
Lilly

Answers That Matter.

Patterns and Pathways: Using Chemistry to Guide the Characterization of Degradation Products

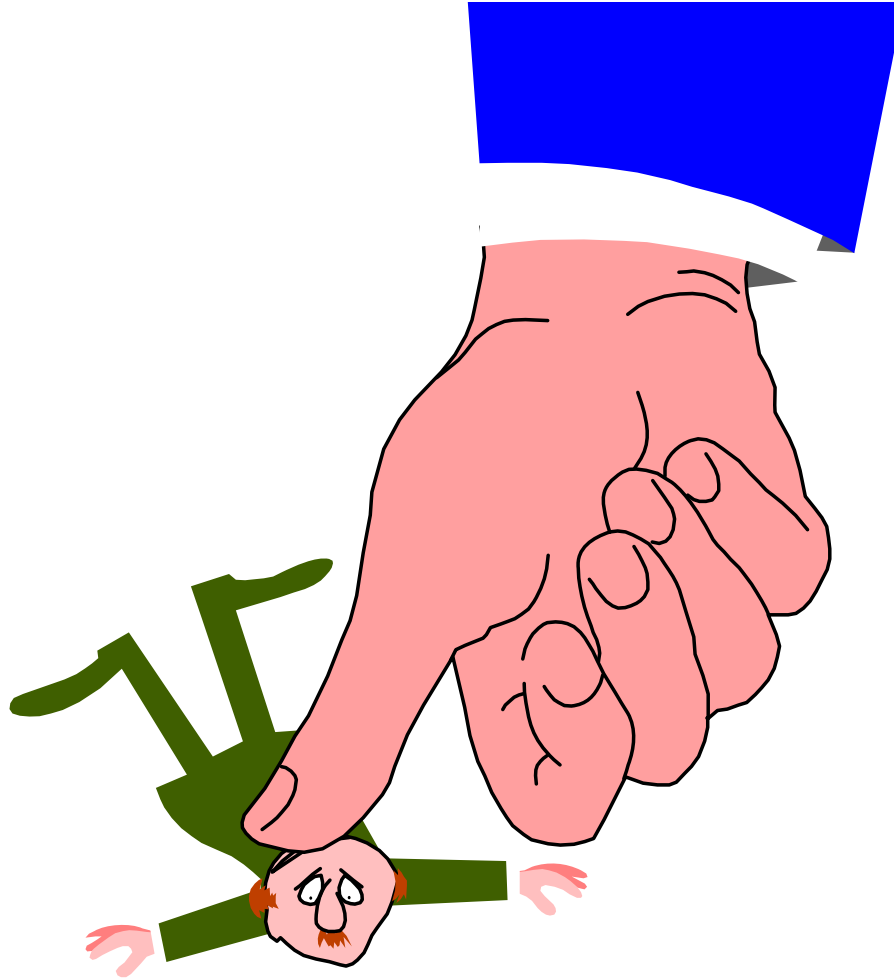
Steven W. Baertschi
Eli Lilly and Company

COSMOS, Boston, MA, August 3-5, 2009

The Lilly logo is written in a red, cursive script font.

Answers That Matter.

Thanks to Doug Kiehl...



Outline

- Introduction
- “Quality-by-Design” Principles
- Processes for identifying of an unknown degradation-related impurity
- Extracting information from Precedent
 - Where to find such information
 - Looking at patterns in the data
 - Examples of using such information
- Conclusions

Stress Testing: The Foundation of Stability Testing

Stress Testing:

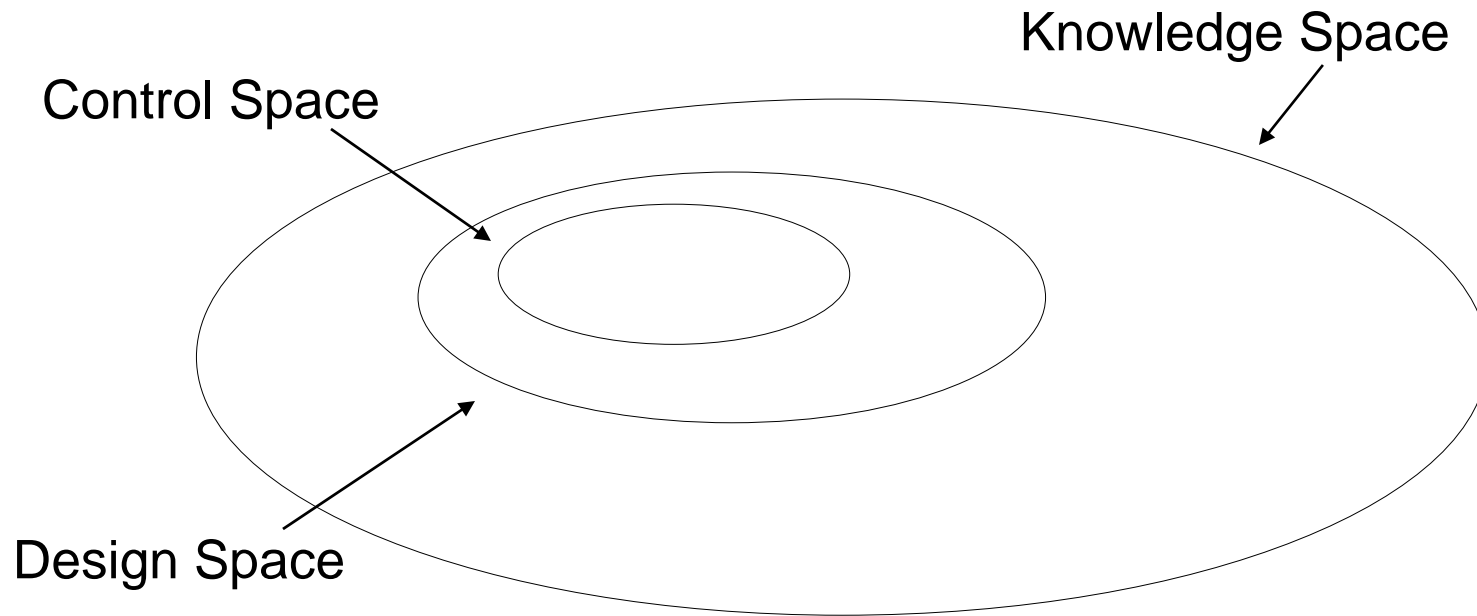
“Stress testing helps to determine the **intrinsic stability** of the molecule by establishing **degradation pathways** in order to identify the **likely degradation products** and to validate the stability-indicating power of the analytical procedures used.”

-ICH Guideline on Stability Testing of New Drug Substances and Products, Q1A(R2), 2003.

Such studies should include:

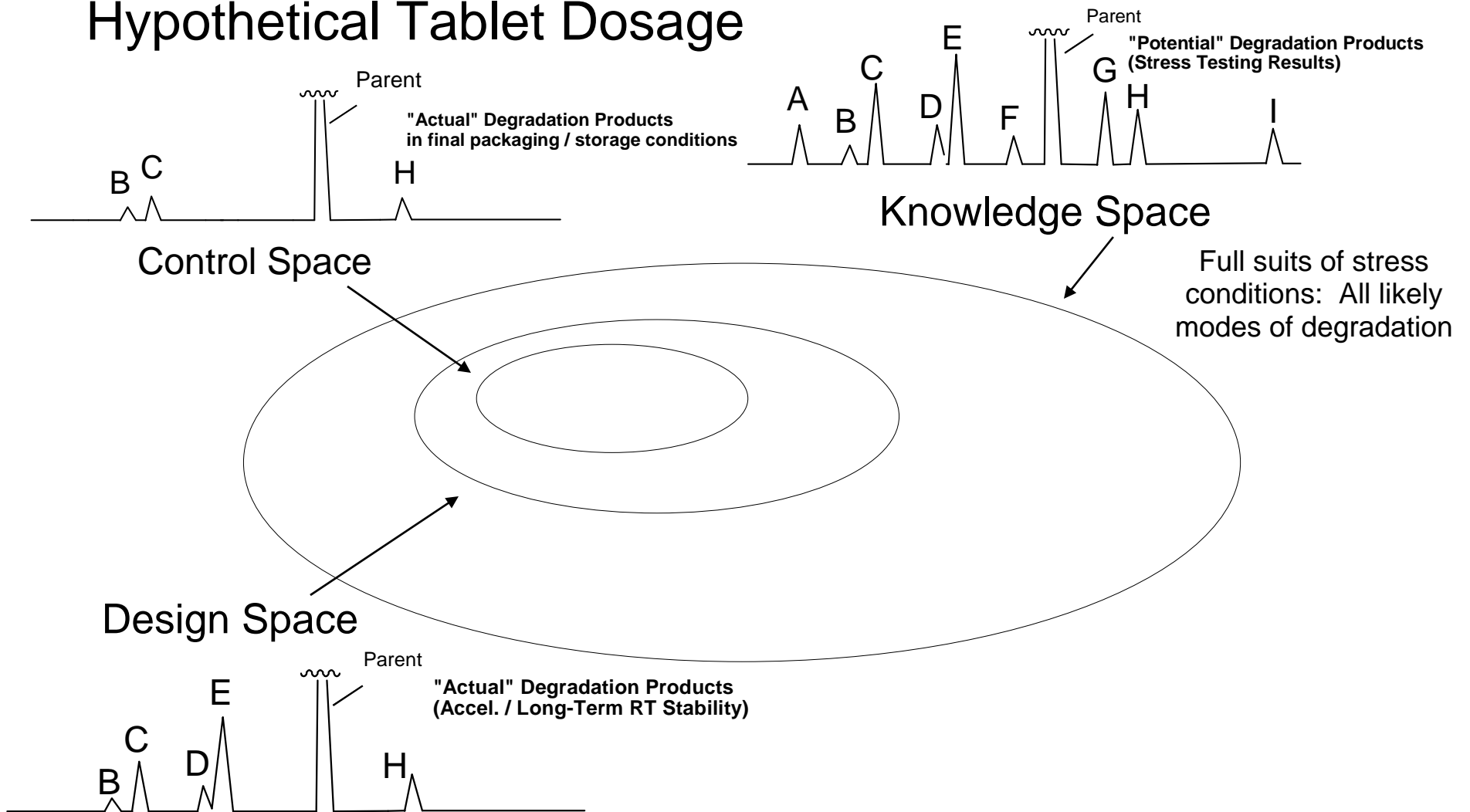
- Effect of elevated temperature and humidity
- Susceptibility to hydrolysis over a wide range of pH values
- Exposure to oxidative conditions
- Photostability testing

Stress Testing Strategy: Quality-by-Design Principles

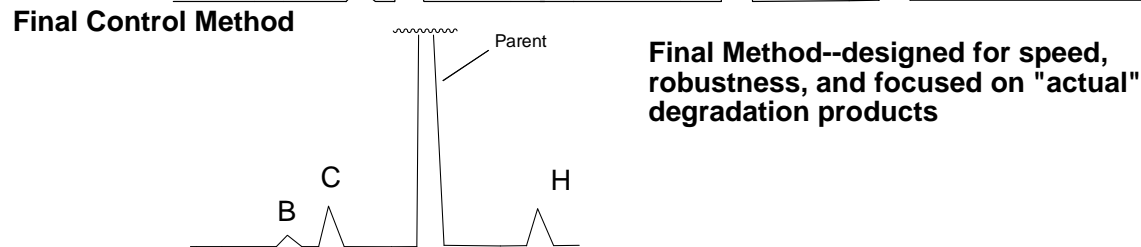
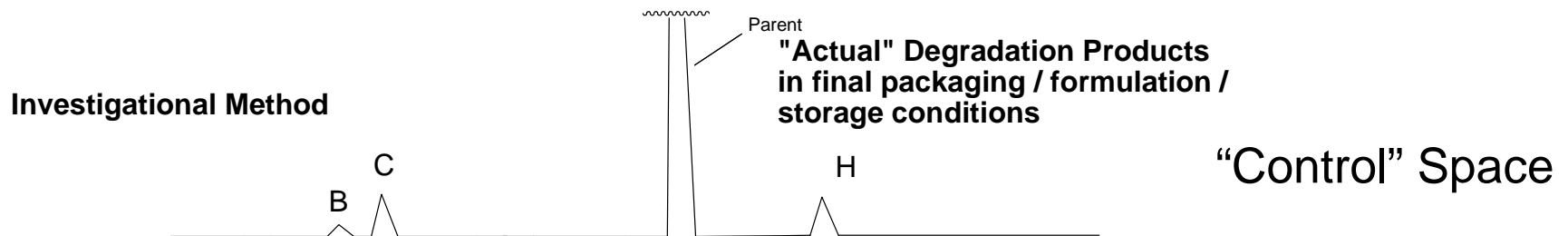
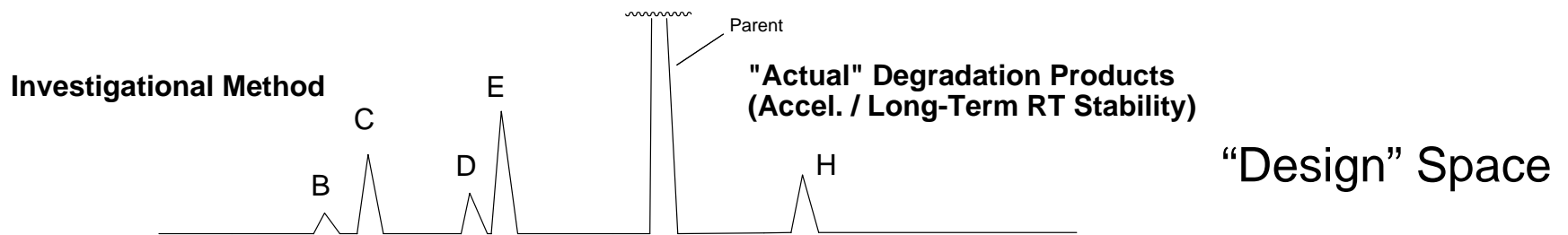
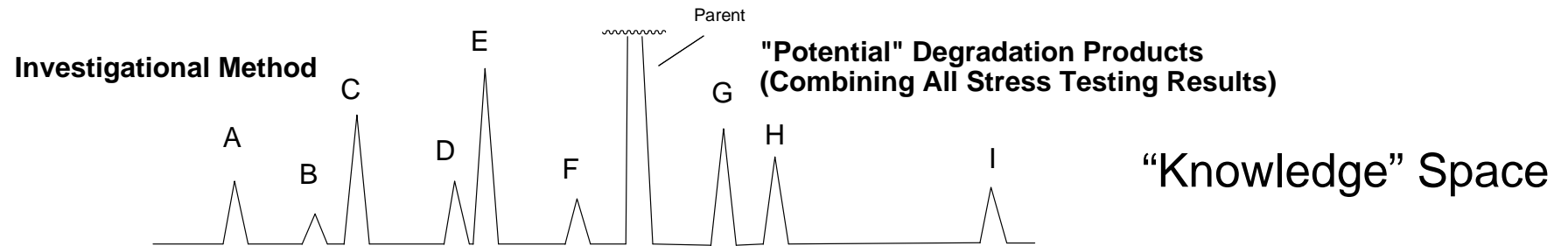


Putting meaning to QbD for Stress Testing and Stability...

Hypothetical Tablet Dosage



Stress Testing Strategy: Chromatographic Representation



Processes for Identifying and Unknown Degradant

- Analytical / spectroscopic approaches
 - Separation and detection
 - Spectroscopic characterization and interpretation
- Chemistry-guided approaches
 - **Use of stress testing screens (e.g., oxidative susceptibility, other specific stress conditions)**
 - Use of personal knowledge of chemistry
 - Use of in silico tools (i.e., computational engines)
 - Use of “Precedence” (i.e., known deg. chemistry in the public domain)

Oxidative Susceptibility Testing

Predictive

Nucleophilic/Direct

- 0.3% H₂O₂ 1 d, RT

Autoxidation

- AIBN (1 equiv, 40C, 1 d), or VAZO52 (25C, 1 d)

Electron-transfer

- Cu²⁺ (1-5 mM, 1 d)
- Fe³⁺ (1-5 mM, 1 d)

Investigative

Peroxides: e.g., Peracetic acid, mCPBA, Oxone (<30 min)

¹O₂ Photosensitizers: e.g., rose bengal, h_v, <30 min

Fenton conditions: Fe²⁺ (1-5 mM) / 0.3-3% H₂O₂, < 10 min

Ozone (?)

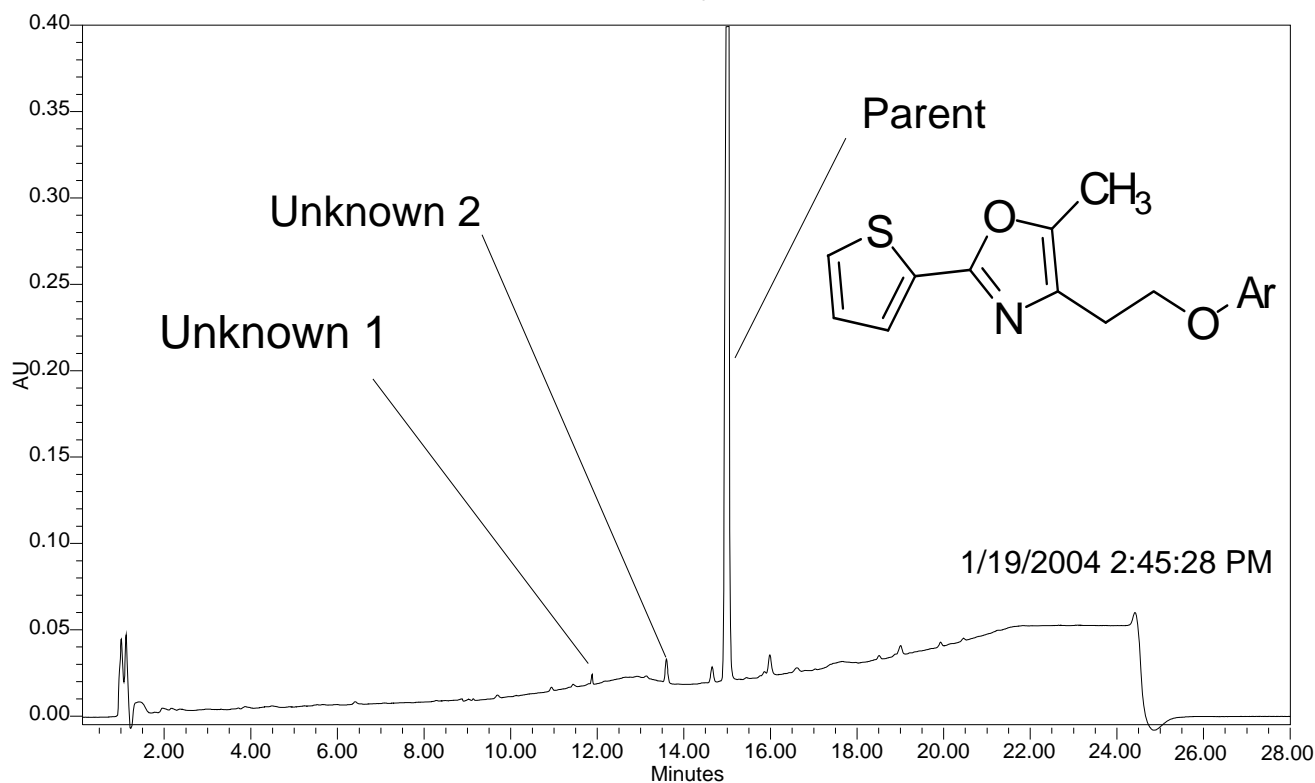
Other Oxidative Reagents (e.g., NaOCl, KMnO₄, etc.)

Impurity Problem #1: New Unknown Impurities Detected During Formulation Process

Low Dose: 10-100 ug active in a 200 mg capsule

Two unknown impurities forming during milling (~0.1 to ~0.8% level)

Analytical Difficulties: Related substances **HPLC method requires injection of almost 2 mL (!)** to achieve needed sensitivity. LC/MS analysis to get MW information on impurity is inconclusive.

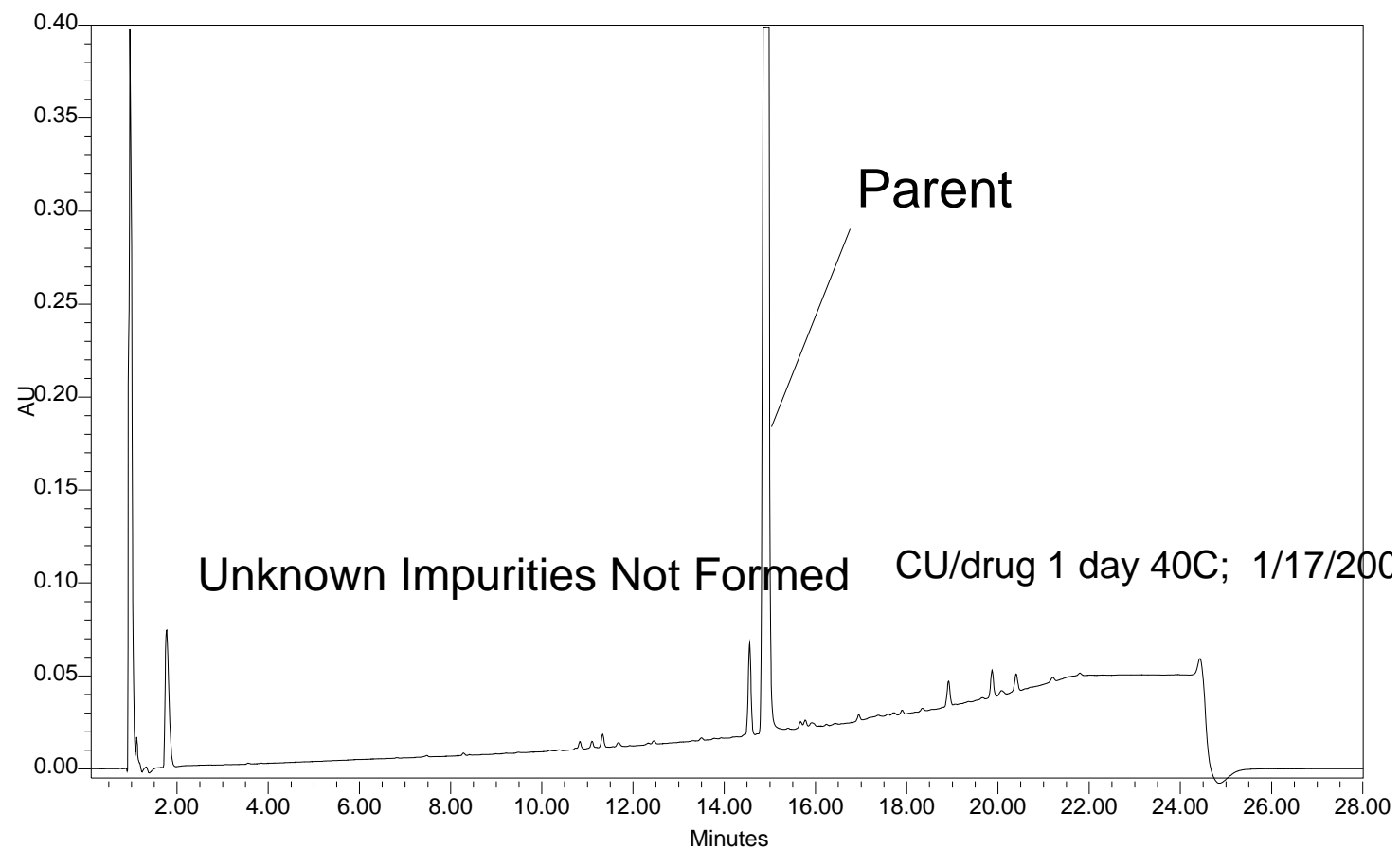


Impurity Problem: Investigational Approach

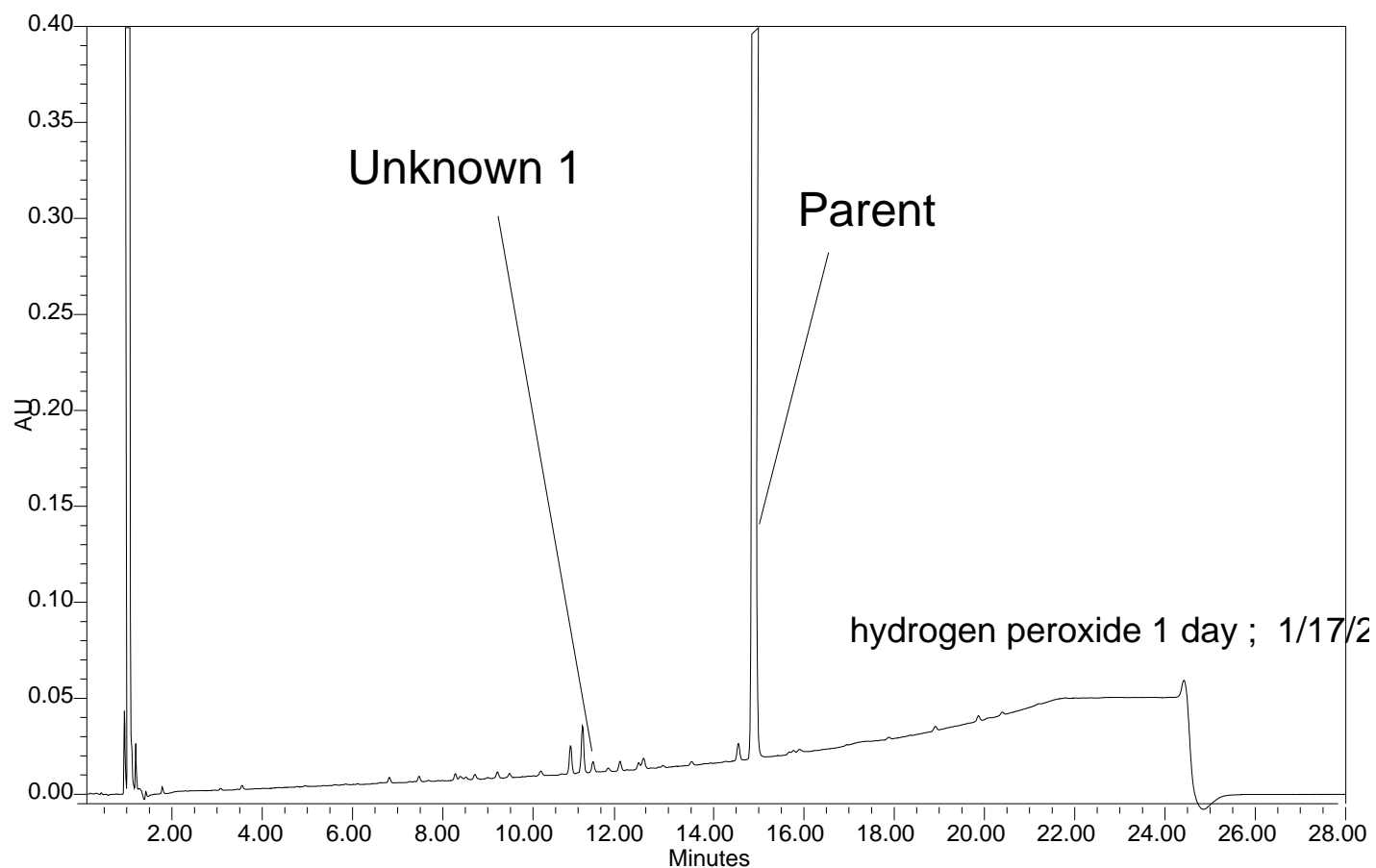
Approach: Try to find some way to make the unknown impurity in larger quantities

- Aggressive / extended milling?
 - Thermal stress? Acid / base stress?
 - Perform oxidative susceptibility screening on drug substance (not product)
 - Cu(II), Fe(III), AIBN, 0.3% hydrogen peroxide, 1 day
 - Rose bengal (singlet oxygen generator), 30 min/light
-

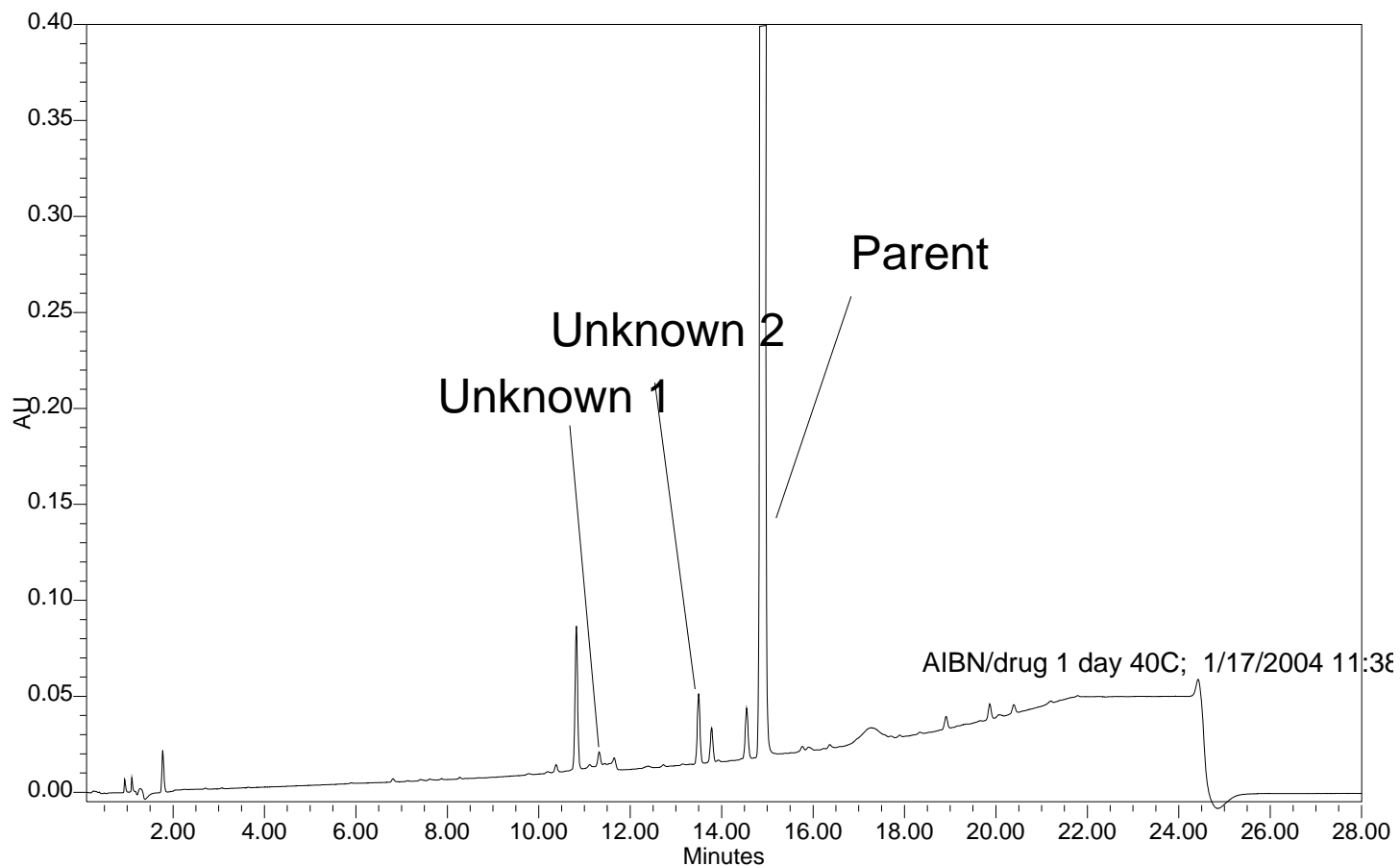
Oxidative Susceptibility: Copper (II), 5 mM, 1 day, 40C



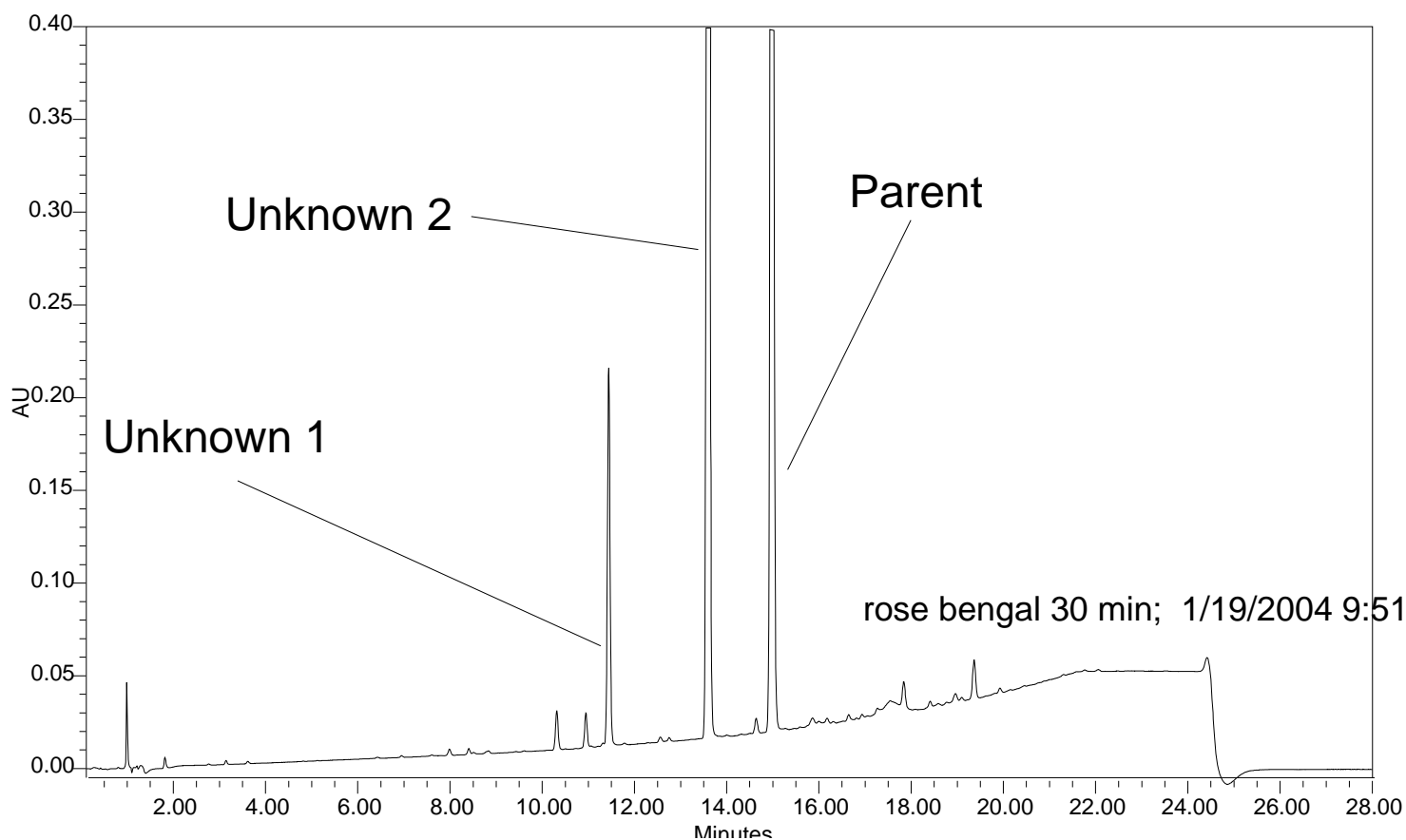
Oxidative Susceptibility: 0.3% H₂O₂, 1 day, RT



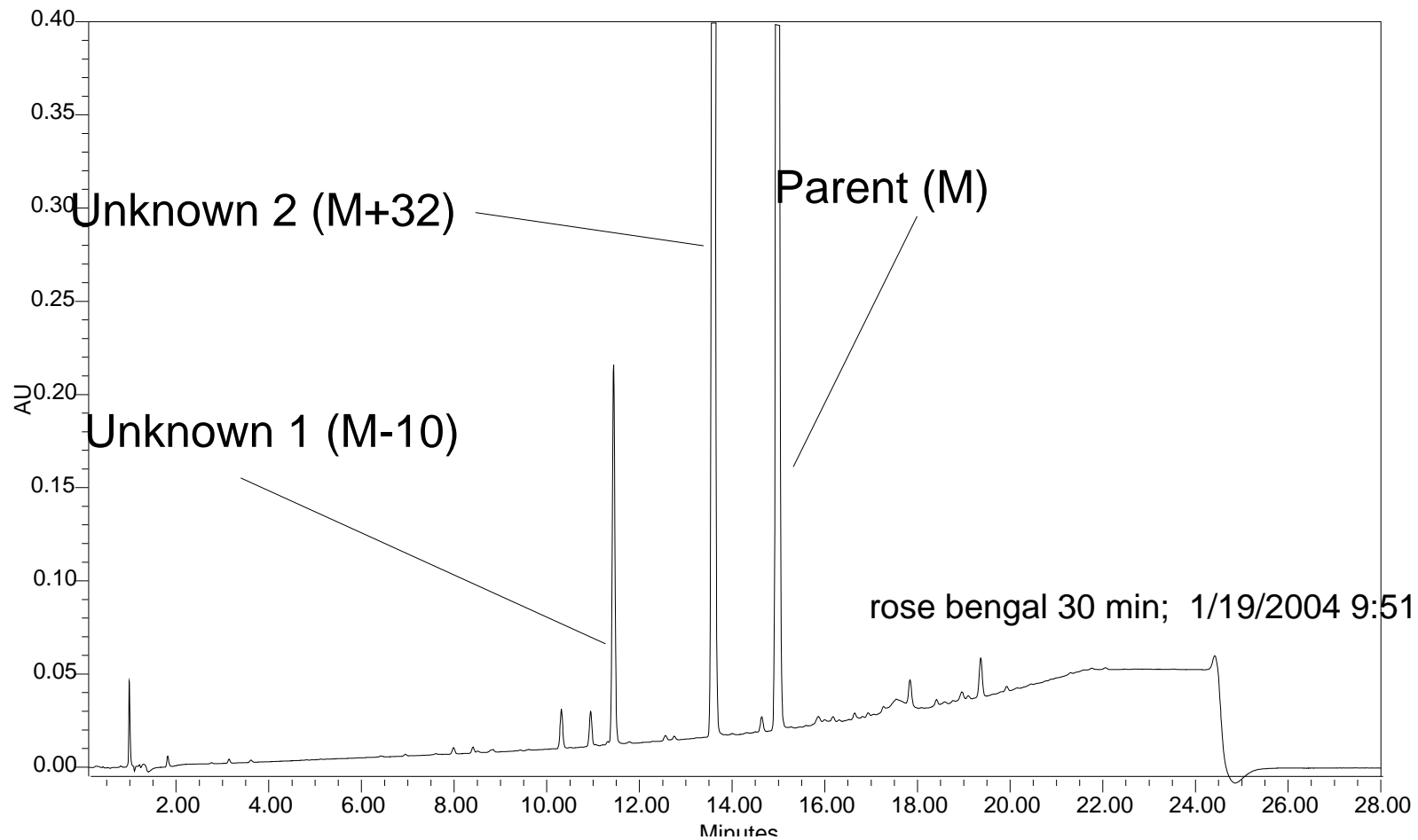
Oxidative Susceptibility: Radical Initiator (AIBN), ~1 eq, 1 day, 40C



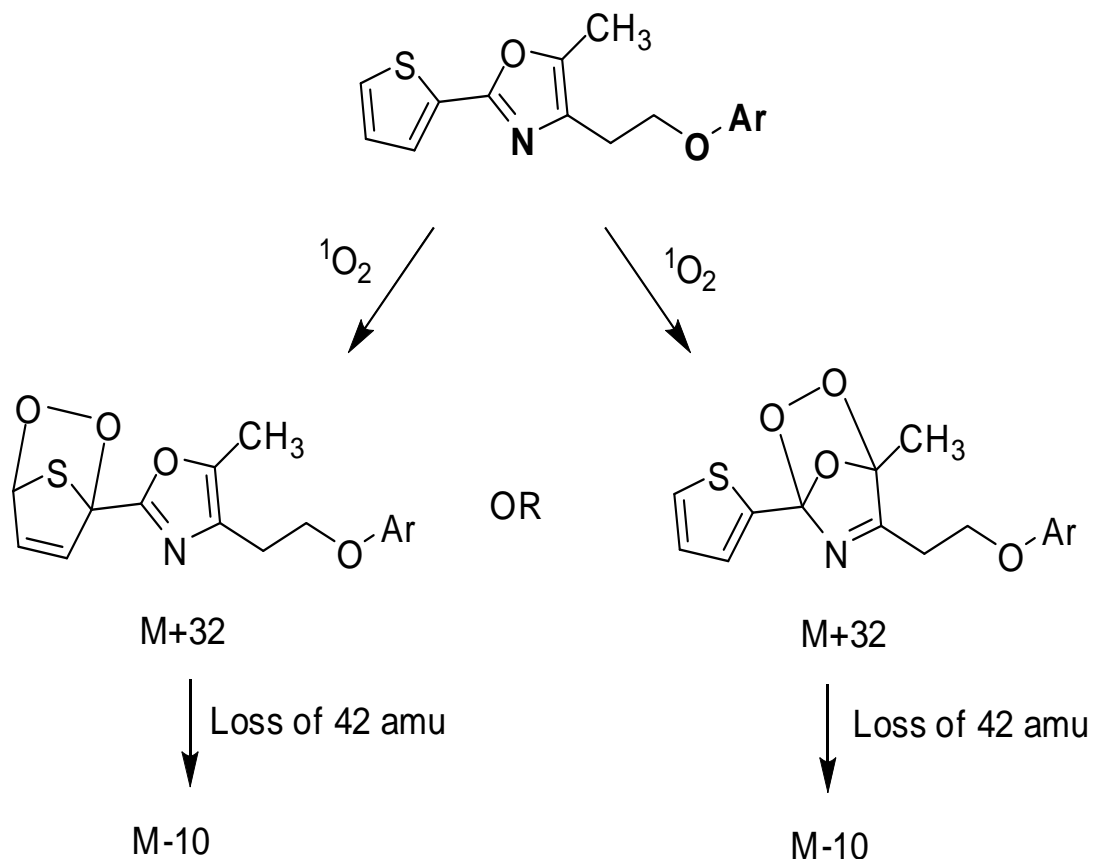
Oxidative Susceptibility: Singlet oxygen sensitizer (rose bengal), 30 min, xenon lamp



LC/MS Results

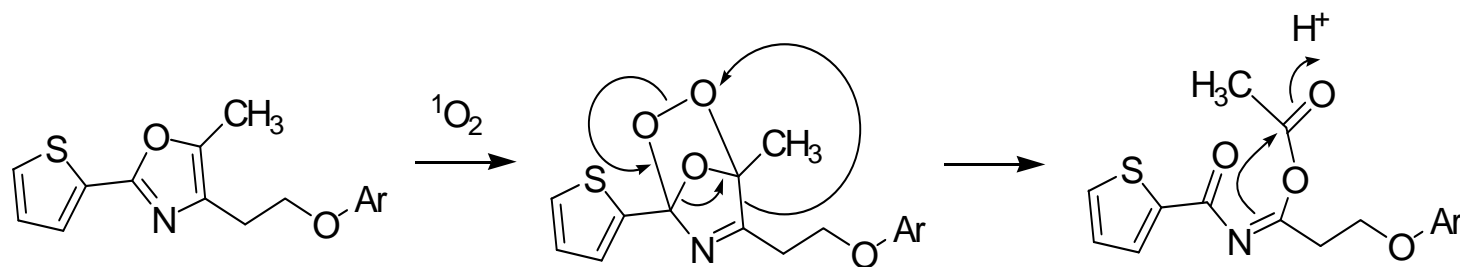


Two Reasonable Hypotheses for Reaction of Singlet Oxygen

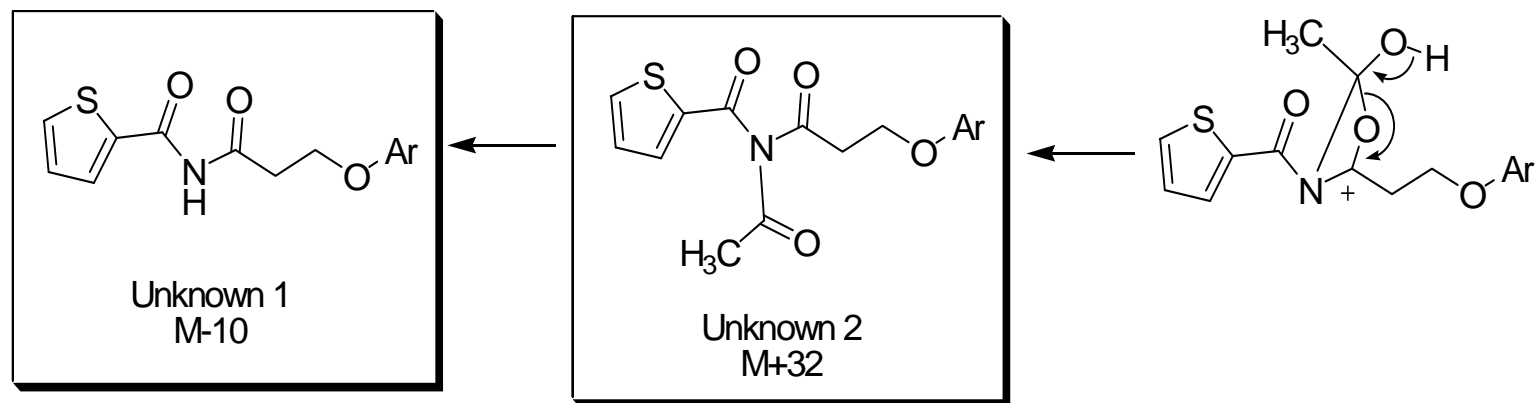


Structures Proposed Based on Literature Precedent

Key Reference: Reaction of $^1\text{O}_2$ with Oxazoles -- Wasserman, H.H, et al., JACS, 94:20 (1972)



Elapsed time: 48 hours to structure proposal!! Isolation and NMR characterization within 1 week of sample receipt.



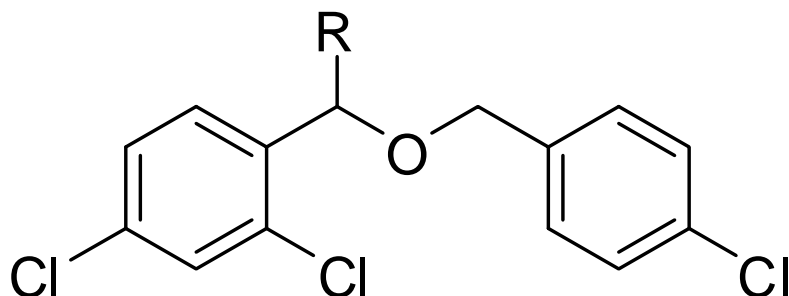
Processes for Identifying and Unknown Degradant

- Analytical / spectroscopic approaches
 - Separation and detection
 - Spectroscopic characterization and interpretation
- Chemistry-guided approaches
 - Use of stress testing screens (e.g., oxidative susceptibility, other specific stress conditions)
 - **Use of personal knowledge of chemistry**
 - Use of in silico tools (i.e., computational engines)
 - Use of “Precedent” (i.e., deg. chemistry in the public domain)

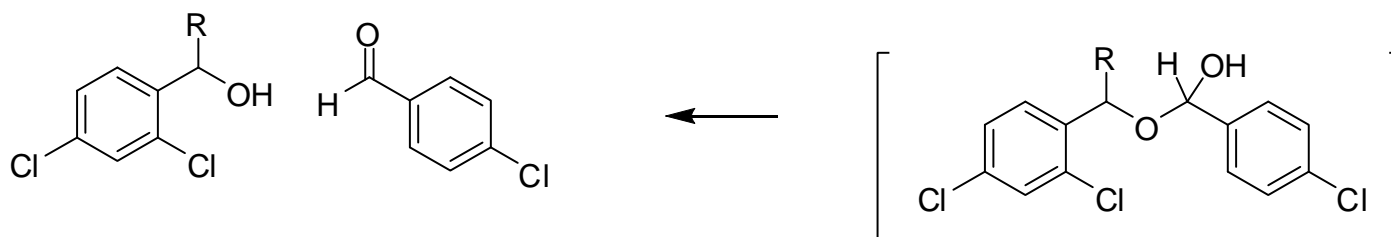
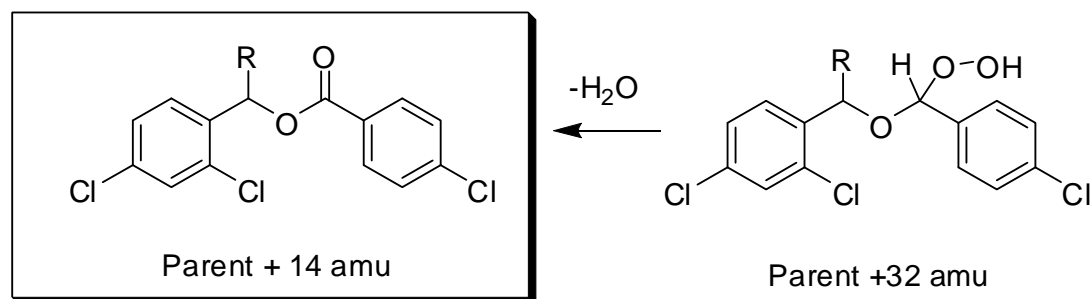
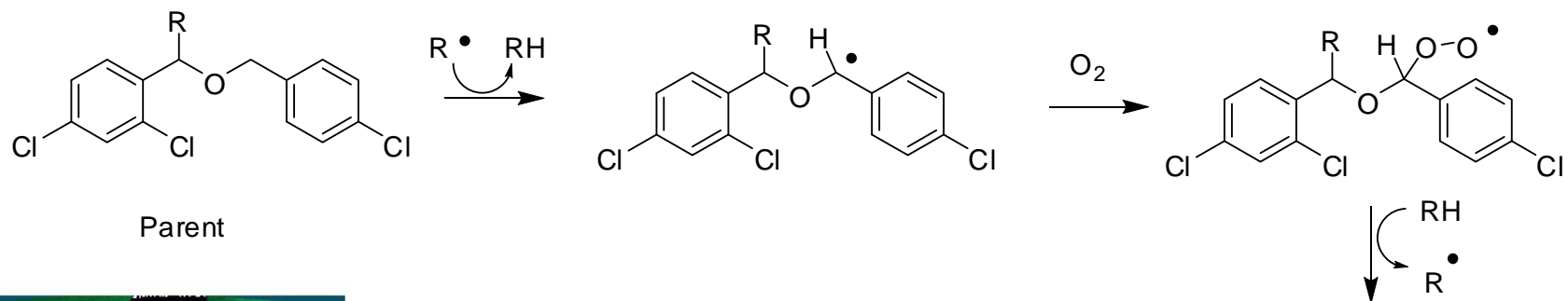
Use of personal knowledge of chemistry...

Situation: New degradant observed in HPLC chromatogram of an aged tablet at level of 0.15%.

- LC/MS/ESI reveals MW of degradant to be 14 amu higher than parent drug.
- Structure of parent:



Use of personal knowledge of chemistry...



Processes for Identifying and Unknown Degradant

- Analytical / spectroscopic approaches
 - Separation and detection
 - Spectroscopic characterization and interpretation
- Chemistry-guided approaches
 - Use of stress testing screens (e.g., oxidative susceptibility, other specific stress conditions)
 - Use of personal knowledge of chemistry
 - **Use of in silico tools (i.e., computational engines)**
 - Use of “Precedent” (i.e., deg. chemistry in the public domain)

In Silico tools...



1. **CAMEO** (Computer Assisted Mechanistic Evaluation of Organic Reactions), A.J. Gushurst and W.L. Jorgensen of Yale University. See series of publications in J. Org. Chem. In the late 1980's.

2. **DELPHI** Prediction of Drug Degradants Using DELPHI: An Expert System for Focusing Knowledge

David L. Pole, Howard Y. Ando,* and Sean T. Murphy

Research Formulations Department and Chemistry Department, Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, Michigan 48105

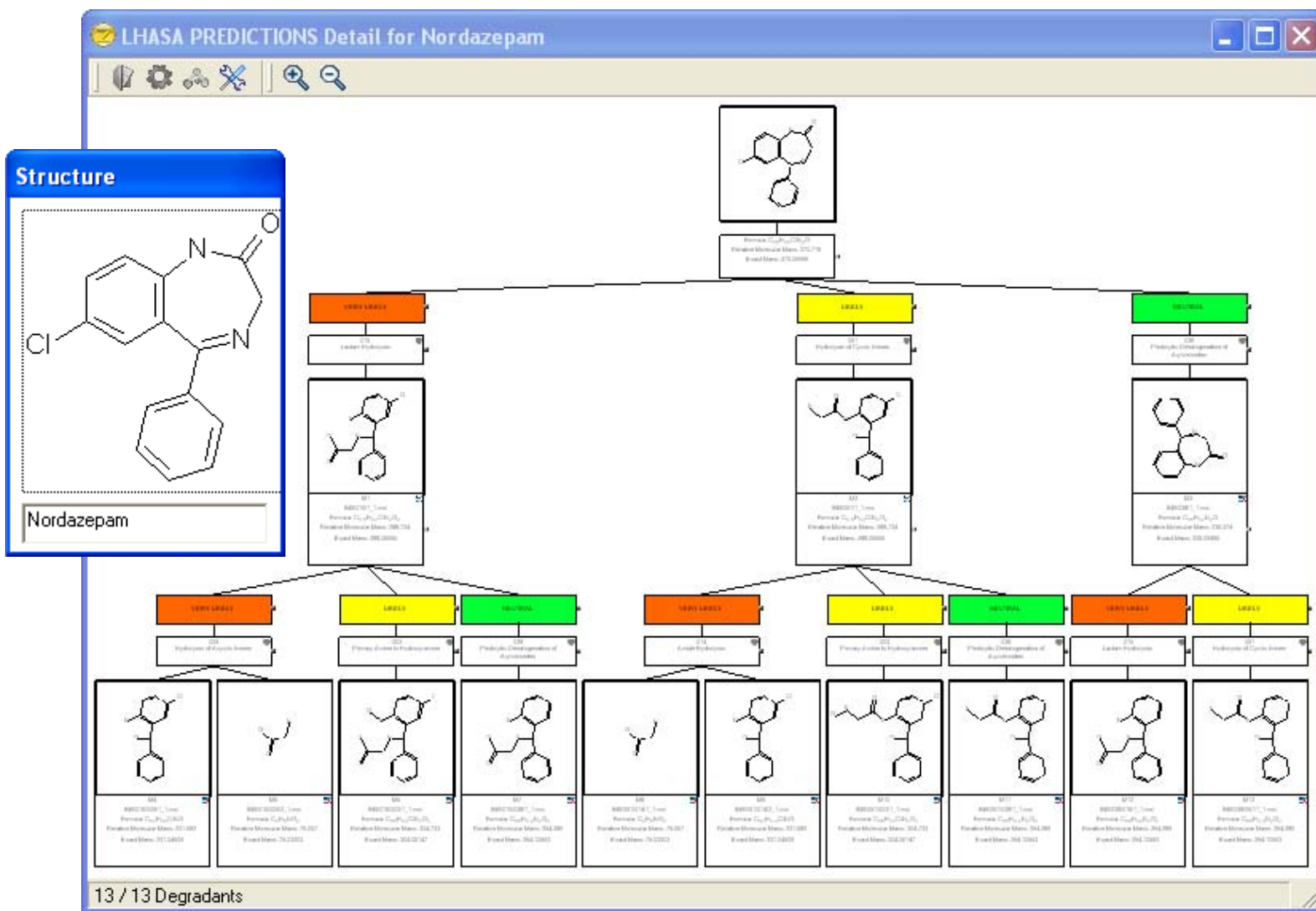
VOL. 4, NO. 4, 539–549 MOLECULAR PHARMACEUTICS

3. **ZENETH**

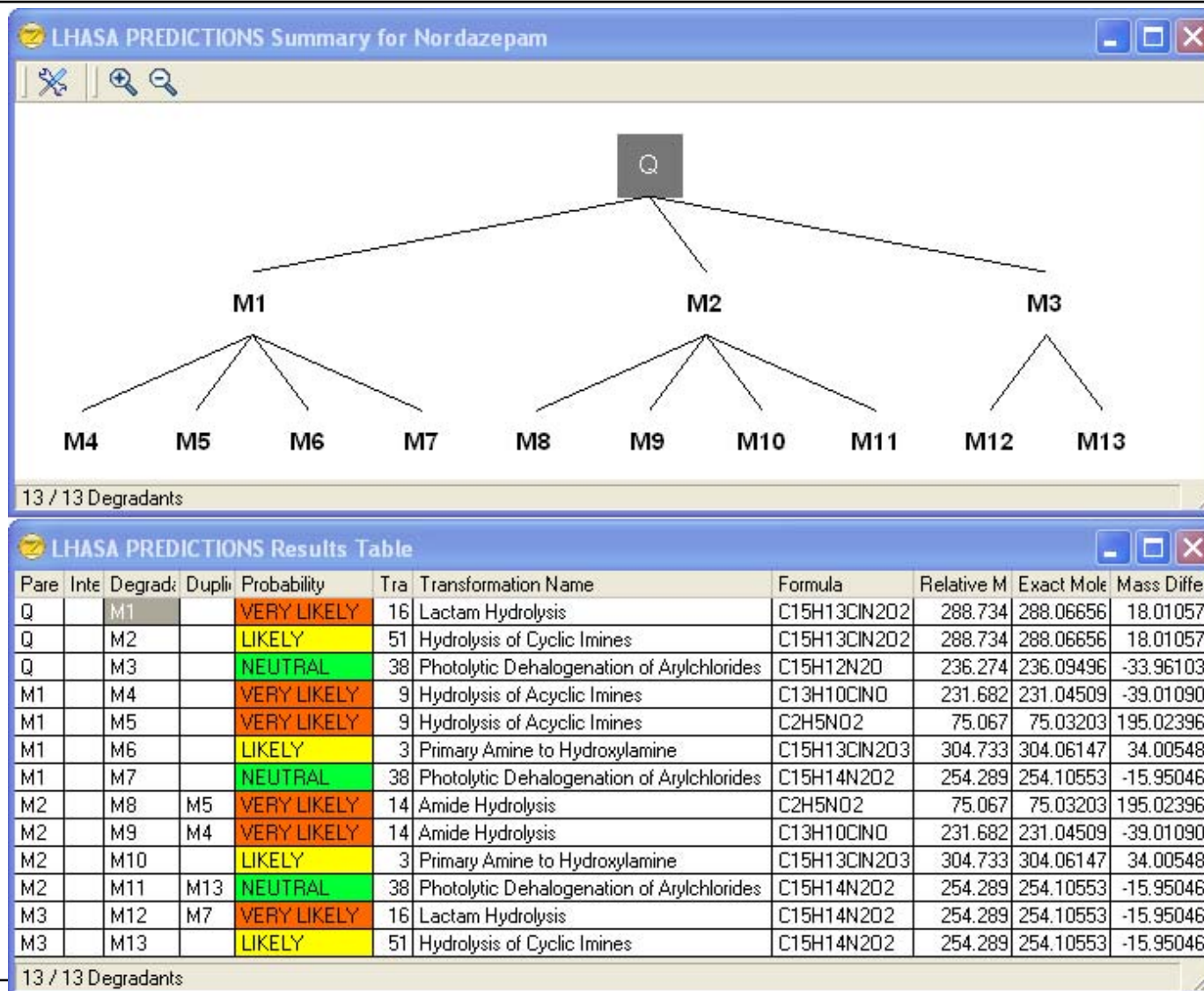


Expert computer system being developed by Lhasa Ltd to predict degradation pathways

Zeneth Chemical Degradation Prediction Software



Zeneth Chemical Degradation Prediction Software



Processes for Identifying and Unknown Degradant

- Analytical / spectroscopic approaches
 - Separation and detection
 - Spectroscopic characterization and interpretation
- Chemistry-guided approaches
 - Use of stress testing screens (e.g., oxidative susceptibility, other specific stress conditions)
 - Use of personal knowledge of chemistry
 - Use of in silico tools (i.e., computational engines)
 - **Use of “Precedence” (i.e., deg. chemistry in the public or private domain)**

Extracting Information from “Precedence”

- Where to find such information? Literature, textbooks, etc. OR...
 - Pharma D3 – Drug Degradation Database at www.d3.cambridgesoft.com . Click on the icon and you are “in”.

Currently 346 drugs;
>1150 degradation
products;
Literature references

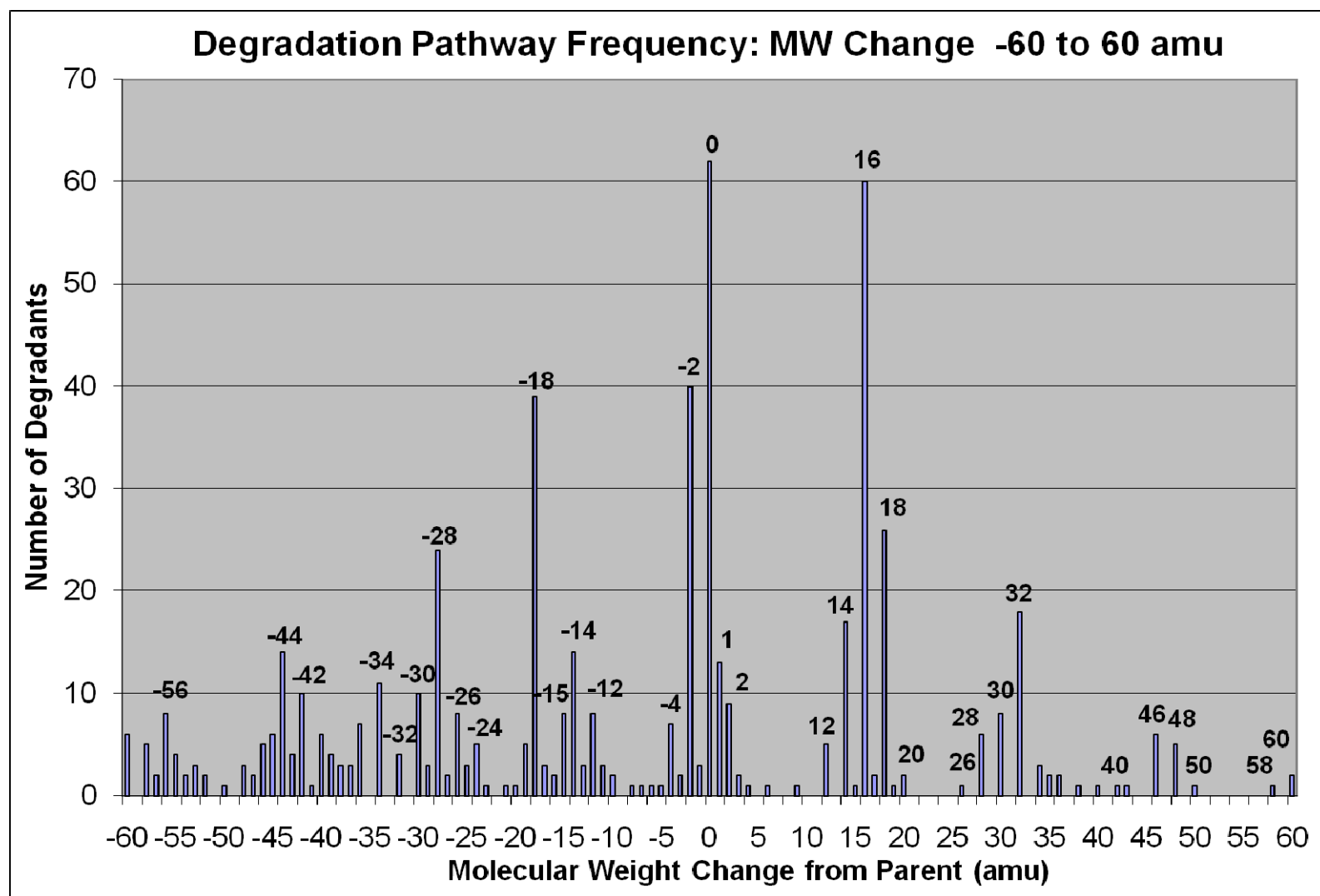


Extracting Information from “Precedence”: Online Drug Degradation Database

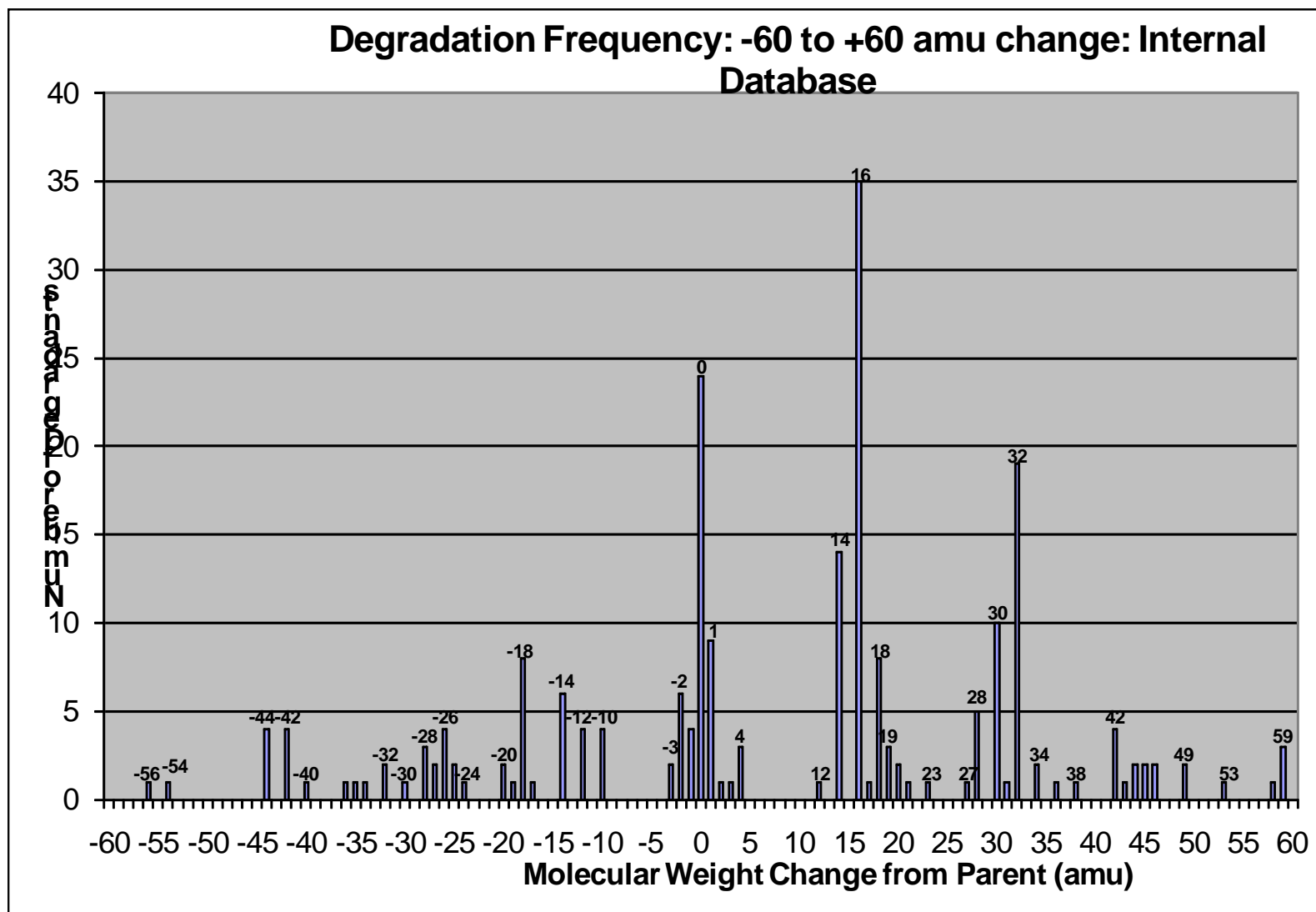
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Trade name	<input type="text"/>	Molecular weight	<input type="text"/>
Common/Other name	<input type="text"/>	Change in Molecular weight	<input type="text"/>
Compound number	<input type="text"/>	Salt	-- Select one -- <input type="button" value="v"/>
Functional Group	-- Select one -- <input type="button" value="v"/>	Conditions	-- Select one -- <input type="button" value="v"/>

Parent	Degradant
SearchType Substructure <input type="button" value="v"/>	SearchType Substructure <input type="button" value="v"/>
<div style="border: 1px solid black; height: 150px;"></div>	<div style="border: 1px solid black; height: 150px;"></div>

Looking for Patterns in Published Information: Mining the Pharma D3



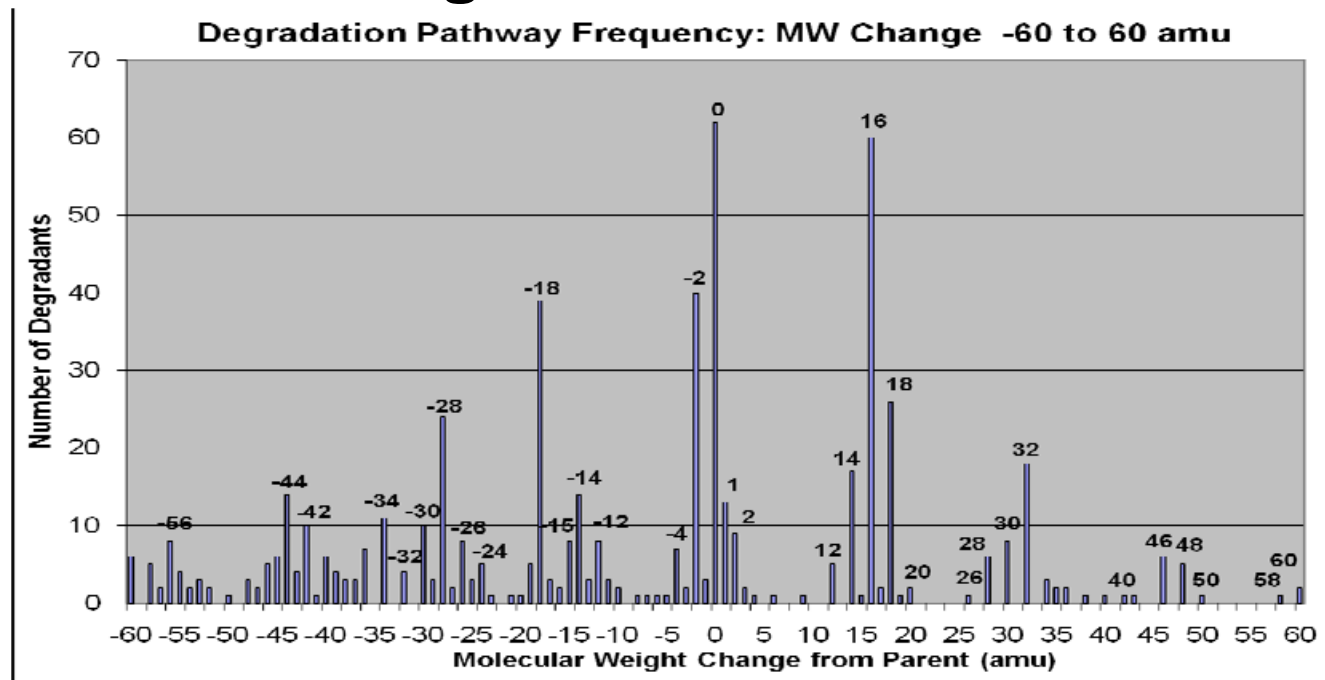
Looking for Patterns in “Unpublished” Information: Mining Lilly Internal Database



Looking for Patterns in Published Information: Mining the Pharma D3

0 amu change = ~60 unique examples.

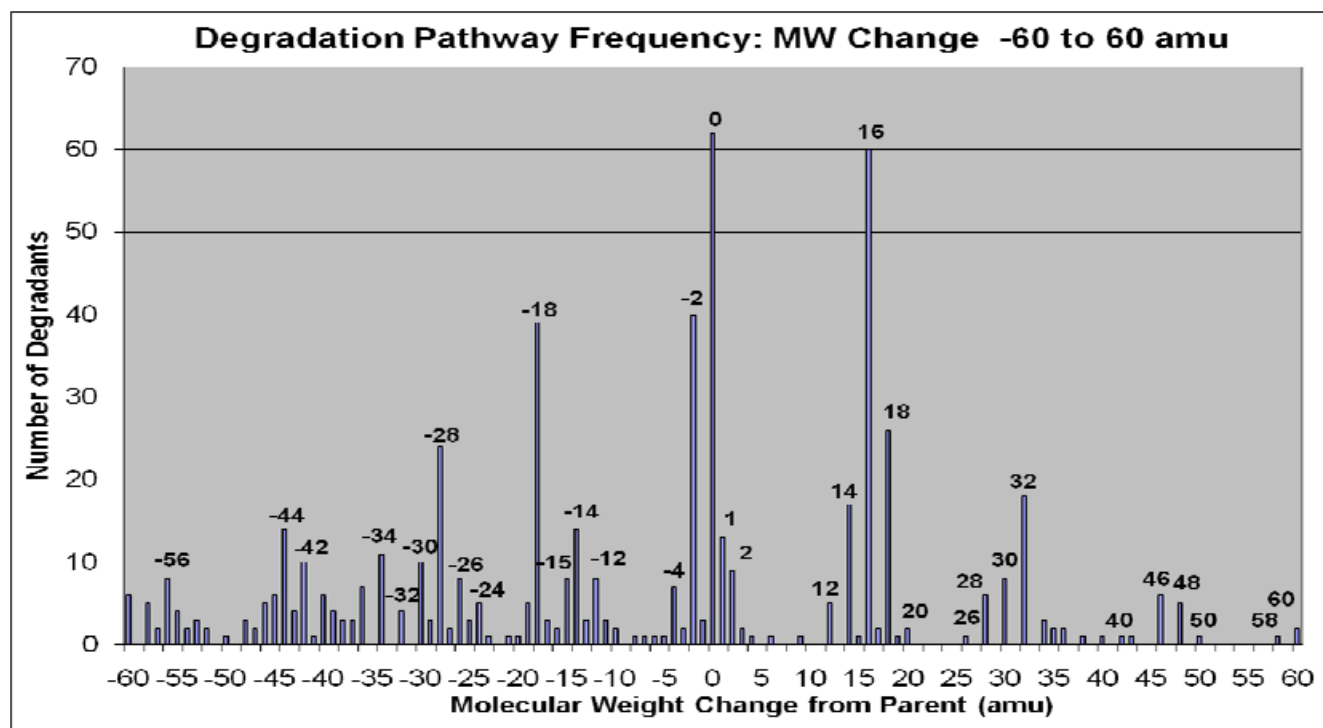
Rearrangement – 31; Epimerization / racemization – 13; cis/trans double bond configuration – 8; double bond migration – 5.



Looking for Patterns in Published Information: Mining the Pharma D3

+16 amu change = 60 examples.

Tertiary amine to N-oxide – 13; thioether to sulfoxide – 9; benzylic oxidation – 6; sulfoxide to sulfone – 4; allylic oxidation – 3; alpha to a heteroatom (N or O) – 3.



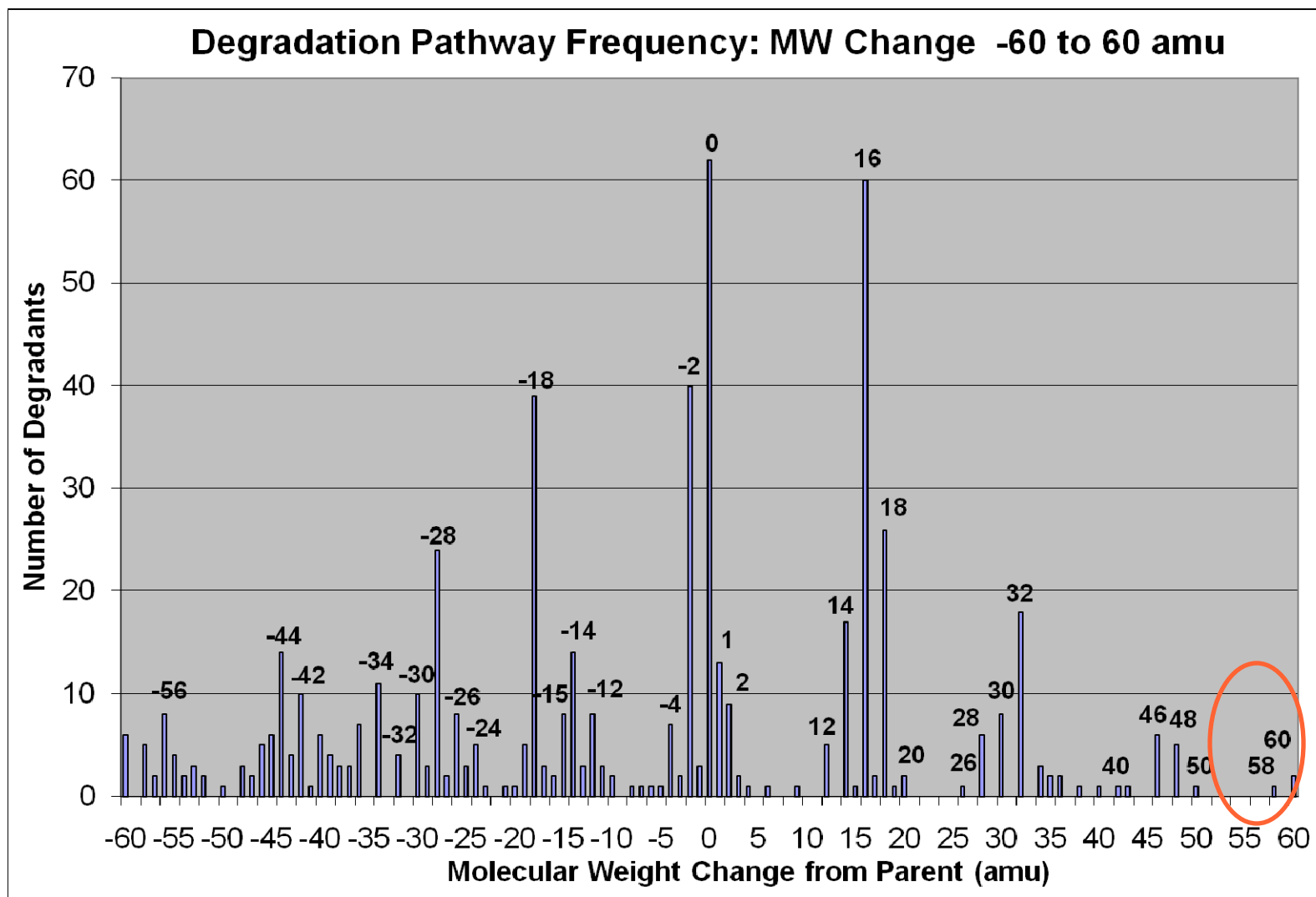
Using Pharma D3 to Speed Up Degradation Product Identification

Situation: New degradant observed in HPLC chromatogram of an aged tablet at level of 0.15%.

- LC/MS/ESI reveals MW of degradant to be 58 amu higher than parent drug(??!!).
- Now what?
 - Accurate mass
 - Fragmentation studies
 - LC/NMR
 - Efforts to isolate / purify

OR...

Looking for Patterns in Published Information: Mining the Pharma D3

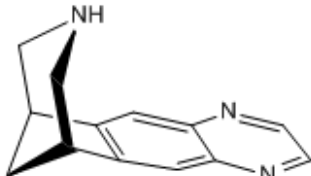
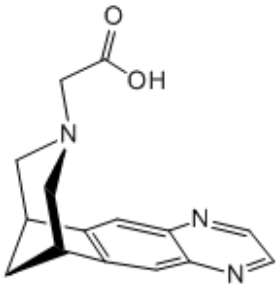


Extracting Information from “Precedence”: Search on “Change in Molecular Weight”: +58

Generic name	<input type="text"/>	Formula	<input type="text"/>
Trade name	<input type="text"/>	Molecular weight	<input type="text"/>
Common/Other name	<input type="text"/>	Change in Molecular weight	<input type="text" value="+58"/>
Compound number	<input type="text"/>	Salt	-- Select one -- <input type="button" value="v"/>
Functional Group	-- Select one -- <input type="button" value="v"/>	Conditions	-- Select one --

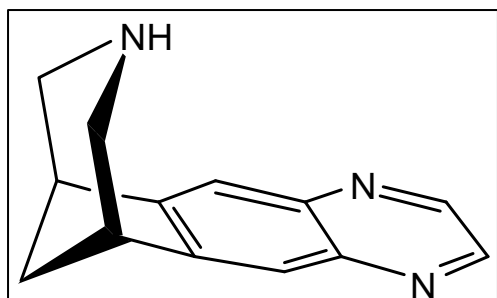
Parent	Degradant
SearchType <input type="text" value="Substructure"/> <input type="button" value="v"/>	SearchType <input type="text" value="Substructure"/> <input type="button" value="v"/>
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Retrieved information...

<p>Record 1 of 1</p> <p>Mark Record</p>	 <p>$C_{13}H_{13}N_3$</p> <p>MW: 211.26</p>	<table border="1"> <tr> <td>Generic Name</td> <td>Varenicline</td> </tr> <tr> <td>Trade name</td> <td>Chantix (Pfizer)</td> </tr> <tr> <td>Compound Id</td> <td>249296-44-4</td> </tr> <tr> <td>Salts</td> <td></td> </tr> <tr> <td>Experiments</td> <td> Photostability Accelerated Stability Studies </td> </tr> <tr> <td>Degradants</td> <td> View Degradants </td> </tr> </table>	Generic Name	Varenicline	Trade name	Chantix (Pfizer)	Compound Id	249296-44-4	Salts		Experiments	Photostability Accelerated Stability Studies	Degradants	View Degradants
Generic Name	Varenicline													
Trade name	Chantix (Pfizer)													
Compound Id	249296-44-4													
Salts														
Experiments	Photostability Accelerated Stability Studies													
Degradants	View Degradants													
<p>Matching Degradants</p>	 <p>$C_{15}H_{15}N_3O_2$</p> <p>MW: 269.30</p> <p>Accelerated Stability Studies</p>													

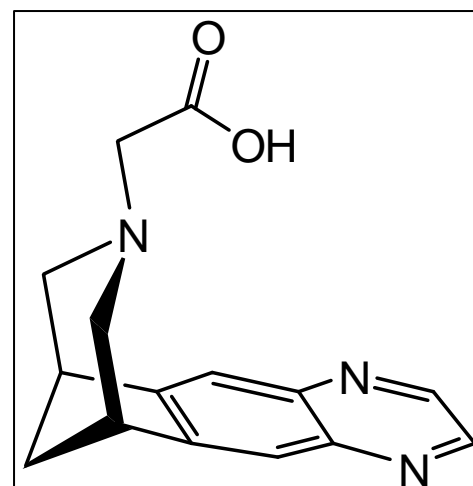
Using Pharma D3 to Speed Up Degradation Product Identification

- Searching on M+58 amu...



Varenicline

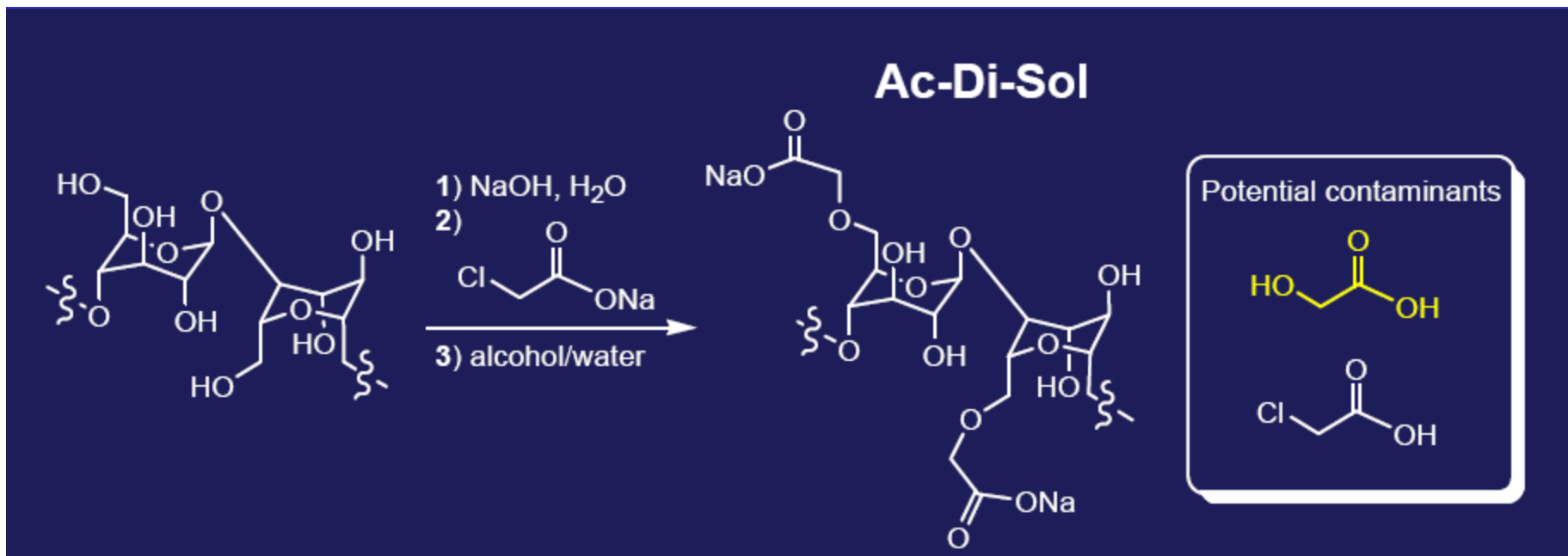
Tablet
formulation
→



M+58 amu

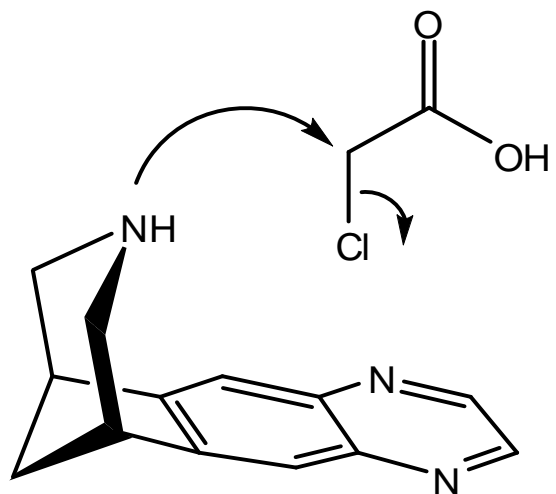
Sodium Starch Glycolate: Chloroacetic acid Contaminant → M+58 Impurity

Varenicline: Excipient Interaction D. Santafianos

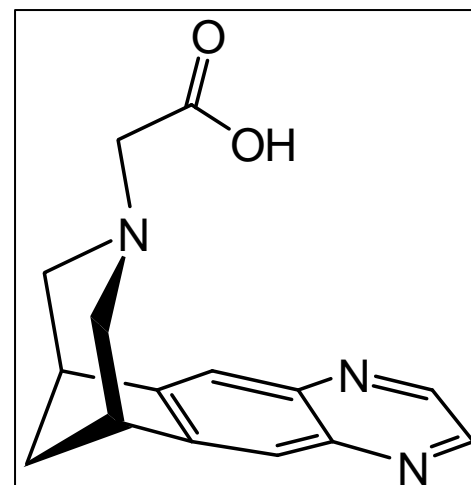


Using Pharma D3 to Speed Up Degradation Product Identification

chloroacetic acid impurity



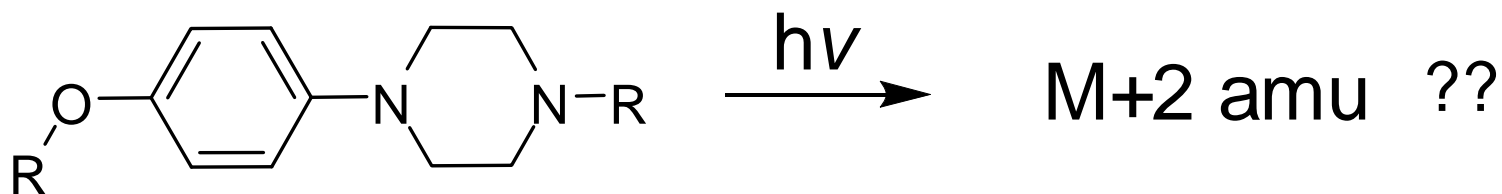
Varenicline



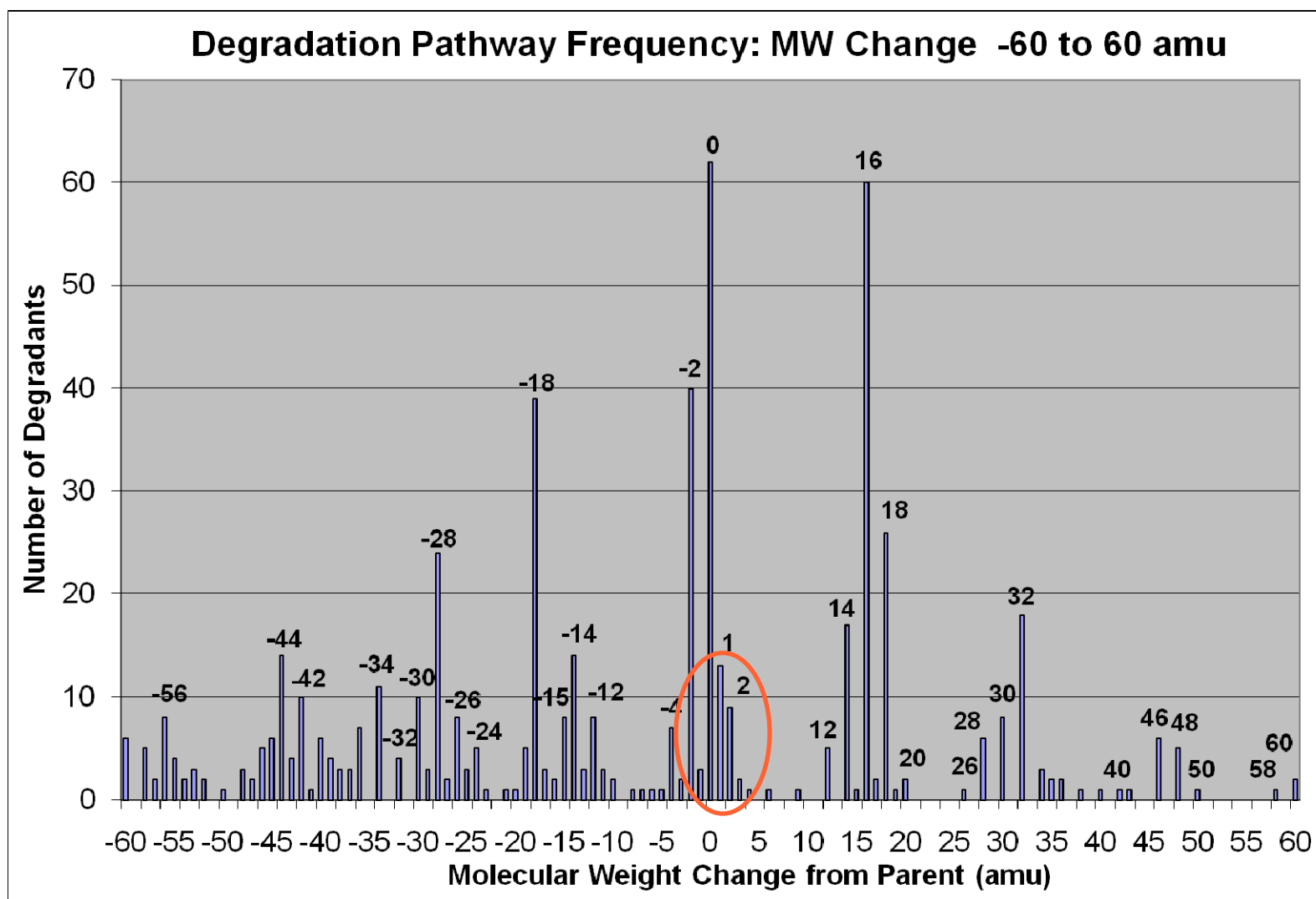
Using Pharma D3 to Speed Up Degradation Product Identification

Situation: New degradant observed in HPLC chromatogram of a photo-stressed API sample.

- LC/MS/ESI reveals MW of degradant to be 2 amu higher than parent drug.



Looking for Patterns in Published Information: Mining the Pharma D3



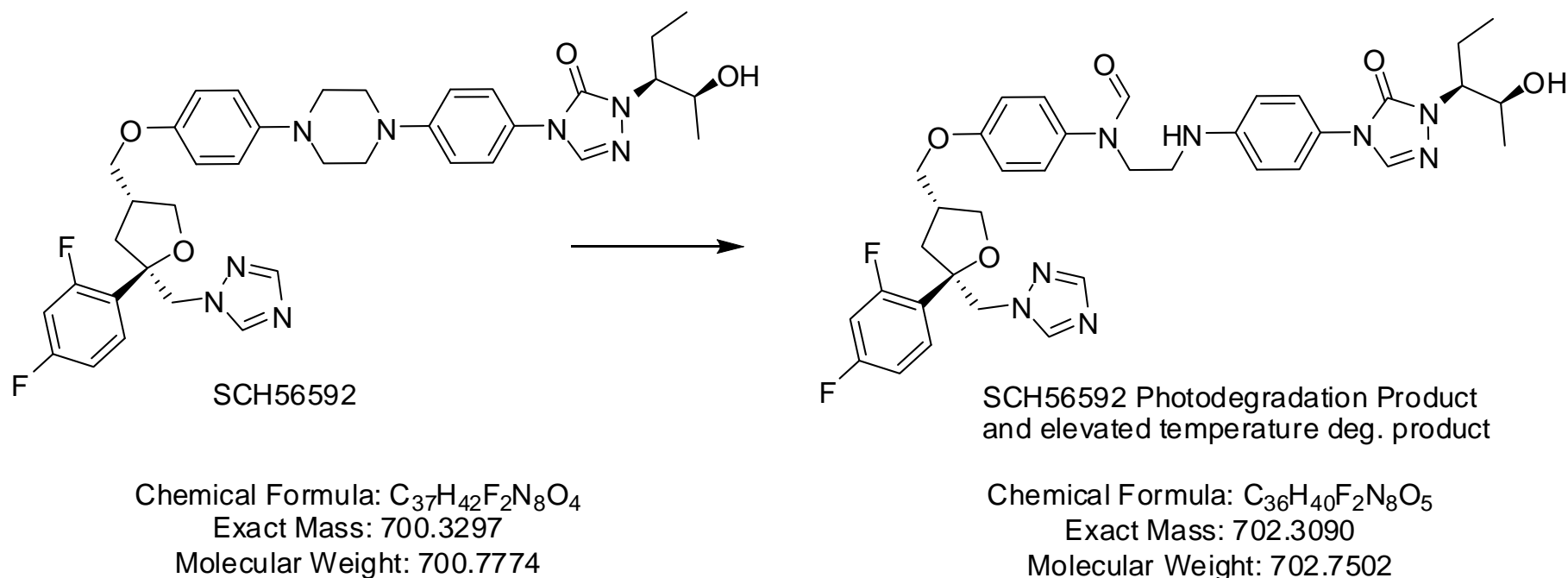
Extracting Information from “Precedence”: Search on “Change in Molecular Weight”: +2

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Trade name	<input type="text"/>	Molecular weight	<input type="text"/>
Common/Other name	<input type="text"/>	Change in Molecular weight	<input type="text" value="+2"/>
Compound number	<input type="text"/>	Salt	-- Select one -- <input type="button" value="v"/>
Functional Group	-- Select one -- <input type="button" value="v"/>	Conditions	-- Select one -- <input type="button" value="v"/>

Parent	Degradant
SearchType <input type="text" value="Substructure"/> <input type="button" value="v"/>	SearchType <input type="text" value="Substructure"/> <input type="button" value="v"/>
<div style="border: 1px solid black; height: 150px;"></div>	<div style="border: 1px solid black; height: 150px;"></div>

Results from Search in Pharma D3

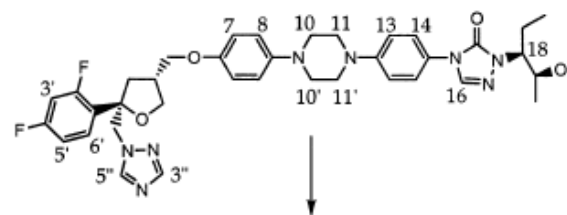
Photodegradation of the piperazine ring leads to an M+2 product, among other products:



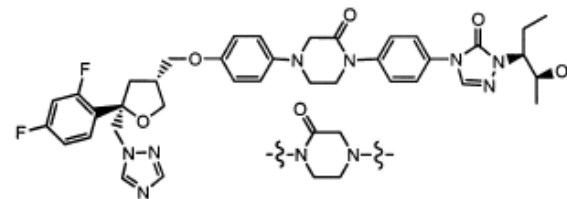
Feng, W.; Liu, H.; Chen, G.; Malchow, R.; Bennett, F.; Lin, E.;
Pramanik, B.; Chan, T. *Journal of Pharmaceutical and
Biomedical Analysis*, 2001(25) 545-557

Other degradation products to look for from this degradation pathway

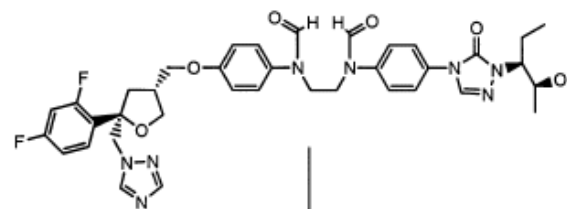
(a) SCH 56592
MW 700
 $C_{37}N_8F_2O_4H_{42}$



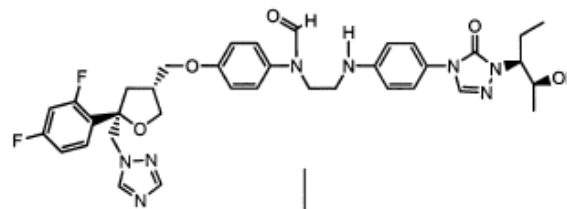
(b) Component A
MW 714
 $C_{37}N_8F_2O_5H_{40}$



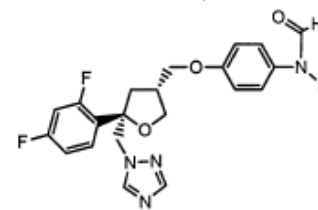
(c) Component B
MW 730
 $C_{37}N_8F_2O_6H_{40}$



(d) Component C
MW 702
 $C_{36}N_8F_2O_5H_{40}$



(e) Component D
MW 414
 $C_{21}N_4F_2O_3H_{20}$



Looking for Patterns in Published Information: Other examples??

Many more examples could be shown.

This database needs to continue to grow...
visit <http://d3.cambridgesoft.com/>



To contribute degradation product examples to PharmaD3, contact any one of these by email:

Dinos Santafianos: dinos.santafianos@pfizer.com

Karen Alsante: karen.m.alsante@pfizer.com

Steve Baertschi: baertschi@lilly.com



Answers That Matter.

Conclusions

- Understanding degradation chemistry is an important aspect of building in Quality (i.e., Quality-by-Design principles)
- Leveraging chemistry knowledge, personal or public, can greatly speed up the process for structure elucidation of unknown degradants and their associated degradation pathways.
- Pharma D3 is a useful tool to elucidate degradation products and pathways.
- Lhasa is developing a chemical degradation prediction program called Zeneth, and is seeking additional consortium member companies.

Acknowledgements



Answers That Matter.

Pharma D3

Karen M. Alsante – Pfizer

Dinos Santafianos – Pfizer

Allen Templeton – Merck

Patrick Jansen – Lilly

Jeffrey Press, Peter Hindmarch,

Jennifer Runyan -- Contractor

Zeneth Degradation Project

Martin Ott – Lhasa Ltd.

