Apply Quality by Design (QbD) Principles in Analytical Method Development

Kangping Xiao

The opinions and conclusions expressed in this presentation are solely the views of the author and do not necessarily reflect those of the Bayer Healthcare.
About me

Wuhan University

東京大学
The University of Tokyo

MICHIGAN STATE UNIVERSITY

MICHIGAN
Molecular Recognition @ The University of Tokyo

Hyperbranched Polymers on Porous Surfaces @ Michigan State University

Drug Release from Coronary Artery Stents @ The University of Michigan
Global Quality Services – Analytical Sciences @ Schering Plough

New Product Development @ McNeil Consumer Care, Johnson & Johnson

Global New Product Development @ Bayer Healthcare
Overview of QbD Principles and Approaches Applied to Analytical Method Development

---- Regulatory Guidance

• ICH Q8 – Pharmaceutical Development
• ICH Q9 – Quality Risk Management
• ICH Q10 – Pharmaceutical Quality System
• ICH Q11 – Development and Manufacture of Drug Substances

Systematic approach that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.

The goal is to embed quality into pharmaceutical products to ultimately protect patient safety.
ICHQ8(R2) does not explicitly discuss analytical method development. However, same concepts apply.

Benefits include:
• Development of a robust method
• Understand, reduce and control sources of variability
• Applicable throughout the life cycle of the method
  – Regulatory flexibility: Movements within “Analytical Design Space” are not considered a change in method

Current Status:
• FDA has approved some NDA applications applying QbD approach to analytical methods (e.g. HPLC and UV)
• Regulatory flexibility has been granted for movements within the defined analytical method design space/ “MODR” (Method Operable Design Region)
Regulatory Guidance, continued

Analytical Target Profile (A TP) – The concept of an ATP parallels the concept of a Quality Target Product Profile described and defined in ICH Q8. The ATP defines the objectives of the test and quality requirements, including the expected level of confidence, for the reportable result that allows the correct conclusion to be drawn regarding the attributes of the material that is being measured.

Risk Management – Quality Risk Management (QRM) for analytical procedures can be defined as a systematic process for the assessment, control, communication, and review of risks to the quality of data across the product lifecycle. Process mapping, Ishakawa (fishbone) diagrams can be employed to ensure a rigorous approach is used in identifying all potential variables that may affect data quality. The variables should include all aspects of the full analytical procedure, i.e., sampling, sample preparation, standards, reagents, facility, and equipment operating conditions.
Knowledge Management – The knowledge gathered to develop the method understanding should be collected in a repository and shared as needed to support implementation of the control strategy across sites that use the analytical procedure. Changes and improvements to an analytical procure should be made with reference to the method knowledge repository, which contains the information from the various stages of the method lifecycle.

Analytical Control Strategy – The variables and their acceptable ranges (from the risk assessment or experimental work) should be explicitly specified in the procedure.

Lifecycle Stages: a three-stage concept that is aligned with current process validation terminology: Stage 1 – Procedure Design (development and understanding); Stage 2 – Procedure Performance Qualification; Stage 3 – Continued Procedure Performance Verification.
Regulatory Guidance, continued

Lifecycle Stage 3 includes:

**Routine Monitoring**, three action triggers: special –cause variation (e.g., new, unexpected phenomenon); unacceptable common cause variation (e.g., expected variability inherent in the procedure); continual improvement.

**Observed Variations**, A variable was not identified or adequately studied during the procedure understanding study (Stage 1), and therefore no proper control was defined; A variable was not identified or adequately studied during Stage 2 (precision study), and therefore no proper control was defined; Series member of a set of categorical variables (not included in the DoE, Stage 1) has been found to have an effect in performance (e.g., a new batch of column packing results in unacceptable performance); A control strategy was defined but not followed; A noise variable has been found to have an impact on routine performance.

**Continual Improvement**, inclusion of an additional control, introducing a new method or technology, changing the intended purpose to incorporate a new impurity or tighten specifications, or alignment with a procedure in a compendial monograph that has been updated. The nature of the change dictates the action that should be taken and a risk assessment should be performed to identify what action is required, and the change should be documented.
Approaches Discussed in Literature, Example

Reversed-phase method development workflow

Wave 0: Plot Log D vs pH for known Compounds

pH range to evaluate

Wave 1: Stationary Phase Solvent, pH, Organic Screen

Set column and organic

Wave 2: Narrow pH Screen

Set pH

Wave 3: Temperature and Gradient Screen

Draft conditions

<table>
<thead>
<tr>
<th>100mm x 2.1mm</th>
<th>0.1% TFA pH 2</th>
<th>0.1% formic acid pH 2.5</th>
<th>10 mM NH₄OAc pH 6.5</th>
<th>0.1% NH₃OH pH 10.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>MeOH</td>
<td>AN</td>
<td>MeOH</td>
<td>AN</td>
</tr>
<tr>
<td>Column 1 (1.7 μm)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Column 2 (1.7 μm)</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Column 3 (1.7 μm)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Column 4 (1.8 μm)</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Wave 2 is a 5-point experiment where the pH or additive concentration of the mobile phase is systematically varied around the pH (±0.5 and ±1.0 pH unit, and set point) or additive concentration (range 0.05 - 0.20%, set point 0.10%) determined from wave 1.

Wave 3 is a six-point experiment where gradient steepness (15 and 45 minute gradient time) and temperature (30°C, 45°C and 60°C) are systematically varied.

Reversed-phase method development workflow
Efficient Method Development Strategy When You Know What You Are Working on

Questions

Have you been taking the approaches from what you can find through literature search?

Are you a designer?

Do you design for the sake of designing?

What if you are in a place that does not have all the time you need, to carry out the QbD for one method?
Analytical Support during Product Development

Product development in the Over-the-Counter (OTC) industry faces constant changes as this business is largely driven by consumer desires, marketing, regulatory, packaging innovations, and manufacturing capability, etc.

Formulation or even process can change at the last minute!

The analytical method development is in parallel with the formulation development!

Product development cycle can be very short, let alone analytical method development cycle.
Modernization of Analytical Methods

- Does not always mean the methods get short and sweet.

- The requirements to a modern method are much higher than before.

- Monitoring potential degradation products has been becoming a must in methods intended for regulatory filings.

- Although the molecules in Over-The-Counter (OTC) medicines are usually very “old”, new degradation products can be generated when new formulations are attempted.
### Impurities Assessment of OTC Monograph Products: Regulatory Challenges

<table>
<thead>
<tr>
<th>OTC Monograph Drug</th>
<th>USP DP Impurity Testing Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>B, C</td>
</tr>
<tr>
<td>Aspirin</td>
<td>B</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>C</td>
</tr>
<tr>
<td>Brompheniramine</td>
<td>C</td>
</tr>
<tr>
<td>Guaifenesin</td>
<td>C</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>C</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>C</td>
</tr>
<tr>
<td>Methyl Salicylate</td>
<td>C</td>
</tr>
<tr>
<td>Menthol</td>
<td>C</td>
</tr>
<tr>
<td>Bisacodyl</td>
<td>C</td>
</tr>
</tbody>
</table>

A. USP Impurity Testing Protocol Adequate for Assessment of OTC Monograph Products  
B. Inadequate  
C. Protocol does not exist

M. Scott Furness, Ph.D., Director Division of Nonprescription Regulation Development Office of Nonprescription Products Center for Drug Evaluation and Research United States Food and Drug Administration Department of Health and Human Services
New Requirements for an Analytical Scientist

- Modernization of analytical methodology for OTC medicines is a regulatory push rather than an voluntary industry action.
- In March 2014, FDA held a public hearing for “Over the Counter Drug Monograph System – Past, Present and Future”.
- FDA proposes that OTC drugs should be subject to the same safety and efficacy standards as other drugs based on risk versus benefit.
- USP has already received a list of OTCs from the FDA prioritized for modernization to address missing or outdated tests for impurities.
- The diversity in OTC formulations, such as various colors, flavors, functional excipients, and combination products with multiple active pharmaceutical ingredients (APIs), poses extreme complexities in developing analytical procedures.
- A “modern” analytical scientist must understand the chemistry among all the compounds in one formula and prepare the method accordingly.
What Are Needed from Analytical?

Pre-formulation stage:
Goal of product development --- identification of possible failure in future development
Goal of analytical support --- achieve maximum information within the shortest period of time

Formulation stage:
Goal of product development and analytical support --- ensure each batch manufactured meets the specifications for identity, strength, quality, and purity

An analytical chemist must be able to provide chemistry knowledge, not just the knowledge of separation science, definitely not just data.
# Quality Attributes in Analytical Support for Product Development

<table>
<thead>
<tr>
<th>Goal of Pre-formulation</th>
<th>Analytical Quality Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go or No Go</td>
<td>• Timely results</td>
</tr>
<tr>
<td></td>
<td>• Insightful</td>
</tr>
<tr>
<td></td>
<td>• Client oriented</td>
</tr>
</tbody>
</table>

## What if no existing method

<table>
<thead>
<tr>
<th>Development platform methods (beforehand or behind the scene) that are versatile for multiple projects / products.</th>
<th>Be flexible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use placebo as control</td>
<td></td>
</tr>
<tr>
<td>Subtract placebo peaks</td>
<td></td>
</tr>
<tr>
<td>Use different wavelengths to minimize interference</td>
<td></td>
</tr>
<tr>
<td>Use multiple methods</td>
<td></td>
</tr>
<tr>
<td>Apply less system suitability requirements</td>
<td></td>
</tr>
<tr>
<td>Not focusing on peaks &lt; 0.05%, or 0.1% at this stage</td>
<td></td>
</tr>
<tr>
<td>Modify the method behind the scene, by a second analyst if necessary</td>
<td></td>
</tr>
<tr>
<td>Perform forced degradation, excipient compatibility studies, etc., behind the scene, to build database / treasure box.</td>
<td></td>
</tr>
</tbody>
</table>
## Quality Attributes in Analytical Support for Product Development

<table>
<thead>
<tr>
<th>Goal of Formulation</th>
<th>Analytical Quality Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability</td>
<td>• Timely results</td>
</tr>
<tr>
<td>Consistent Performance</td>
<td>• Accuracy</td>
</tr>
<tr>
<td></td>
<td>• Trend indicating</td>
</tr>
<tr>
<td></td>
<td>• Identify unknown peaks</td>
</tr>
<tr>
<td></td>
<td>• Client oriented</td>
</tr>
</tbody>
</table>

### What if the method is not “perfect”

- Apply the flexibility
- Modify the method behind the scene, by a second analyst if necessary
- Make sure bridging the results obtained from “old” and “new” methods
- Apply forced degradation to prepare for more difficult sample extraction, instead of waiting for 3 months or 6 months
Think QbD Beyond Developing One Method

- The numbers of the molecules used as the active pharmaceutical ingredients in OTC products are limited. Extensive studies on the properties of the molecules are possible and/or available.

- Build knowledge space – from Organic Chemistry to Analytical Chemistry, and even Physical Chemistry (super individual or a team work).

- Build database – whenever possible, conduct API forced degradation, API-Excipient and API-API compatibility studies and keep the knowledge in a central place.

- Keep up with new technology – including column technology, chromatographic technology, and new software development
Building Knowledge Space – Degradation Chemistry

Common degradation pathways of a drug molecule

- **Hydrolytic:** *high humidity and solution stressing*
- **Thermolytic:** *heat/humidity*
- **Photolytic:** *exposure to appropriate light source(s)*
- **Oxidative:** *oxidative degradation can be complex*

Common functional groups to watch out

- Carboxylic group
- Amine group
- Hydroxyl group

Common “culprits” in excipients

- *Peroxide*
- *Aldehyde*
- *Trace metals*
Degradation Chemistry – Autoxidation

- Oxidation is loss of electrons from a molecule. In other words, it is a gain in O or a loss of H.
- Autoxidation of a drug molecule occurs by an initial abstraction of a labile hydrogen atom followed by reaction with molecular oxygen.
  - Therefore, oxidative degradation should be linked to the liability of hydrogen atoms within the molecular framework.
  - As a rule of thumb, the more stable the resulting radical, the easier for the hydrogen atom to be abstracted from the corresponding position of the molecule.

\[
\text{ROOH} + \text{Fe}^{3+} \rightarrow \text{ROO}^\cdot + \text{Fe}^{2+} + \text{H}^+
\]
Degradation Chemistry – N-Oxide Formation

Formed by oxidation of tertiary amines by Peroxide

Common peroxide containing excipients
• Povidone; Tween 80; PEG;

- The nitrogen atom acts as nucleophile in this reaction by attacking an oxygen atom of the peroxide to form amine oxide.

- So keep the electron lone pair on N busy by protonation, i.e., maintain a low local pH microenvironment. Wet-n-Dry process can to some degree remove the counter acid such as HCl, and make the N liable to oxidation during storage.
Degradation Chemistry – Esterification

- Carboxylic acid group containing compounds reacting with sugars or sugar alcohols, R.
  \[ R = \text{Mannitol, Lactose, Dextrose, Isomalt, Sorbitol, etc.} \]

- Esterification can happen between API-Excipient or API-API.

- Esterification can also happen between API and sample solvent such as methanol.
Degradation Chemistry – Aldehyde, Amine, Amide

Primary and secondary amines are well known to react with formaldehydes and formic acid

\[
\text{R}\text{NH}_2 + \overset{\text{H}}{\overset{\text{C}}{\overset{\text{O}}{\text{H}}}} \rightleftharpoons \text{RHN} = \overset{\text{C}}{\overset{\text{H}}{\text{H}}} + \text{H}_2\text{O} \rightleftharpoons \overset{\text{RN}}{\overset{\text{C}}{\text{H}}} \rightleftharpoons \overset{\text{R}}{\overset{\text{N}}{\overset{\text{C}}{\text{H}}}} + \overset{\text{H}_3\text{O}^+}{\text{O}}
\]

iminium ion

\[
\text{R}_1\text{NH} + \overset{\text{H}}{\overset{\text{C}}{\overset{\text{O}}{\text{H}}}} \rightleftharpoons \overset{\text{R}_2\text{R}_1}{\overset{\text{N}}{\overset{\text{C}}{\text{H}}}} + \overset{\text{HO}}{\overset{\text{C}}{\text{O}}}
\]

imine

If \( R_2 \) is H, then two methyl groups will be added, i.e., \( \text{R}_1\text{N}(\text{CH}_3)_2 \)

Formaldehyde is present as a trace impurity in materials containing polyoxyethylene chains, such as poly(ethylene terephthalate) for plastic bottles and various surfactants such as polyethylene glycols and polysorbate 80, povidone, croscamemellose sodium, colors, flavors, etc.

*Chem. Pharm. Bull. 57(10) 1096—1099 (2009)*
Degradation Chemistry – Aldehyde, Amine, Amide

Example of formaldehyde reacting with phenylephrine, a Pictet Spengler reaction:

\[
\text{HO-CH}_2\text{NH-CH}_2\text{OH} + \text{CHO} \rightarrow \text{HO-CH}_2\text{NH-CH}(-\text{CHO})_\text{OH} \rightarrow \text{HO-CH}_2\text{NH-CH}(-\text{NCO})_\text{OH} + \text{H}^+ 
\]

4,6 - isoquinoline

4,8 - isoquinoline
Degradation Chemistry – Aldehyde, Amine, Amide

Carboxylic acid and esters can react with amines to form amides:

\[
\text{R}_1\text{C}\text{OH} + \text{R}_2\text{NHR}_3 \xleftrightarrow{\text{Amide hydrolysis}} \text{R}_1\text{C}-\text{N} \text{R}_2\text{R}_3
\]

Amide hydrolysis reverse the reaction. Example, acetaminophen hydrolysis by strong acids forms PAP.

Acetaminophen \[\rightarrow\] 4-aminophenol (PAP)
Degradation Chemistry – TransEsterification

Prostaglandins are a family of molecules that promote a wide range of biological processes, including inflammation. Acetylsalicylic acid, commonly known as aspirin, acts by transferring - through a transesterification reaction - an acetyl group to a serine residue on the enzyme responsible for the biosynthesis of prostaglandin H$_2$ (one member of the prostaglandin family).

Acetylation of the serine blocks a channel leading to the active site, effectively shutting down the enzyme, impeding prostaglandin production, and inhibiting the inflammation process that causes headaches.

http://chemwiki.ucdavis.edu/Organic_Chemistry
Degradation Chemistry – Total Moisture and Free Moisture/Water Activity

- Loss on drying (LOD) or total moisture content of pharmaceutical products can include both bound (e.g. water of hydration) and free water.

- It is the **free water** that is responsible for degradation of moisture sensitive materials resulting in poor stability profiles.

- Humidity sensitivity is dominated by physical effect such as plasticization rather than any direct reaction with water. Physical changes include polymorphic transition, hydrate/solvate formation, dehydration/desolvation, crystallization of amorphous material, vaporization, etc.

- The role of moisture in causing physical changes are related to water activity of the system rather than the moisture content of either the dosage form or the surrounding air. Drug product water content will directly correlate with the degradation rate only when the water content correlates directly with the water activity.

- Free Moisture: moisture free to interact with the surroundings, can be quantified by determining the water activity (aw), which is a comparison of a %RH generated by a material compared to that of pure water.

- Bound Moisture: moisture that is not freely available because it is bound or absorbed onto another material (e.g. a hydrate or absorbed moisture on very hygroscopic material). Therefore sometimes it is counter intuitive that more hygroscopic material stabilizes the product. For example, Starch has a high LOD/total moisture but has low water activity; Cyclodextrin has a high LOD and high water activity.
Degradation Chemistry – Excipients and Packaging

- Excipients are defined as inert ingredients, which is contradictory to their actual functions.
- Excipient is always a mix of the claimed entity and other components and impurities.
- Excipients can be a source for extra moisture and alter the pH of the moisture layer.
- Low drug excipient ratio = higher reaction risk

- Packaging: a WVTR = 0.12 g mm/(m² day) (e.g., HDPE), and the initial moisture content in the bottle is zero, the relative humidity inside the bottle will rise to over 50% (in a 60cc bottle) within about ONE DAY.
- Not only WVTR but also OVTR should be taken into consideration.
- HDPE is a better moisture barrier than PVC, while a poor barrier for Oxygen.
Building Knowledge Space – Sample Preparation

- Different from prescription medicinal products, the compositions of over-the-counter (OTC) drug products tend to be complicated due to the presence of a variety of ingredients (multiple drug substances, inert but functional excipients, flavors, dyes, etc.).

- Many excipients used in OTC products are intended to achieve fast onset effects, provide pleasant experiences, and improve consumer compliance. However, the added “inert” ingredients not only can sometimes cause product instability, but also give big challenges to analytical scientists.
Sample Preparation – Placebo Impact and Instrument Limitations

- A common practice for HPLC quantitation of pharmaceutical products is to measure a sample response against an external standard response.

- For routine analysis, the standard solution is usually prepared by using solutions that do not contain a sample matrix, i.e., the placebo of the product.

- The assumption is that the sample matrix does not cause any difference in the responses between the analyte in the sample and the external standard solutions.

- For this assumption to hold true, the sample matrix and chromatographic instrumentation play very important roles.
Sample Preparation – Placebo Impact and Instrument Limitations, *Example*

- Differences between placebo concentrations, autosampler temperatures, injector sample withdrawing speeds, and injection volumes on Agilent HPLC, Waters Alliance HPLC, and Waters Acquity UPLC were investigated in one study.

- In extreme cases, a sample response can be 90% less than the standard response, which obviously causes attention.

- But in some cases, the difference in responses is less than 4% and maybe overlooked.

- Product development endeavors can be wrongly focused on product stability, content uniformity, etc., without knowing that the undesired analytical recoveries are actually caused by the method which overlooks the sample matrix effect or does not take into account the delicate nature of chromatographic instrumentation.
The peak distortion in 10x placebo-API injection (black line) is very obvious since it is chosen for illustration purposes. In some cases, the peak distortion is not quite as obvious and might mislead the chromatographer to believe there is some “co-elution” resulting in efforts to “improve” the “separation”.

Overlaid chromatogram of a 10x placebo-API (bottom black line), a 1x placebo-API (middle blue line), and an API standard (top red line) injections. The injections were made on a Waters 2695 Alliance HPLC.
Sample Preparation – Peak Shape Does Not Necessarily Tell the Truth

Overlaid chromatogram of a 10x placebo-API (bottom black line), a 1x placebo-API (top blue line), and an API standard (middle red line) injections. The injections were made on a Waters Acquity UPLC (0.3 mL injection, 104% recovery for 10x placebo-API and 99.8% recovery for 1x placebo-API).
### Sample Preparation – Impacts on Different Instruments

<table>
<thead>
<tr>
<th>10x Placebo-API in Water : Methanol</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>10 µL</td>
</tr>
<tr>
<td></td>
<td>15 µL</td>
</tr>
<tr>
<td>Fast</td>
<td>104.1%</td>
</tr>
<tr>
<td>Normal</td>
<td>105.3%</td>
</tr>
<tr>
<td>Slow</td>
<td>106.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1x Placebo-API in Water : Methanol</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>10 µL</td>
</tr>
<tr>
<td></td>
<td>15 µL</td>
</tr>
<tr>
<td>Fast</td>
<td>100.7%</td>
</tr>
<tr>
<td>Normal</td>
<td>100.9%</td>
</tr>
<tr>
<td>Slow</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10x Placebo-API in Water : Acetonitrile</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>10 µL</td>
</tr>
<tr>
<td></td>
<td>15 µL</td>
</tr>
<tr>
<td>Normal</td>
<td>103.4%</td>
</tr>
</tbody>
</table>

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<th>1x Placebo-API in Water : Acetonitrile</th>
<th>Injection volume</th>
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<tr>
<td>Syringe Withdraw Speed</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>10 µL</td>
</tr>
<tr>
<td></td>
<td>15 µL</td>
</tr>
<tr>
<td>Normal</td>
<td>100.8%</td>
</tr>
</tbody>
</table>

Recoveries of spiked API in 10x placebo and 1x placebo samples on a Waters Alliance HPLC. Autosampler temperature 4°C
## Sample Preparation – Impacts on Different Instruments

<table>
<thead>
<tr>
<th>10x Placebo-API in Water : Methanol</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Syringe Withdraw Speed</strong></td>
<td>5 µL</td>
</tr>
<tr>
<td>1000 µL/minute</td>
<td>6.3%</td>
</tr>
<tr>
<td>200 µL/minute (Default)</td>
<td>17.6%</td>
</tr>
<tr>
<td>10 µL/minute</td>
<td>107.8%</td>
</tr>
</tbody>
</table>

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</tbody>
</table>

<table>
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<tr>
<th>10x Placebo-API in Water : ACN</th>
<th>Injection volume</th>
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<tbody>
<tr>
<td><strong>Syringe Withdraw Speed</strong></td>
<td>5 µL</td>
</tr>
<tr>
<td>200 µL/minute (Default)</td>
<td>101.0%</td>
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</table>

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<th>1x Placebo-API in Water : ACN</th>
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<tr>
<td><strong>Syringe Withdraw Speed</strong></td>
<td>5 µL</td>
</tr>
<tr>
<td>200 µL/minute (Default)</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Recoveries of spiked API in 10x placebo and 1x placebo samples on an Agilent 1260 HPLC. Autosampler temperature 4°C
### Sample Preparation – Impacts on Different Instruments

<table>
<thead>
<tr>
<th>10x Placebo-API in Water : Methanol</th>
<th>Injection volume (autosampler at 4(^\circ)C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>100 µL/minute (Default)</td>
<td>97.7%</td>
</tr>
<tr>
<td>20 µL/minute</td>
<td>103.9%</td>
</tr>
<tr>
<td>50 µL/minute</td>
<td>103.2%</td>
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</tbody>
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<thead>
<tr>
<th>1x Placebo-API in Water : Methanol</th>
<th>Injection volume (autosampler at 4(^\circ)C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>100 µL/minute (Default)</td>
<td>100.9%</td>
</tr>
<tr>
<td>20 µL/minute</td>
<td>99.8%</td>
</tr>
<tr>
<td>50 µL/minute</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10x Placebo-API in Water : Methanol</th>
<th>Injection volume (autosampler at 25(^\circ)C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>100 µL/minute (Default)</td>
<td>106.5%</td>
</tr>
<tr>
<td>20 µL/minute</td>
<td>103.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1x Placebo-API in Water : Methanol</th>
<th>Injection volume (autosampler at 25(^\circ)C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Withdraw Speed</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>100 µL/minute (Default)</td>
<td>101.7%</td>
</tr>
<tr>
<td>20 µL/minute</td>
<td>100.2%</td>
</tr>
</tbody>
</table>

Recoveries of spiked API in 10x placebo and 1x placebo samples on a Waters Acquity UPLC. Autosampler temperature 4\(^\circ\)C and 25\(^\circ\)C

J. Li Dec 2012 American Pharmaceutical Review, v.15 issue 7
## Sample Preparation – Impacts on Different Instruments

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Injection volume</th>
<th>0.5 μL</th>
<th>1.0 μL</th>
<th>1.5 μL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10x Placebo-API in Water : Acetonitrile</strong></td>
<td>Syringe Withdraw Speed at 100 μL/minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 μL</td>
<td>1.0 μL</td>
<td>1.5 μL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>104.9%</td>
<td>92.6%</td>
<td>111.8%</td>
</tr>
<tr>
<td><strong>1x Placebo-API in Water : Acetonitrile</strong></td>
<td>Syringe Withdraw Speed at 100 μL/minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 μL</td>
<td>1.0 μL</td>
<td>1.5 μL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>114.7%</td>
<td>95.6%</td>
<td>102.1%</td>
</tr>
<tr>
<td><strong>0.1x Placebo-API in Water : Acetonitrile</strong></td>
<td>Syringe Withdraw Speed at 100 μL/minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 μL</td>
<td>1.0 μL</td>
<td>1.5 μL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.4%</td>
<td>99.9%</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

Recoveries of spiked API in 10x placebo, 1x placebo, and 0.1x placebo samples on a Waters Acquity UPLC. Autosampler temperature 4°C.
Based on the ICH / FDA guidelines:

- Forced degradation / stress testing is a study carried out to determine the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedures used.

- Forced degradation can be performed on drug substances and the final products to examine the effects of temperatures, humidity, oxidation, hydrolysis, and photolysis.

- Forced degradation is not the accelerated stability testing, such as the formal stability study.
Forced Degradation – Challenges

- With invasive forced degradation, such as mixing the finished products with acid, base, or oxidants, not only little degradation kinetic information can be obtained, but also irrelevant degradation products may form.

- The obtained information may not reflect real life chemistry and may not sound relevant to the formulators.

- For prediction purpose, Arrhenius equation is the only well accepted model, while Arrhenius theory requires that the kinetic models for degradation are the same for all the temperatures.

- In reality, multiple mechanisms with different rate constants and activation energies often co-exist, which render the Arrhenius equation not quite useful.

- Even if the kinetics is simple, to make the extrapolation from Arrhenius equation meaningful, the experimental results have to be accurate, which sometimes is difficult due to small/slow degradation.
“put-it-up-on-stability-for-3-months”
And
“let’s do it at 25/60, 30/65, 40/75”
And
“package it in blisters, pouches, 20ct bottles, 40ct bottles, 80ct bottles”
And
“there are ten formulas”
And
“actually let’s put them up on stability for 2 years just in case”

Excipient compatibility

Formulation trials-and-errors to find lead prototypes

Various packaging configurations for the lead prototypes

Final Formulation in final packaging configurations for long term stability

40°C/75%RH
3~6 months
several rounds of iterations

40°C/75%RH
3~6 months
Forced Degradation – Objectives

- Forced degradation at the early stage of the product development is to generate knowledge space for both formulation and analytical method development.

- Mild to harsh approaches should be applied to the API molecule of interest.

- Smart approaches should be carried out for API-excipient compatibility studies, since it requires a lot of resources and it is direction indicating.

- When pursuing stability-indicating power of the analytical procedures on finished products, formulation relevancy is the key.

- Early read on impacts of product aging on sample extraction is critical.
Forced Degradation – Excipient Compatibility

- Generate impurities for method development.

- It is not to say the excipient is not compatible, it is to say use with caution.

- It has to be formula and/or process relevant

Forced Degradation – Finished Products

- Direct contact of the products with acid or base is less meaningful. Non-invasive/non-destructive approach is preferred.

- To assess the effect of heat and humidity, create controlled relative humidity by using saturated salt solutions.

- Data generated is used qualitatively and for comparison and directional purpose. Therefore, minimum product samples can be used which not only reduce the sample sizes, but also reduce the costs/resources used in the studies.

- Ovens can be used but the temperatures should not exceed 80°C, preferably below 70°C.
Forced Degradation – Finished Products

- A final check for stability-indicating power of the analytical procedures. It would be too late to develop a method based on the final product.

- Forced degradation on the finished products can help evaluate different formulations at their last step, and to select most suitable packaging materials. This is especially useful prior to or during the informal stability studies.

- Quick assessment on the long term drug product stability based on experiments carried out within a truly short period of time, such as less than 10 days or at most 2 weeks.

- The prediction can be performed based on comparison of the new product to the historical data of some similar products (which may readily available for over-the-counter medicines).
Forced Degradation on Finished Products – Experimental Setup

- Grease sealed
- Tablets in Petri Dishes
- Saturated Salt solution

Data analysis
Examples of Some Saturated Salt Solutions and Relative Humidity

<table>
<thead>
<tr>
<th>Salt</th>
<th>25°C %RH</th>
<th>30°C %RH</th>
<th>40°C %RH</th>
<th>50°C %RH</th>
<th>60°C %RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>NaBr</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>NaCl</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>KCl</td>
<td>85</td>
<td>85</td>
<td>85</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>65</td>
</tr>
</tbody>
</table>
Product Development with Forced Degradation

**Excipient compatibility**

50°C/75%RH

Prototype **Trials-and-Errors**

2~3 weeks

X rounds of iterations

Lead Prototypes Stability Confirmation

40°C/75%RH

3~6 months

X rounds of iterations

Final Formulation in Various Packaging Configurations for Long Term Stability

The stress study at 50°C/75%RH does not provide a shelf-life prediction, rather, it provides a ranking of relative stability among the prototypes.
Accelerated Stability Assessment Program (ASAP)

- A software available commercially;

- This software can predict such as 2, 3, or 5 years of shelf-life for solid drug substances and drug products;

- The prediction is based on carefully designed forced degradation studies that are completed within typically less than three weeks;

- The software can largely reduce packaging screenings based on embedded database and mathematic algorithm;

- The software can simulate excursions without performing the actual tests.
ASAP – Humidity Corrected Arrhenius Equation

\[ \ln k = \ln A - \frac{E_a}{RT} + B(RH) \]

\textbf{A}: reflects collision frequency;

\textbf{k}: reflect reaction kinetics that connects to stability specification limit;

\textbf{E}_a: reflects activation energy;

\textbf{B}(RH): humidity sensitivity factor at certain RH
“stress the formulas for up to 3 weeks”  
And  
“no packaging needed”  
And  
“run the ASAP model to predict shelf-lives”  
And  
“Excipient compatibility study is more for information and not for restriction”
Forced Degradation – A Few More References

- Organic Chemistry of Drug Degradation, by Min Li (Author)
- Pharmaceutical Stress Testing: Predicting Drug Degradation (Drugs and the Pharmaceutical Sciences), by Steven W. Baertschi (Editor)
Building Knowledge Space – Separation Science

**Analytical Chemistry** is a science about Separation and Detection.

Therefore, it is not possible to talk about Separation Science in much detail in this presentation. It is a knowledge you must obtain before you even think about Quality by Design.

**Method development objective:**

A stability-indicating HPLC method must be developed to provide stability information on the API /formulation /product, based on structure elucidation, forced degradation and drug-excipient compatibility studies.

This method shall properly retain any compounds that may be formed as a result of hydrolysis and are more polar and less hydrophobic than the API; also, the same method must be able to elute additional hydrophobic species, such as dimers of the API.
Method Development Considerations

• If any regulatory requirement are to be met

• If the method will be transferred to product sites or other QC sites

• Additional requirements such as sample throughput, analysis time, and instrument limitations

• Critical study of an API to assess the likely degradation pathways

• Collection of information on physicochemical properties
Method Development Considerations

• Perform separation studies based on stress and/or forced degradation studies, or using available known impurity compounds

• Compatibility studies with excipients

• Evaluation of completeness of extraction based on recovery (spiking), kinetic (change in time), and thermodynamic (change in volume) studies

• Mass balance for assay (similar results shall be obtained by peak area normalization and by weight percent / label claim)
Method Development Considerations

• Analyte structure and pKa

• Solubility of compounds and Diluent effects (avoid possible reaction of the analyte with diluent and mobile phases, especially for compounds containing keto functionalities, active aldehyde, active esters, and enolate intermediates, which may react with protic solvents)

• Choice of detector

• Solution stability and sample preparation

• Choice of stationary phase (black magic or science)

• Choice of mobile phase pH, buffers

• Isocratic versus gradient separations
# HPLC Column Selection

**Physicochemical parameters:**
- type of silica synthesis (A/B)
- spec. surface
- pore volume
- pore size
- particle size
- particle size distribution

**Surface modification parameters:**
- Silane (functionality, reactivity)
- type of bonding (monomeric, polym.)
- carbon content
- ligand density
- endcapping (number of residual silanols)

**Column pool:**
- ACE columns: C8, C18, Phenyl
- YMC columns: Hydrosphere C18, Basic,
- Agilent columns: Zorbax XDB C8, Phenyl
- Waters column: XBridge, SymmetryShield

... to infinity and beyond!...
Items Preferred and Not Preferred in Methods

Preferred:
• Short run time
• Robust and Rugged
• Reproducible on any brand of HPLC systems
• At least two brands of columns that are true equivalent
• Long column life time
• Baseline separation of all named impurities
• Easy on peak integration/quantitation
• Column temperature controlled
• Mobile phase compatible with LC-MS

Not Preferred:
• Method that requires QC analysts to adjust experimental conditions to meet system suitability
• Filtration of mobile phases after mixing of organic solvents
• Instrument specific
• Vendor specific reagents, solvents, etc.
• THF
Analytical Method Development
Chromatography Optimization – Summary

To achieve maximized separation power

- Materials: APIs and excipients
- Knowledge of separation science
- Knowledge of literature work
- Forced Degradation of APIs
- API-API compatibility
- API-Excipient compatibility
Analytical Method Development
Sample Preparation – Summary

Robust sample preparation

Materials: hundreds of dosage units

Knowledge of material science

Knowledge of sample preparation techniques

Study matrix effect

Automation feasibility

Short term high temperature / high moisture stress
Analytical Method Development
Dissolution Optimization – Summary

- Knowledge of API solubility and stability
- Various apparatus
- Various rotation speeds
- Various media with various pHs
- Knowledge of dissolution techniques
- Materials: hundreds of dosage units

Discriminative dissolution

Materials: hundreds of dosage units
Knowledge of dissolution techniques
Various media with various pHs
Various rotation speeds
Various apparatus
Knowledge of API solubility and stability
Other Important Things

- Utilizing method development software
- keeping up with new column technology
- Keeping your clients in mind

*Interactive exercise with audience*
“Degradation Peak” Generated by Analytical Method, Aspirin Anhydride

This is actually a simplified reverse route of Aspirin synthesis.
“Degradation Peak” Generated by Analytical Method, Ibuprofen Methyl Ester

Investigation Into the Formation of Ibuprofen Methyl Ester in Aqueous Methanol Solutions of Different pH
American Pharmaceutical Review, 2010, Aug
THANK YOU!