An *in silico* tool to aid identification of degradants from forced degradation studies

Dr Rachel Hemingway
Senior Research Scientist

rachel.hemingway@lhasalimited.org
Aim of the talk

➢ To provide an overview of the current Zeneth program

➢ To outline how the knowledge base is developed

➢ To detail the new scientific features being added into the re-developed software
Contents

• Introduction to Lhasa Limited

• The Zeneth program

• Developing the knowledge base in Zeneth

• New scientific developments

• Future aspirations

• Concluding remarks
INTRODUCTION
Lhasa Limited

➢ Established 1983, headquarters in Leeds, UK

➢ Not-for-profit organisation and educational charity
  - “Shared Knowledge, Shared Progress”

➢ It is controlled by its members (more than 350 organisations worldwide)
  - Pharmaceutical companies
  - Chemical and agrochemical companies
  - Personal product and cosmetic companies
  - Food/Flavour companies
  - Tobacco companies
  - Universities
  - Contract research organisations
  - Government (including regulatory) bodies
  - Consultants
In silico approach

- Forced Degradation
- Stability-Indicating Method
- Degradation Mechanism
- Degradant Identification
- Formulation Development
- Packaging
- Final Formulation

Chemical reaction: $\text{R-COOH} + \text{NaOCl} \rightarrow \text{R-H}$
**In silico assumption**

- **Real Space**
- **Potential Space**
- **Hypothetical Space**

Hypothetical degradation products as in stress testing conditions and *in silico* methods

Potential degradation products as in accelerated and stability studies

Real degradation products in final packaging and storage conditions
Zeneth: an *in silico* tool

**Z** is for…

- Expert knowledge based system for the prediction of forced degradation\(^1,\ 2\)
- Zeneth contains a ‘library’ of chemical transformations

- The program uses its knowledge base to:
  - find ‘matching’ transformations,
  - predict chemical degradation,
  - assess the likelihood of a given degradant

---

Chemical patterns

Chemical degradation reactions are represented as patterns in the knowledge base

1. Degradation reaction

\[
\text{Cefuroxime} \rightarrow \begin{array}{c}
\text{O} \\
\text{H}_2\text{N} \\
\text{O} \\
\text{Na}^+ \\
\text{O} \\
\text{O} \\
\text{O} \\
\end{array} + \begin{array}{c}
\text{O} \\
\text{O} \\
\text{H}_2\text{N} \text{O}^-. \text{Na}^+ \\
\end{array}
\]

2. Determine the ‘degradophore’

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\end{array} \rightarrow \begin{array}{c}
\text{O} \\
\text{O} \\
\end{array} + \begin{array}{c}
\text{O} \\
\end{array}
\]

3. Define the scope of the transformation, masking any proprietary nature

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{R}_1 \\
\text{R}_2 \\
\text{R}_3 \\
\end{array} \rightarrow \begin{array}{c}
\text{O} \\
\text{O} \\
\text{R}_2 \text{R}_3 \\
\end{array} + \begin{array}{c}
\text{O} \\
\text{R}_1 \text{O}^- \\
\end{array}
\]

R1-R3 = any atom
The R2-C=C-R3 bond is cis or aromatic
Excipients

- Potential API-excipient interactions can be predicted by Zeneth
- Important use case for many members
- Database of 188 structures (excipients, counterions and their associated degradants and impurities)

![Cefuroxime](image)

Esterification with primary aliphatic alcohol

![D-Mannitol](image)
Methodology

- **Query**
  - Likelihood Threshold
  - Environmental conditions

- **Knowledge base Transformation Library**

- **Reasoning**
  - Relative: Compares the likelihood of competing transformations
  - Absolute: Evaluates the likelihood that a degradation will occur

- **Degradant**

Input:
- API (+ excipients)

Transformation Engine
Environmental conditions

*Information from Q3B guideline

**PROCESSING CONSTRAINTS**

<table>
<thead>
<tr>
<th>Set</th>
<th>Enabled</th>
<th>Temperature °C</th>
<th>pH</th>
<th>Water</th>
<th>Oxygen</th>
<th>Metal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>✔</td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>20</td>
<td>7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

**PROCESSING CONSTRAINTS**

- **Hydrolysis**
  - 0.1N HCl: 40 °C, 60 °C, 1, 3, 5 days
  - 0.1N NaOH: 40 °C, 60 °C, 1, 3, 5 days
  - Acid Control (no API): 40 °C, 60 °C, 1, 3, 5 days
  - Base Control (no API): 40 °C, 60 °C, 1, 3, 5 days
  - pH: 2, 4, 6, 8
  - 3% H₂O₂: 25 °C, 40 °C, 1, 3, 5 days
  - Peroxide Control: 25 °C, 40 °C, 1, 3, 5 days
  - Azobisisobutyronitrile (AIBN): 40 °C, 60 °C, 1, 3, 5 days
  - AIBN Control: 40 °C, 60 °C, 1, 3, 5 days
- **Oxidative**
  - Light, 1 X ICH: NA, 1, 3, 5 days
  - Light, 3 X ICH: NA, 1, 3, 5 days
  - Light control: NA, 1, 3, 5 days
- **Photolytic**
  - Heat Chamber: 60 °C, 1, 3, 5 days
  - Heat Chamber: 60 °C / 75% RH, 1, 3, 5 days
  - Heat Chamber: 80 °C, 1, 3, 5 days
  - Heat Chamber: 80 °C / 75% RH, 1, 3, 5 days
  - Heat Control: Room Temp., 1, 3, 5 days

*Information from Q3B guideline*
Methodology

Input:
- Likelihood Threshold
- Environmental conditions

Transformation Engine:
- Knowledge base
- Transformation Library

Query

Reasoning
- Relative: Compares the likelihood of competing transformations
- Absolute: Evaluates the likelihood that a degradation will occur

Degradant

API (+ excipients)
Worked example

**Query**

**Likelihood Threshold**

**Environmental conditions**

**Knowledge base**

**Transformation Library**

**Reasoning**

**Degradant**

**Input**

Very Likely

**Conditions:** Water, pH 4

R1, R2 = Carbon or Hydrogen

R3 = Aliphatic or aromatic carbon

C=N bond cannot be in a ring

Transformation 009

\[ \text{Transformation Engine} \]

If [conditions: water] is [certain] then [Hydrolysis variable] is [pH variable]

If [8 <= pH <= 10] is [certain] then [pH variable] is [likely]

If [pH > 10] is [certain] then [pH variable] is [very likely]

If [pH < 8] is [certain] then [pH variable] is [very likely]
DEVELOPING THE KNOWLEDGE BASE
Knowledge base status

Knowledge base Transformation Library

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of transformations</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>300</td>
<td>-</td>
</tr>
<tr>
<td>2014</td>
<td>367</td>
<td>+67</td>
</tr>
<tr>
<td>2015</td>
<td>400</td>
<td>+33</td>
</tr>
<tr>
<td>2016</td>
<td>446</td>
<td>+46</td>
</tr>
<tr>
<td>2017</td>
<td>492</td>
<td>+46</td>
</tr>
</tbody>
</table>

- Oxidation (152)
- Hydrolysis (110)
- Condensation (93)
- Elimination (59)
- Isomerisation (36)
- Photolysis (42)

Total = 492
Industry influence

- Run brainstorming sessions with a group of Zeneth users
- We put forward proposals for inclusion in the knowledge base
- Industry can (and do) suggest proposals

These decisions directly influence the chemistry that goes into the knowledge base
Industry proposals

Recent additions to the Knowledge base Transformation Library

**Boron chemistry**

1. $\text{PhB(OH)}_2 \xrightarrow{\Delta, \text{H}_2\text{O}} \text{PhH}$
2. $\text{F_B(OH)}_2 \xrightarrow{\Delta, \text{H}_2\text{O}} \text{FH}$
3. $\text{X_B(OH)}_2 \xrightarrow{\Delta, \text{H}_2\text{O}} \text{XH}$

where $X = O, S$ (divalent) or $X = C$(univalent), $R1$ is any atom.

The atoms marked * are boron, and the atoms marked $\bullet$ are oxygen.

**Norrish-Yang cyclisation**

1. $\text{R}_1\text{N} = \text{R}_2\xrightarrow{\text{hv}} \text{R}_3\text{O} = \text{R}_4$

where $R1 = \text{aromatic carbon}$, $R2 = \text{aliphatic carbon (not multiply bonded and not attached to another heteroatom), aromatic carbon or hydrogen}$, $R3 = \text{aliphatic carbon (not multiply bonded), aliphatic carbon (multiply bonded to a heteroatom), aromatic carbon or S-R5}$, $R4 = \text{aliphatic carbon (not multiply bonded), aromatic carbon or hydrogen}$, $R5 = \text{carbon}$.

The carbonyl groups cannot be in a ring.

+ hydrolysis of borinic, boronic and boric esters
NEW SCIENTIFIC DEVELOPMENTS FOR ZENETH 8
Re-development

What’s new?

- Degradants will be ‘scored’ instead of being given a defined likelihood level
- Inclusion of a property calculator to calculate pK\textsubscript{a} values
- Improvements in treatment of stereochemistry
- Ability to handle trimolecular reactions
Scoring methodology

Moving from likelihood levels to scores → lead to more granular predictions

**scale used for illustrative purposes only, subject to change**

Formula = \( \min\left(\frac{(pH-1)}{10} + 0.1, 0.9\right) \)

pH profile 10 (base catalysed)

What are my top 20 most likely degradants?
• $pK_a$ of a molecule determines its protonation state at a given pH
• $pK_a$ tends to be governed by electronic effects that decrease with the distance from the centre of protonation
• Generate a distance spectrum for each atom-type from the $pK_a$ centre
pK$_a$ performance

Preliminary results using 5-fold cross-validation

RMSE: 1.163
How will this calculator improve predictions?

pH = basic

Score: 0.85  Score: 0.71
Stereochemistry

- Have to define stereochemistry to garner stereochemical information in any resulting degradants
  - No longer get individual isomers as degradants from an epimerisation reaction unless the query had its stereochemistry defined

- $S_N2$-type reactions will now demonstrate inversion of configuration

Transformation 134: Nucleophilic ring opening of aziridine
Trimolecular reactions

The new platform gives us the capabilities to express trimolecular reactions.

\[ 2 \text{Ph} \text{CO}_2\text{H} + \text{HO:B:OH} \rightarrow \text{Ph:C:O:O:Ph} \]

\[ 2 \text{tryptophan:CO}_2\text{H} + \text{CHO} \rightarrow \text{H}_2\text{N:CH:N:CO:OH} \]
FUTURE ASPIRATIONS FOR ZENETH
Other calculators

**BDE calculator**

- Calculating BDEs is not trivial
- Balance between accuracy and speed of performance is a critical factor
- QM based calculation..................a cheap one!

**Chromophore calculator**

- Assess (the important) wavelength
- Applied as a whole-molecule property
- Modulate the prediction of photochemical reactions
Reducing false positives

- Zeneth predicts in the hypothetical chemical space
- There will always be a level of overprediction in comparison to the potential (or real) space
- Assessing how to reduce the number of false positives using a validation study

(Small) dataset of ~30 compounds with experimental data
(forced deg, accelerated and stability studies)

➢ Scope amendment?
➢ Lower the score given?
➢ Knowledge gaps
➢ Expand knowledge base
Ideal *in silico* scenario

- **Real Space**: Real degradation products in final packaging and storage conditions
- **Potential Space**: Hypothetical degradation products as in stress testing conditions and *in silico* methods
- **Hypothetical Space**: Potential degradation products as in accelerated and stability studies

**API**: Represents the active pharmaceutical ingredient.
Zeneth is our expert knowledge based system for the prediction of forced degradation.

Helps understand mechanistic pathways as well as aid degradant identification.

Industry helps drive the development of the knowledge base and directly influences the content.

Zeneth is undergoing a complete re-design with the aim of improving usability as well as the quality and speed of predictions.

Scientific developments include a granular scoring method, improved stereochemical behaviour and inclusion of a calculator for pK_a.

Zeneth generates predictions from its knowledge base which currently contains 492 transformations.
Come see us!

Booth number: 10

Lisa Leivers
Product Owner for Zeneth

Maggie Coombs
US Account Manager for Zeneth

Alison Reeves
Sales executive
Acknowledgements

Special thanks to:

Dr Martin Ott
Principal Scientist

Dr Jeff Plante
Senior Research Cheminformatician

Dr Carlos Cunha
(ex)-US Account Manager

Thank you all for your attention
Questions?