Quantification of Composite β-Lactam/ β-Lactamase Inhibitor Antibiotics with 60 MHz Benchtop NMR

Brief History of Penicillin

1896 – Ernest Duchesne noted antibacterial properties of mould

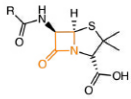
1928 – Alexander Fleming was studying staphylococci & found a culture died after contaminated with mould. Attributed this to penicillin.

1930 – Initial testing of drugs on selected patients

1940s – Merck & Co. mass production of penicillin for war

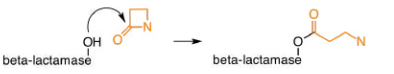
1945 – Bacterial resistance began to be observed

Penicillin Activity



penicillin core

Pencillin is an active antibiotic due to it's 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.[1] However, there is a naturally occurring enzyme (β-lactamase) that can hydrolyze the β-lactam ring, leaving it ineffective.



[1]Lewis, K. *Nature Rev Drug Disc*, **2013**, *12*, 371; Aminov, R. I. *Front. Microbiol*, **2010**, *134*, 1

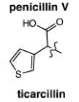
Penicillin Development

1) Derivatization - alter sterics, bioavailability, etc.

R =

penicillin G

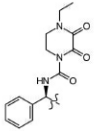
penicillin V



ticarcillin

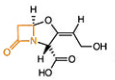
ampicillin

amoxicillin



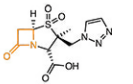
piperacillin

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



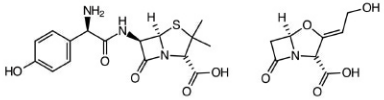
clavulanic acid

salbactam



tazobactam

Composite Penem Antibiotics



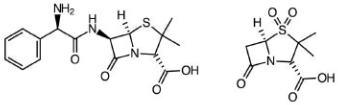
Amoxicillin & Clavulate

(Augmentin – GSK)

Routine ear, lung and sinus infections

Highest bioavailability of penicillin drugs

On market since 1981



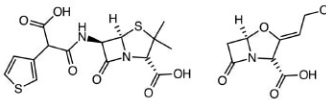
Ampicillin & Salbactam

(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

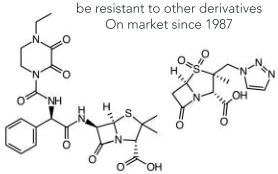


Ticarcillin & Clavulate

(Timentin - GSK)

Blood, bone, respiratory, urinary tract infections

On market since 1998



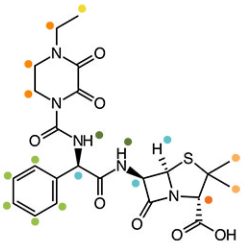
Piperacillin & Tazobactam

(Zobactin - GSK, Zosyn - Pfizer)

Most active antibiotic against *Klebisella* bacteria in pneumonia, Urinary tract infections (UTIs), meningitis, blood diseases etc.

On market since 1993

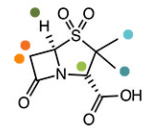
Example ^{1}H NMR Data from Penicillin Family



Piperacillin

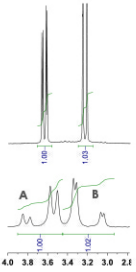
^{1}H NMR (d_6-DMSO):

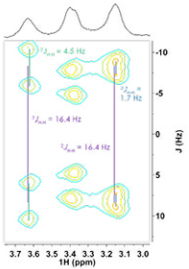
^{1}H NMR Data cont.



Tazobactam

^{1}H NMR (d_6-DMSO):





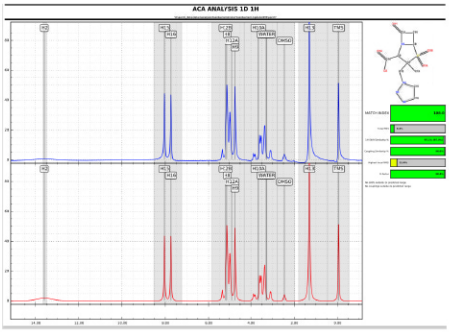
$$J_{AB} = (\nu_1 - \nu_2) = (\nu_3 - \nu_4)$$

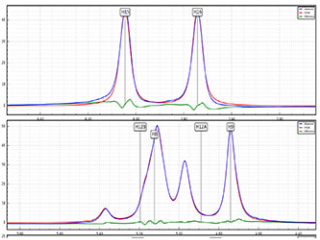
$$\Delta\nu_{AB} = \sqrt{(\nu_1 - \nu_4)(\nu_2 - \nu_3)}$$

	60 MHz	400 MHz
$\Delta\nu_{AB}/J_{AB}$ (Hz)	1.4	9
J_{AB} (Hz)	17	18
$\Delta\nu_{AB}$ (Hz)	23.8	162.11
$\Delta\delta_{AB}$ (ppm)	0.40	0.41
δ_A (ppm)	3.63	3.64
δ_B (ppm)	3.23	3.23

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).

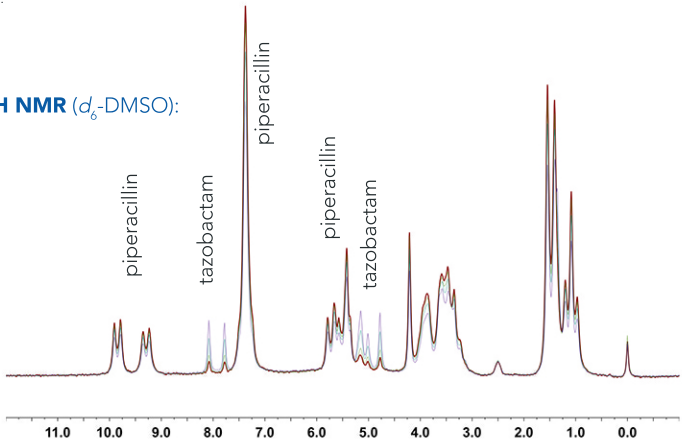




courtesy of Perch Solutions at perchsolutions.com

Relative Compositional Analysis

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

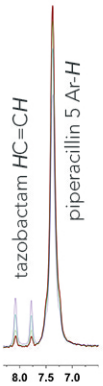


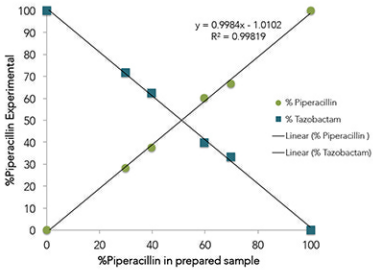
Manual Integration

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 (δ = 7.6595-8.2236 ppm) and the 5 aromatic CH's from the monosubstituted benzene ring in piperacillin (δ = 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.

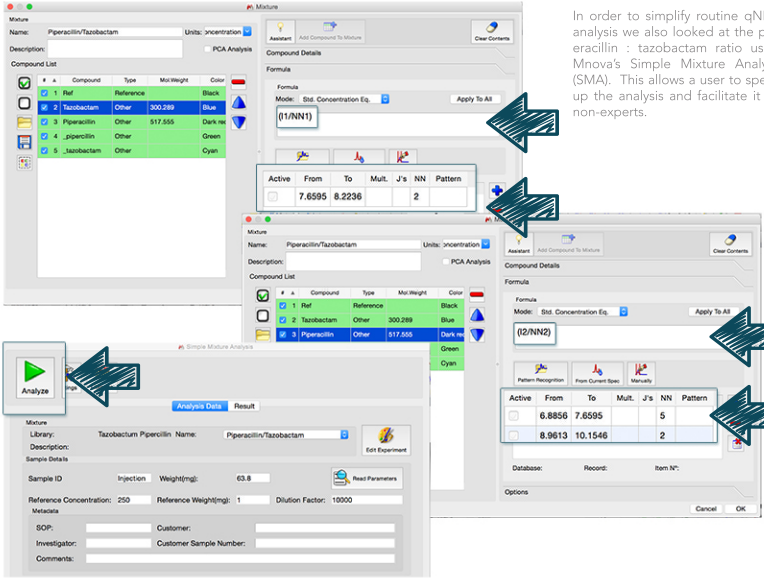
The injection mixture was found to be 83.11% piperacillin : 16.88% tazobactam. This is in very good agreement with the known ratio in the prepared injection mixture 82.3 : 17.7%.





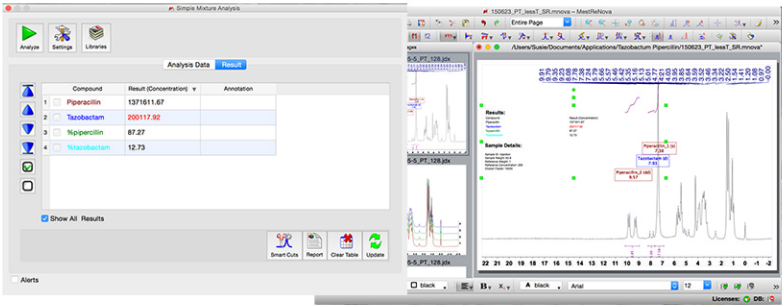
$$\%\ pip = \left[\frac{(1/5)Int_{pipAr-H}}{((1/5)Int_{pipAr-H} + (1/2)Int_{tazoHC=CH})}\right] 100$$

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