Brief History of Penicillin

1896 – Ernest Duchesne noted antibacterial properties of mould
1928 – Alexander Fleming was studying staphylococci & found a culture died after contaminated with mould. Attributed this to penicillin.
1930 – Initial testing of drugs on selected patients
1940s – Merck & Co. mass production of penicillin for war
1945 – Bacterial resistance began to be observed

Penicillin Activity

Penicillin is an active antibiotic due to its ability to weaken bacterial cell walls. The cell wall is composed of alternating N-acetylglucosamine and N-acetylmuramic acid linkages. The muramic acid linkages have dangling tetrapeptides composed of (L-alanine, D-glucosamine, L-lysine and D-alanine) that can form cross-links between rows. β-lactam antibiotics prevent crosslinking, leaving the bacterial cells vulnerable to attack. However, there is a naturally occurring enzyme (β-lactamase) that can hydrolyze the β-lactam ring, leaving it ineffective.

Penicillin Development

1) Derivatization - alter sterics, bioavailability, etc.

R =

- penicillin G
- ampicillin
- penicillin V
- amoxicillin
- ticaricillin
- piperacillin

2) β-lactamase inhibitors - bind irreversibly to β-lactamase

- clavulanic acid
- salbactam
- tazobactam


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Composite Penem Antibiotics

Amoxicillin & Clavulate
(Augmentin – GSK)
Routine ear, lung and sinus infections
Highest bioavailability of penicillin drugs
On market since 1981

Ampicillin & Salbactam
(Unasyn - Pfizer)
Skin, bone, gynecological and abdominal infections
Typically second line defense when bacteria found to be resistant to other derivatives
On market since 1987

Ticarcillin & Clavulate
(Timentin - GSK)
Blood, bone, respiratory, urinary tract infections
On market since 1998

Piperacillin & Tazobactam
(Zobactin - GSK, Zosyn - Pfizer)
Most active antibiotic against Klebsiella bacteria in pneumonia, Urinary tract infections (UTIs), meningitis, blood diseases etc.
On market since 1993

Example 1H NMR Data from Penicillin Family

Piperacillin
1H NMR (d6-DMSO):

400 MHz

60 MHz
**1H NMR Data cont.**

_Tazobactam_  
**1H NMR** (d<sub>6</sub>-DMSO):

400 MHz

60 MHz

\[
\begin{align*}
J_{AB} &= (\nu_1 - \nu_2) = (\nu_3 - \nu_4) \\
\Delta \nu_{AB} &= \sqrt{(\nu_1 - \nu_4)(\nu_2 - \nu_3)}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Frequency</th>
<th>60 MHz</th>
<th>400 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>(J_{AB}) (Hz)</td>
<td>1.4</td>
<td>9</td>
</tr>
<tr>
<td>(\Delta \nu_{AB}) (Hz)</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>(\Delta \delta_{AB}) (ppm)</td>
<td>23.8</td>
<td>162.11</td>
</tr>
<tr>
<td>(\delta_A) (ppm)</td>
<td>3.63</td>
<td>3.64</td>
</tr>
<tr>
<td>(\delta_B) (ppm)</td>
<td>3.23</td>
<td>3.23</td>
</tr>
</tbody>
</table>

**Structural Confirmation Software Tools**

Despite second order effects there are a number of software packages that can used to verify the structure and identify impurities (e.g., Mnova Verify, ACD/Labs Spectrus Processor, or Perch Solutions, shown here where the blue trace is experimental, the red trace simulated and the green the difference).

courtesy of Perch Solutions at perchsolutions.com
Relative Compositional Analysis

Overlaid $^1$H NMR spectra of piperacillin-tazobactam mixtures of varying relative concentration for use in manual and automated quantification.

$^1$H NMR ($d_6$-DMSO):

![NMR spectra diagram]

Manual Integration

While $^1$H resonances of piperacillin and tazobactam overlap, there are distinctive regions that can be integrated for each component. We have chosen the 2 CH=CH peaks from the tazobactam triazole ring ($\delta = 7.6595-8.2236$ ppm) and the 5 aromatic CH's from the monosubstituted benzene ring in piperacillin ($\delta = 6.8856-7.6595$ ppm).

The normalized integral of each region can be used to determine the percent piperacillin in a $d_6$-DMSO solution. This was done for a known concentration series (0, 30, 40, 60, 70 and 100% piperacillin) as well as with an unknown injection mixture to determine the accuracy and linearity of this qNMR experiment.

The injection mixture was found to be 83.11% piperacillin : 16.88% tazobactam. This is in very good agreement with the known ratio in the prepared injection mixture 82.3 : 17.7%.

\[
% \text{pip} = \left[ \frac{\frac{1}{5} \text{Int}_{\text{pip Ar-H}}}{\left(\frac{1}{5} \text{Int}_{\text{pip Ar-H}} + \frac{1}{2} \text{Int}_{\text{tazo HC=CH}}\right)} \right] \times 100
\]
Automated Analysis

In order to simplify routine qNMR analysis we also looked at the piperacillin : tazobactam ratio using Mnova’s Simple Mixture Analysis (SMA). This allows a user to speed up the analysis and facilitate it for non-experts.

Once the method is established anyone can determine concentration or relative percentage by simply hitting the green ‘Analyze’ button.
For more information about pharmaceuticals, qNMR or other benchtop NMR application inquiries please visit

www.nanalysis.com