may form alkoxy radicals



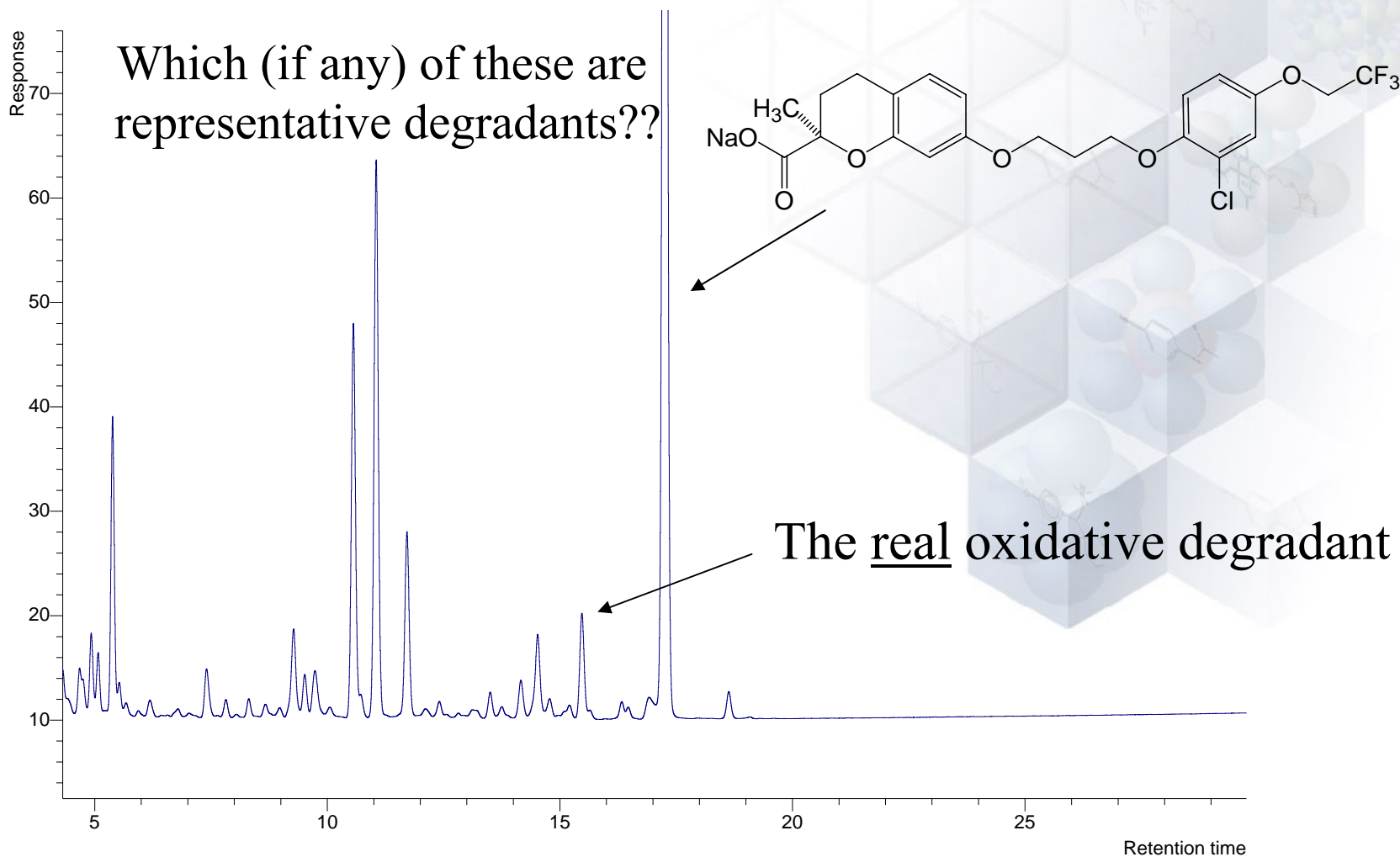
The Pernicious Pitfalls of Peroxide Practices



From K. M. Alsante, L. Martin, and S. W. Baertschi, "A *Stress Testing Benchmarking Study*", *Pharmaceutical Technology*, February 2003, 60-72.



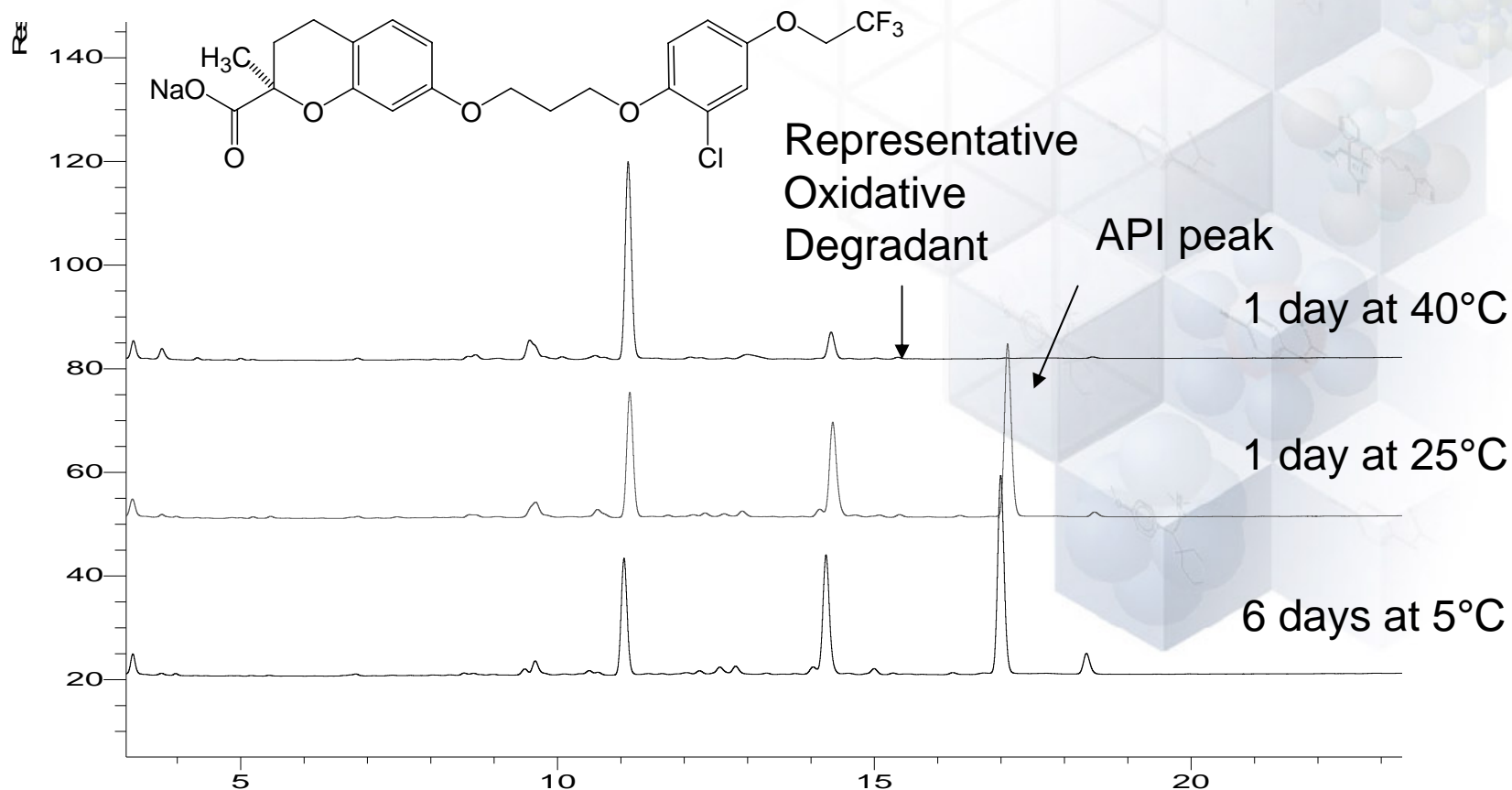
Heated H₂O₂ stress leads to bewildering diversity of products



Stress conditions: 2 days at 40°C in 1% H₂O₂(aq)



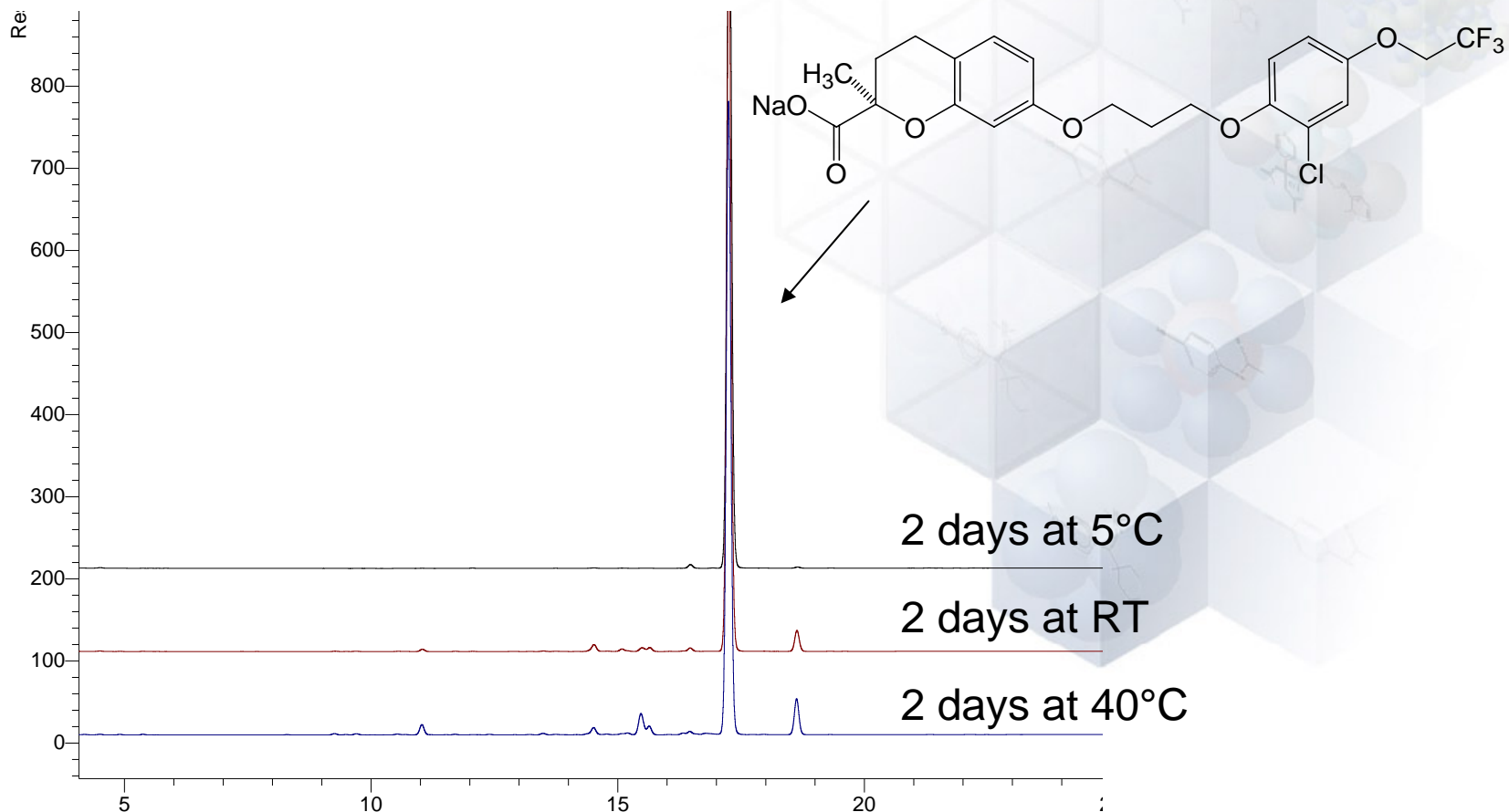
H₂O₂ and acetonitrile don't mix: Peroxycarboximidic acid formation



All solutions stressed in 50/50 acetonitrile/water with 1% H₂O₂



In methanol / water solution, the results are very different.



All solutions stressed in 50/50 methanol/water with 1% H₂O₂

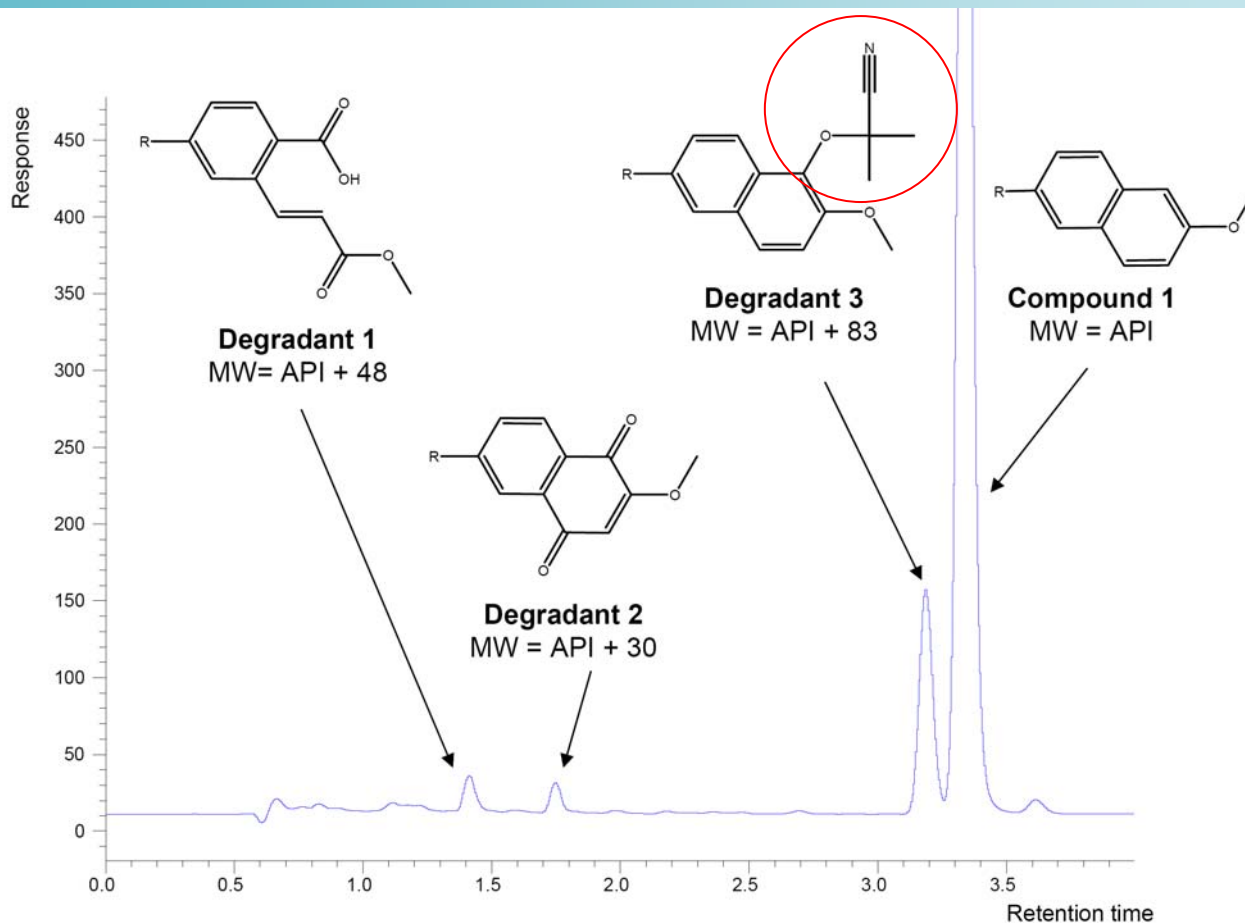


AIBN: RO• issues

- Under some experimental conditions (e.g., low drug concentration, unreactive drug and solvent) alkoxy radicals (RO•) can come to dominate the observed degradation.
- Adding a bit of methanol in a confirmatory stress experiment quenches RO• and rules out species dependent on this more aggressive oxidizing agent.

Nelson *et al.* submitted to *J. Pharm. Sci.*

RO• can actually become part of the observed reactivities in extreme cases



Degradation Artifacts. **Compound 1** at 0.05 mg/mL in 50/50 ACN/water diluent stressed for 3 day at 40°C in the presence of 5 mM AIBN radical initiator.

Figure adapted from “Revision to the AIBN Stress Testing Method: Identification and Elimination of Alkoxyradical-Mediated Drug Degradation Artifacts”, M. A. Watkins, P. A. Harmon, S. M. Pitzenberger, V. Van Nostrand, L. C. Bass, A. C. Templeton, article in preparation.



Validation

- The same forced degradation that was done to provide method development samples can provide validation of specificity—the data is already in your notebook!
- Remember that just like method development, validation is a phase-appropriate activity and also evolves.



Conclusions

- The biggest challenge of early method development is often deciding what to separate.
- Critical thought and understanding of the mechanisms and extent of forced degradation is part of the job.
- The investment of understanding your forced degradation will be greatly rewarded in the efficiency of your method development efforts.



Acknowledgements

• Paul Harmon  **MERCK**

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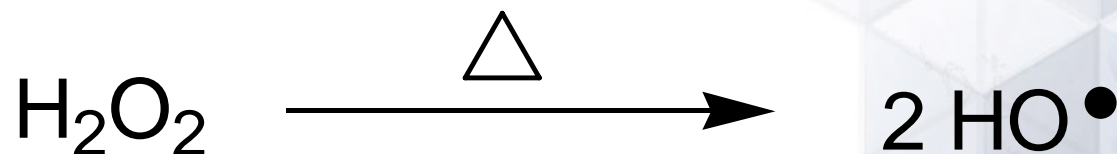
Bibliography of useful papers

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Heat and H₂O₂ don't mix

- Heating H₂O₂ solutions increases the homolytic cleavage of the HO-OH bond to form 2 HO•.



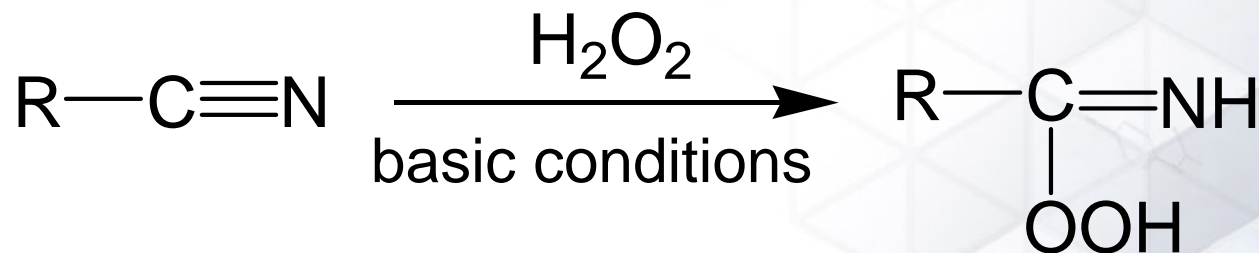
BDE (HO-H) = 120 kcal/mol !!

BDE (ROO-H) = ~89 kcal/mol

- Because HO• is undesirably harsh, it is advisable to conduct H₂O₂ stress \leq room temperature.
- The presence of cosolvents (*e.g.*, MeOH) help to quench undesired HO• to ROO•.



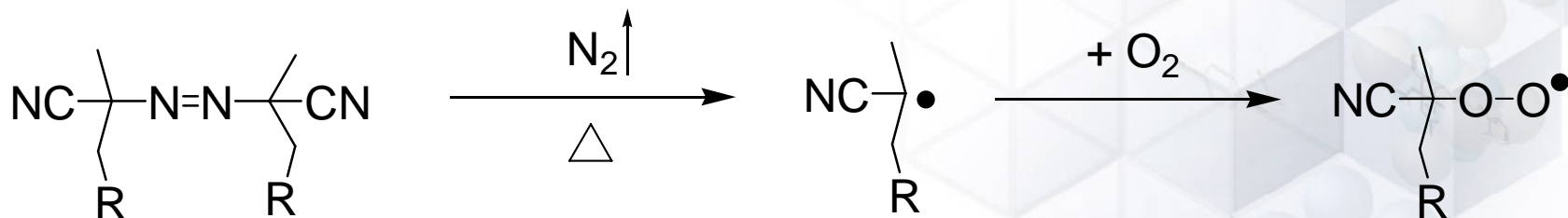
A caution about peroxycarboximidic acid formation in acetonitrile



- H_2O_2 stress in basic acetonitrile solutions may well induce peroxycarboximidic acid formation.
- The reaction can be catalyzed by base, and buffered solutions or basic APIs can increase the peroxycarboximidic acid formation.
- The peroxycarboximidic acid has activated hydroxylation reactivity not representative of HOOH or ROOH .



Azonitrile radical initiators are another convenient source of peroxy radicals.



AIBN: R= H

ACVA: R= CH₂COOH

- Oxygen quickly adds to the primary cyanoalkyl radicals to produce peroxy radicals.
- Ambient air provides sufficient oxygen for most purposes.