Powerful Software Solutions for Drug Metabolite Quantitation and Identification

Powerful Software Solutions for Drug Metabolite Quantitation and Identification

In this webinar we will discuss LC/MS and NMR software workflows, including new algorithms to add power to your drug metabolite quantitation and identification

Today's Presenters from Agilent Technologies:

Steve Madden
Software Product Manager, MassHunter

Doug McIntyre
Software Product Manager, MassHunter

Frank Delaglio
Marketing Manager, Magnetic Resonance Software

Dave Russell
Applications Scientist, NMR Division

Today's Host:

Lauren Constable
Head of Commissioning, FSG publishers of *Bioanalysis*

Steve Madden

Software Product Manager, MassHunter

Agilent Technologies

Doug McIntyre

Software Product Manager, MassHunter

Agilent Technologies

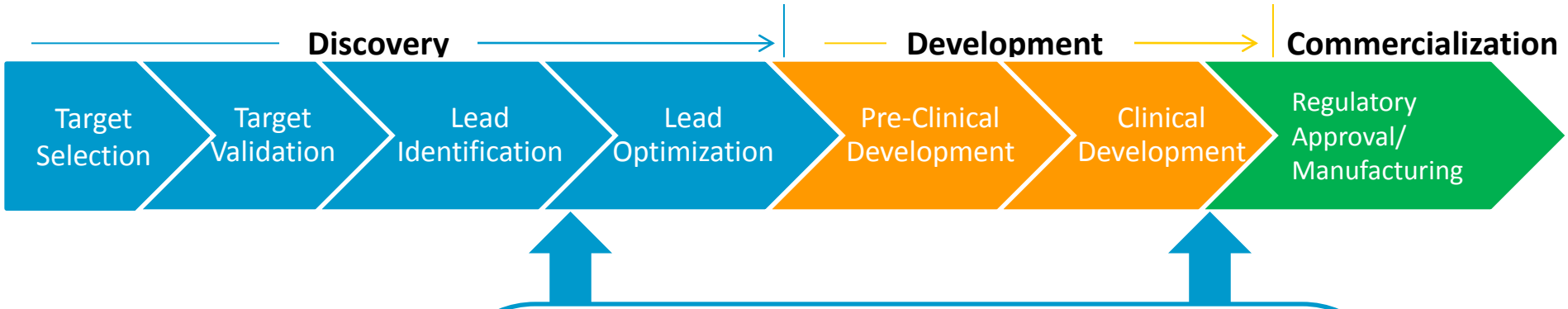
Powerful Software Solutions for Drug Metabolite Quantitation and Identification

- Metabolite Identification
- Quantitative Analysis
- Qual/Quan

June 25, 2013

Steve Madden
Doug McIntyre
Agilent Technologies Inc.
California, USA

Metabolite ID in the Drug Discovery & Development



Study Aim

- Optimize metabolic stability of lead compounds
- Avoid drug-drug interactions
- Profiling metabolite across species
- Help select safety species

Soft spot analysis

- modulate metabolic clearance

Identify isozyme specific metabolites

Identify pharmacologically active metabolites

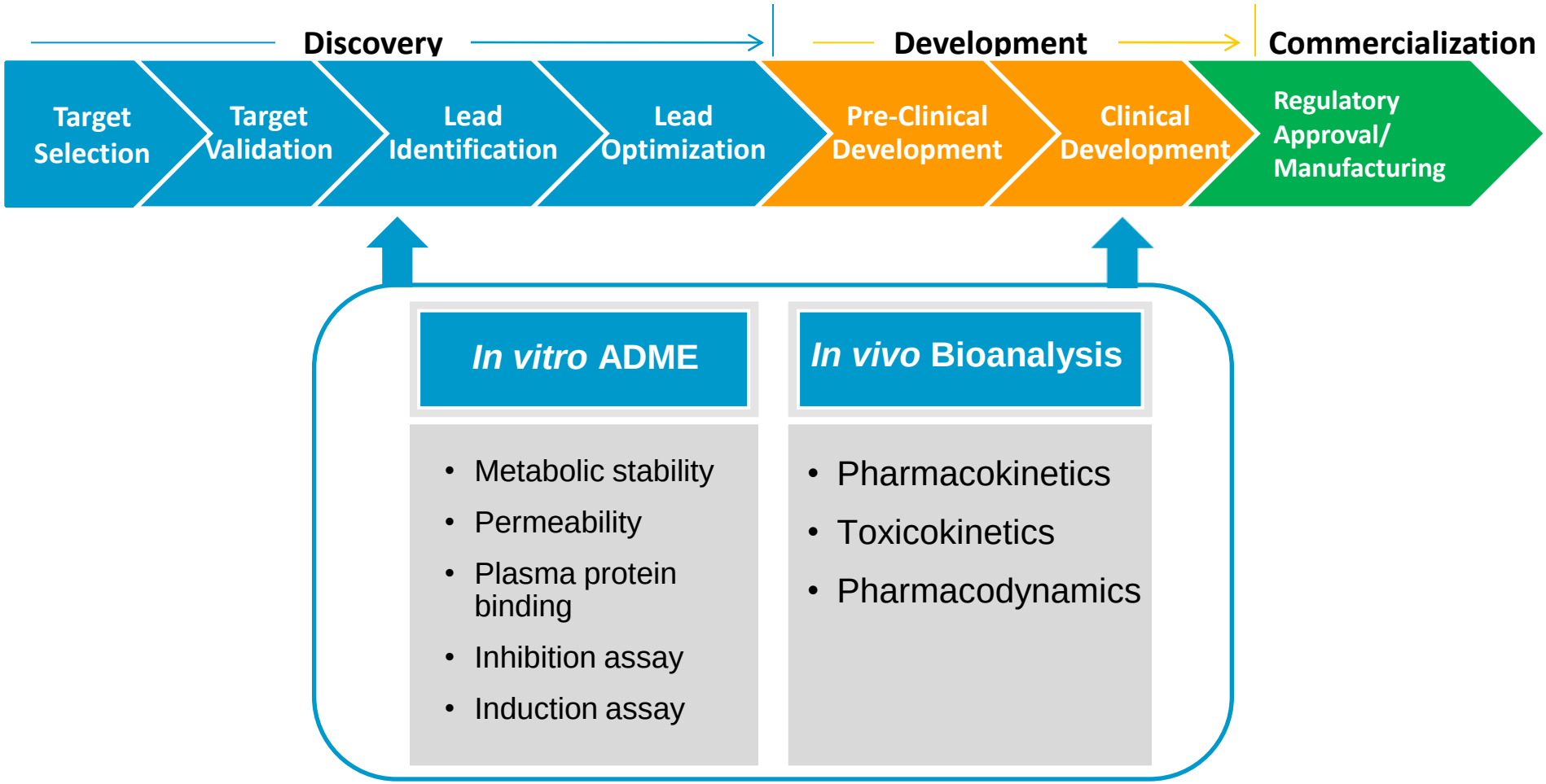
- secure IP protection, understand PK/PD and identify new leads

Identify reactive metabolites

•minimize potential IDRs

Identify toxic metabolites

Quantitation in ADME/PK



Agenda

- Metabolite Identification
  - MassHunter Metabolite ID workflow determining biotransformations
  - Molecular Discovery's Mass-MetaSite workflow for structure prediction and batch processing
- Quantitative Analysis

  --Study Manager and Optimizer for automating Acquisition

  --Compliance features

  --LIMS connectivity
- Qual/Quan Application

Metabolite Identification Software Tools

- Remove the bottleneck of data analysis and interpretation

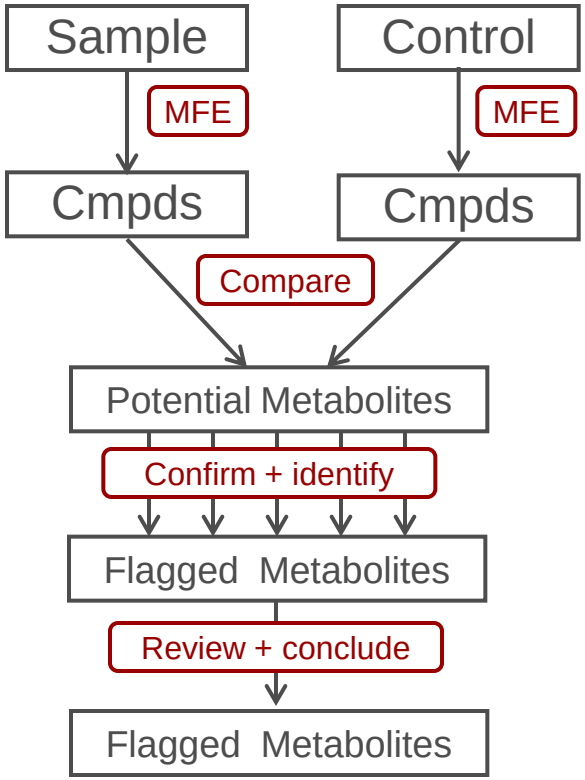
- MassHunter Metabolite ID
- Molecular Discovery's Mass-MetaSite
  - Many Pharma companies use Mass-MetaSite as a global software tool for metabolism studies

Goal

- Increase throughput for metabolite identification
- Provide rapid confident metabolite identification

MassHunter Metabolite ID: Untargeted approach based on MFE comparison

Best approach for expected and unexpected metabolites

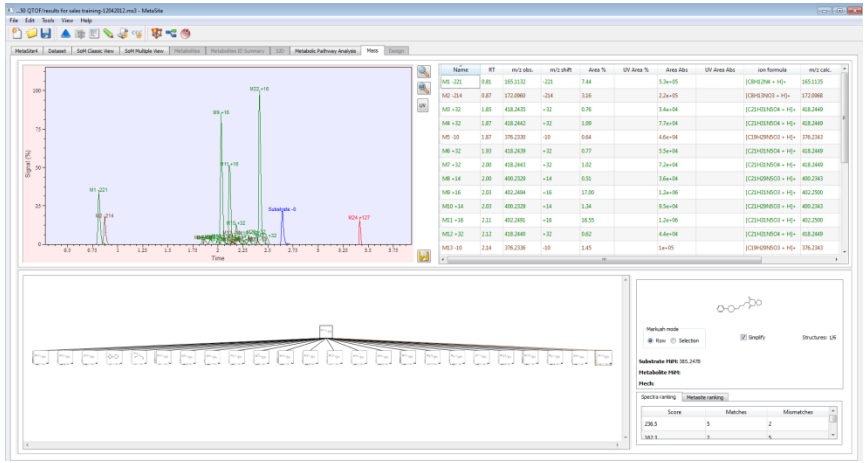


- Untargeted approach via comparison of MFE Compound lists
  - Sophisticated Sample-Control Comparison Algorithm based on Molecular Feature Extraction (MFE) allows more comprehensive detection of differences via exploitation of mass and RT resolution.
  - Best suited to find and confirm expected and unexpected metabolites
  - Find all differences and THEN confirm and identify
  - Confirm expected and identify unexpected with multiple and more sophisticated algorithms

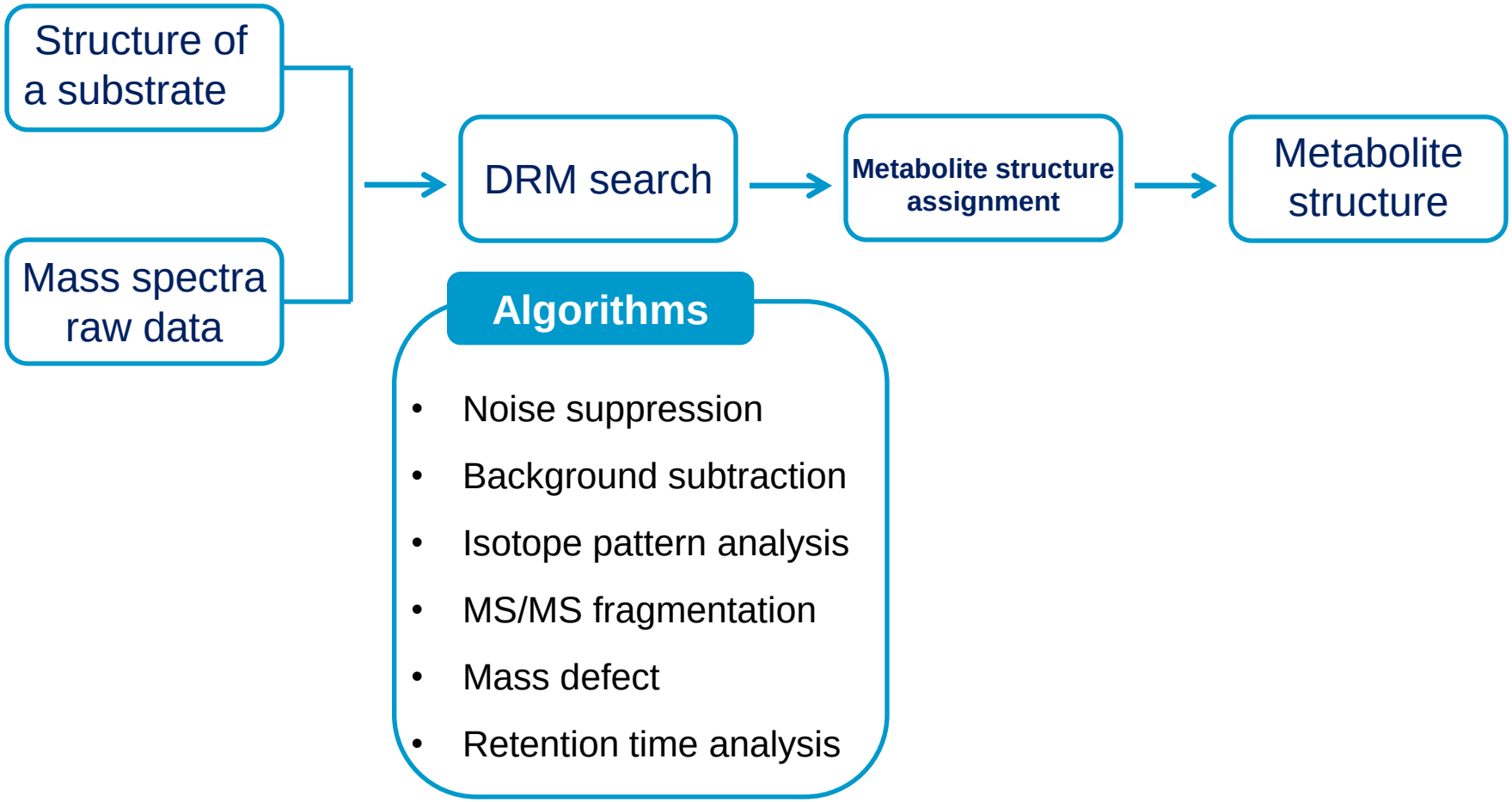
Mass-MetaSite Software

- A desired solution for rapid metabolite Identification

- Fully automated data analysis and structure elucidation
- Site of metabolite localization based on MS/MS data
  - Not on *in silico* predictions as in Metasite
  - Metasite predictions used to designate the most likely SoM within a Markush structure
- Metabolite structure proposal relies on included reaction mechanism (Phase I & II biotransformations)
- Batch processing capability for high throughput application



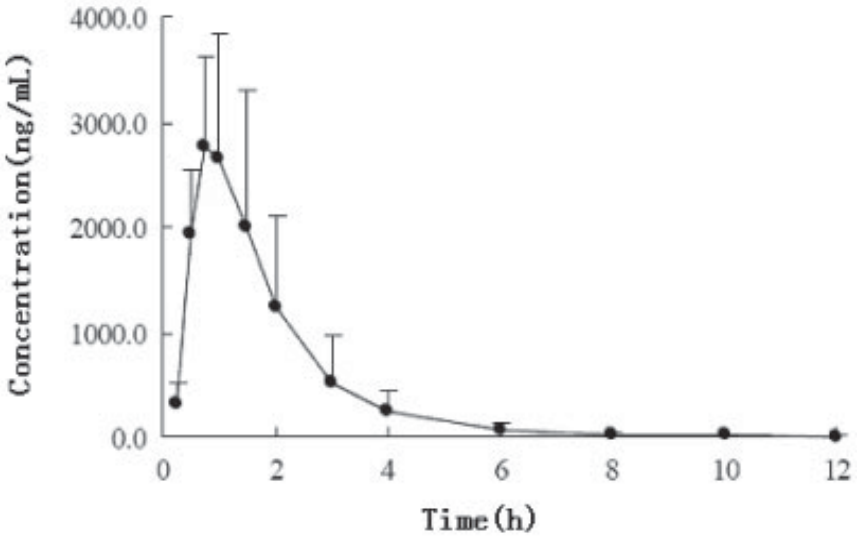
Mass-MetaSite Data Analysis Workflow



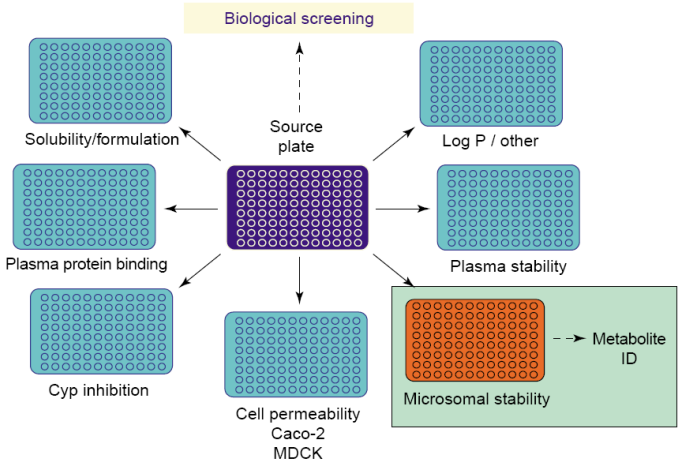
The entire process only takes several minutes

LC/MS Quantitation: Two Major ADME/PK Applications

- Bioanalysis (PK, BA/BE)
  - Measure drug candidate concentration (and metabolites) over time in animals or humans
  - May require compliance option



- (ADME) Drug Discovery Screening
  - Determine properties of lead molecules (permeability, metabolic stability, …)
  - Often 500–1000 compounds/week

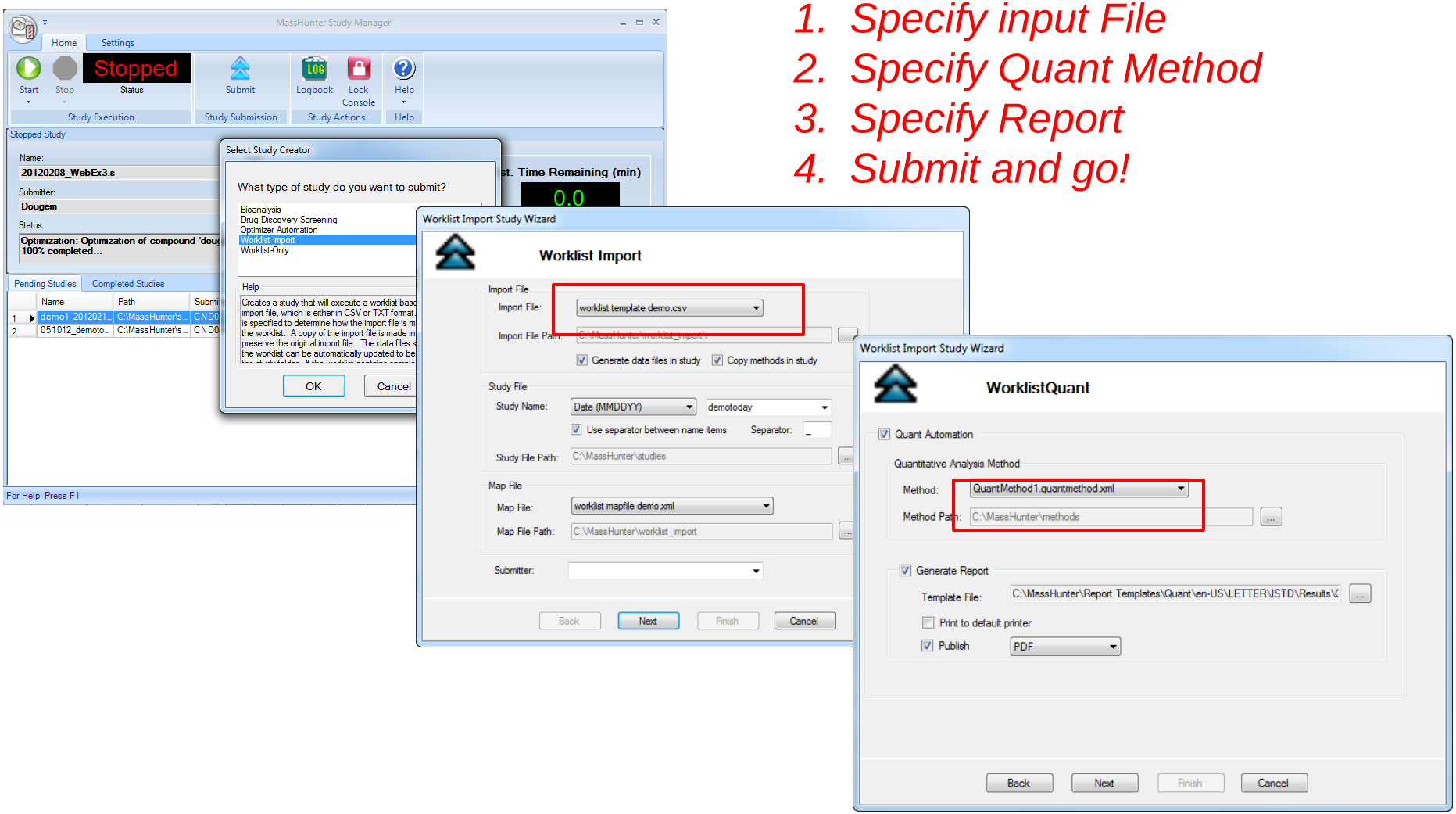


MassHunter Study Manager for Automating Workflow

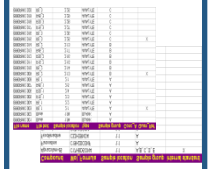
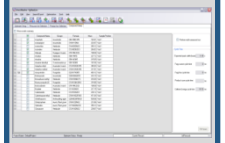
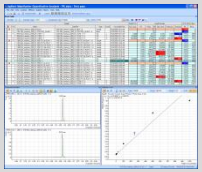
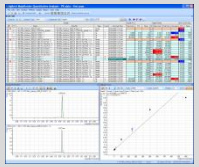
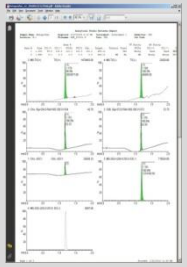
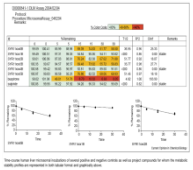
- User selects workflow and submits sample sets (studies) using Excel or text files
- Coordinates acquisition, compound optimization, quantitation and report generation
- Uses different Study Creators for each workflow
  - Generic BioAnalysis
  - Watson LIMS connectivity
  - ADME Screening
- Can be used in compliant mode

Fully Automated Quantitation Workflow

1. *Specify input File*
2. *Specify Quant Method*
3. *Specify Report*
4. *Submit and go!*



HT Targeted Quant in Drug Discovery Screening – "*in vitro*"

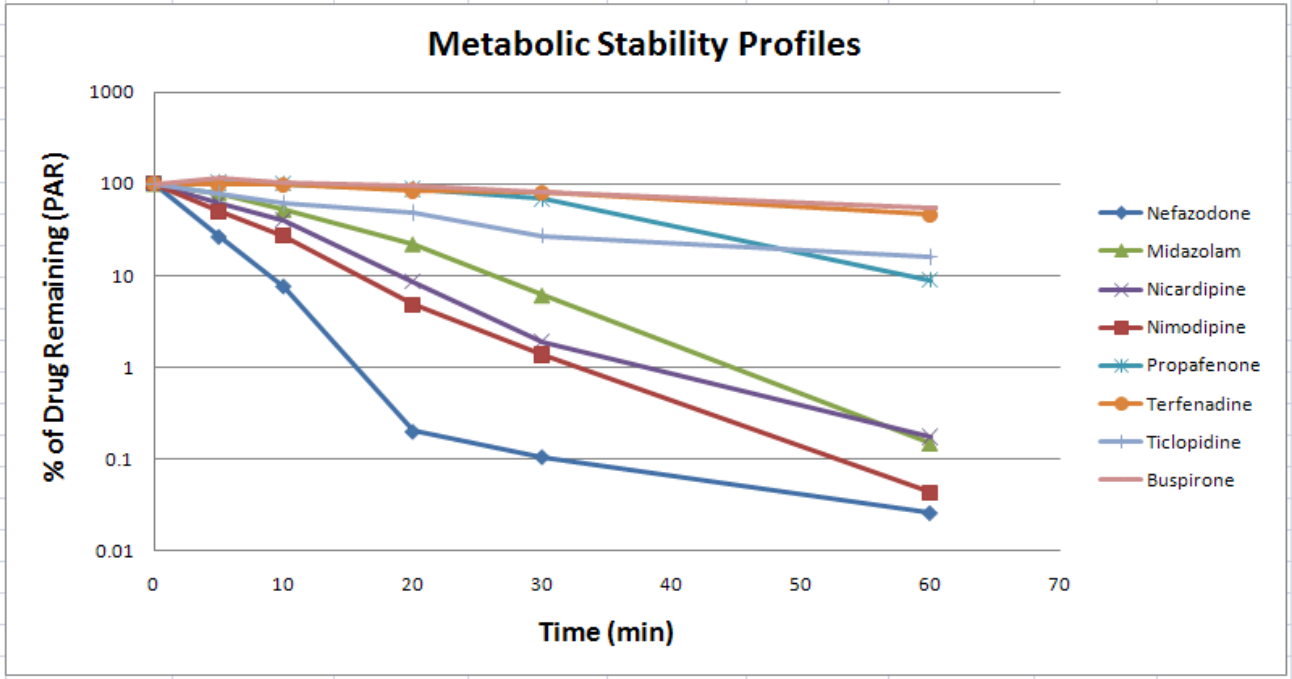
Import Sample Set	Submit Study	Optimize Compounds	Acquire Samples	Analyze Batch	Review Results	Report	Calculate Values
							
Excel or other Electronic Source	MassHunter Study Manager	MassHunter Optimizer	MassHunter Acquisition	MassHunter Quant	MassHunter Quant	MassHunter Quant Reporting	End User Application Software

Study Manager schedules and automates these steps

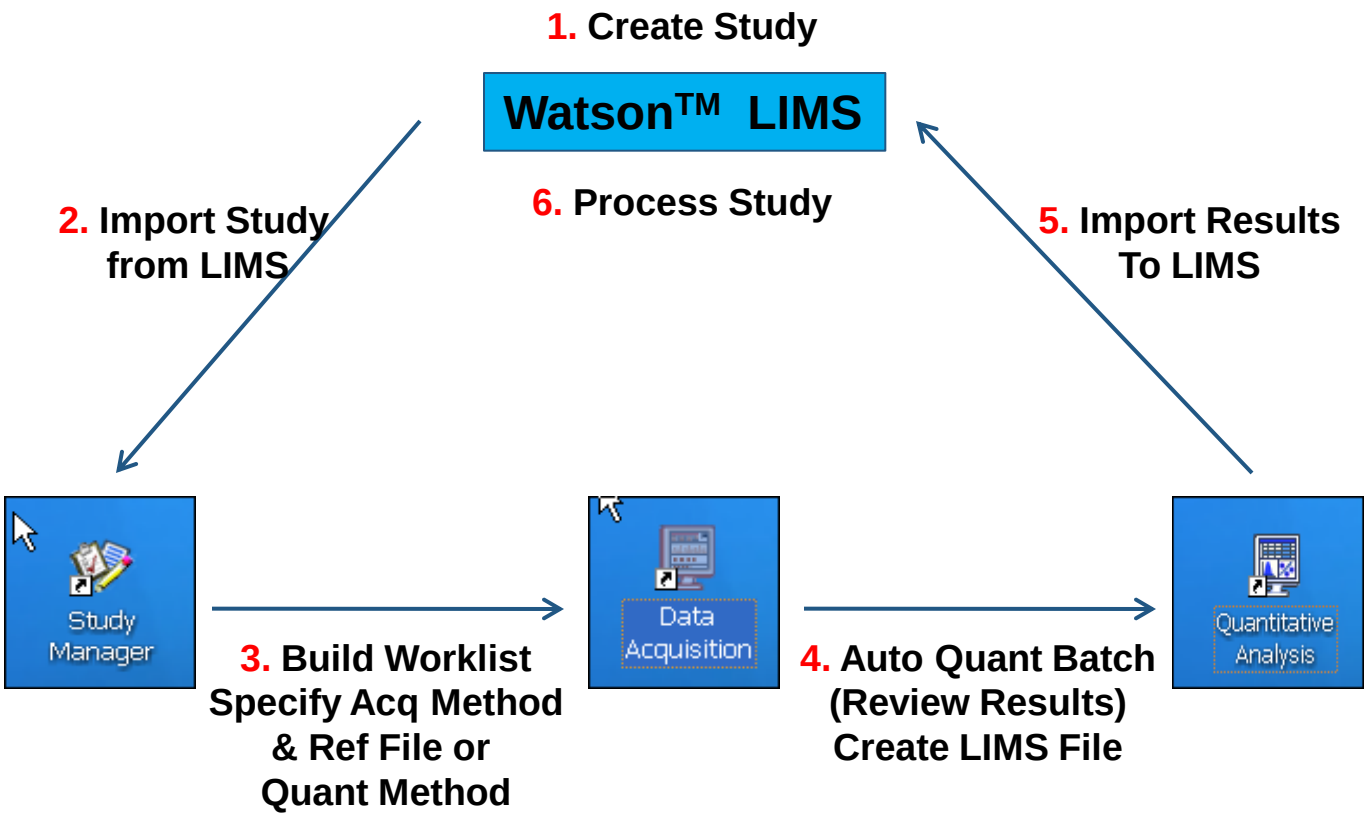
Which of the synthesized compounds will be viable drug candidates?

Metabolic Stability Profiles

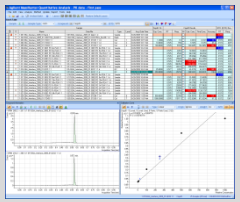
Time (min)	Nefazodone	Nimodipine	Nicardipine	Midazolam	Propafenone	Terfenadine	Ticlopidine	Buspirone
0	100	100	100	100	100	100	100	100
5	26.5	49.9	62.7	78.5	103	97.3	78.1	115
10	7.66	27.2	40.3	52.0	101	97.8	61.5	102
20	0.202	4.86	8.60	22.0	88.5	85.1	48.8	93.9
30	0.107	1.37	1.91	6.09	69.6	80.1	27.1	80.2
60	0.026	0.043	0.177	0.150	8.92	45.8	16.0	54.5



Bioanalysis Using Watson LIMS Workflow



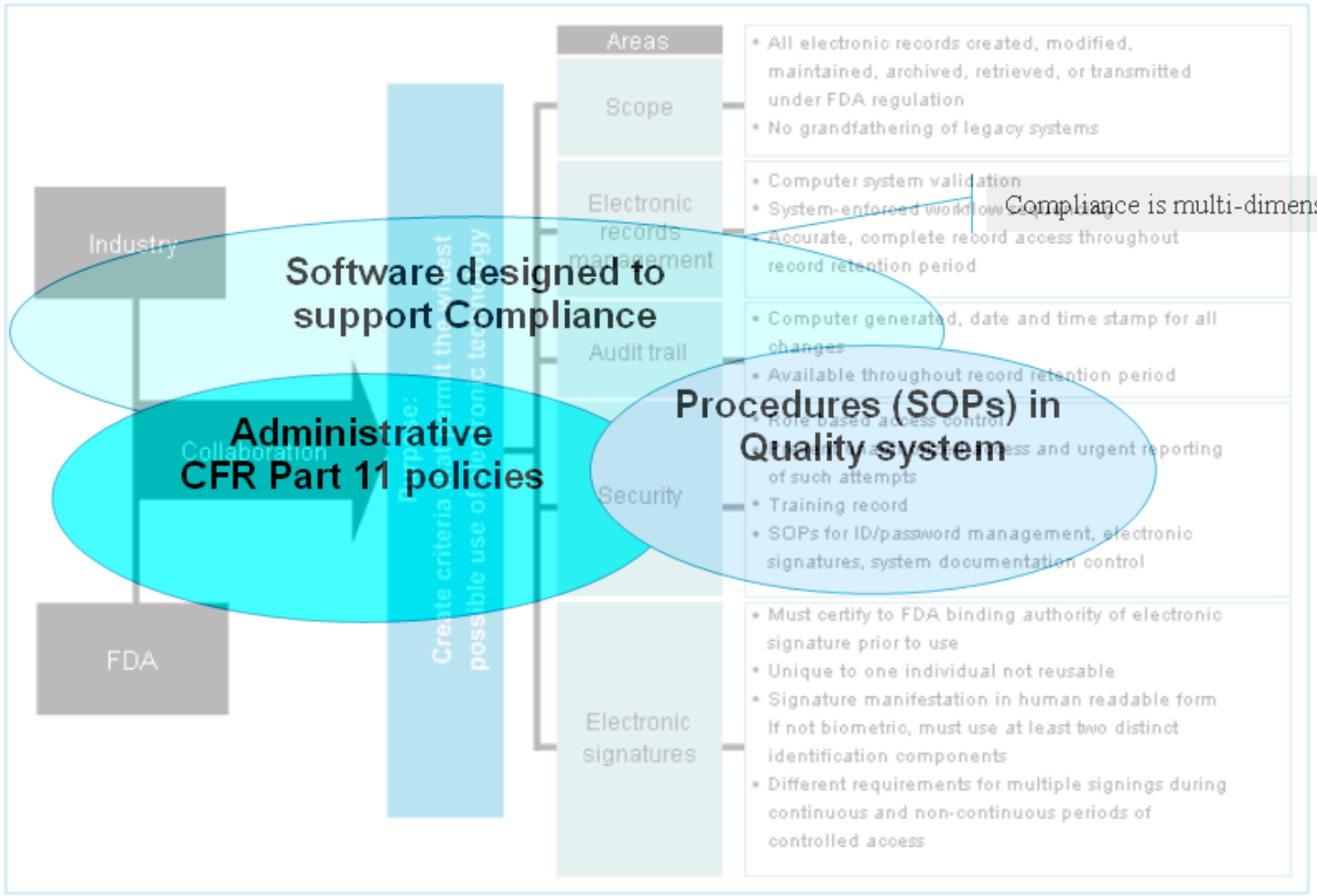
Targeted Quant in Bioanalysis

Watson LIMS	Submit Study	Run Worklist	Analyze Batch	Review Results	Report	Watson LIMS
						
Generate Studies (Sample lists)	MassHunter Study Manager	MassHunter Acquisition	MassHunter Quant	MassHunter Quant	MassHunter Quant Reporting	Upload results (Conc. / Area values)

Study Manager schedules and automates these steps

What are the pharmacokinetic properties of the drug?
What does the body do to the drug?

“Software features that support Compliance ”



Compliance Key Features

Acquisition/Study Manager

- ✓ Mandatory Login
- ✓ Pre-defined Role Based Security
- ✓ UI operations disabled based on Privileges
- ✓ Data file overwrite protection
- ✓ Tamper Detection Capability for Methods and Data files
- ✓ Can require authentication and reason for allowed actions
- ✓ Method versioning
- ✓ Audit Trail on method changes

Quantitative Analysis

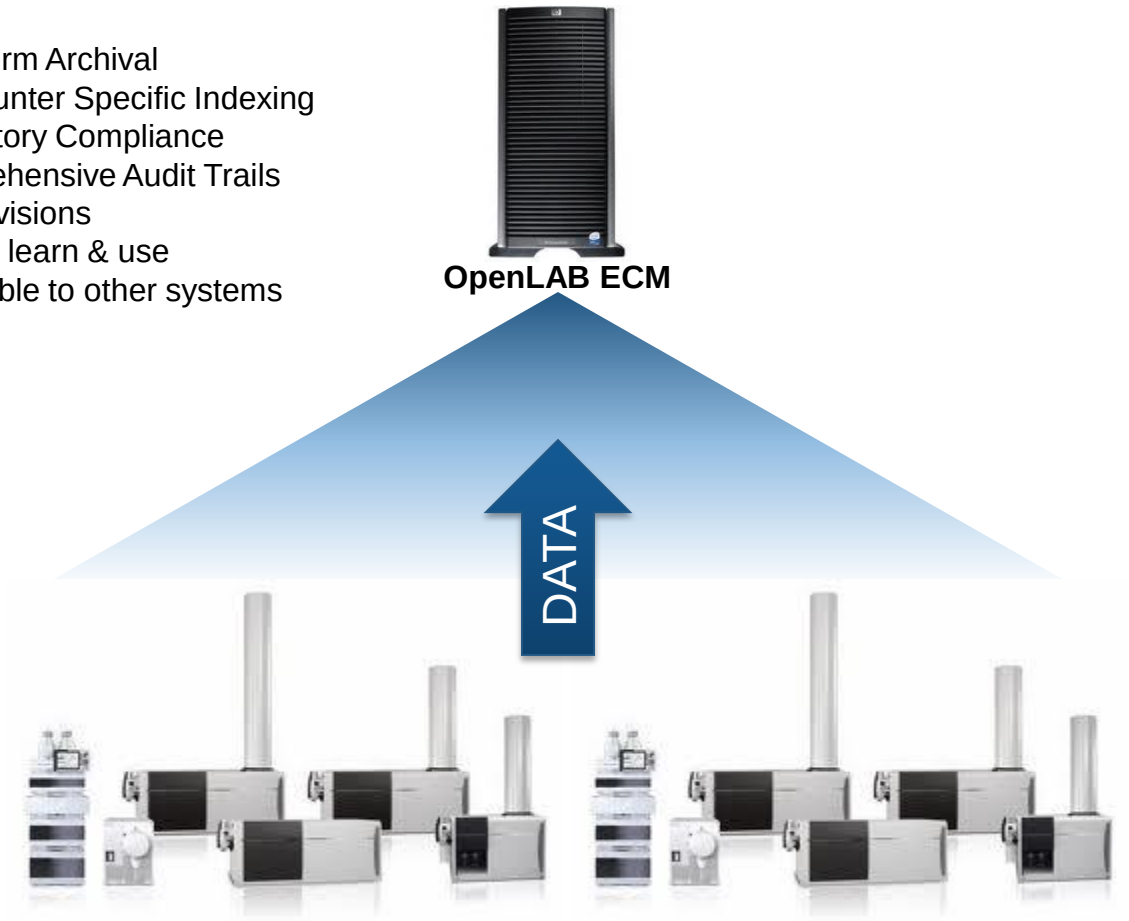
- ✓ Mandatory Login
- ✓ Configurable Role Based Security
- ✓ Operations can be disallowed based on defined capabilities
- ✓ Tamper Detection Capability for Results
- ✓ Password locked reports
- ✓ Can require authentication and reason for allowed actions
- ✓ Audit Trail on all quant actions

OpenLAB ECM

The ultimate scientific data management solution for the laboratory

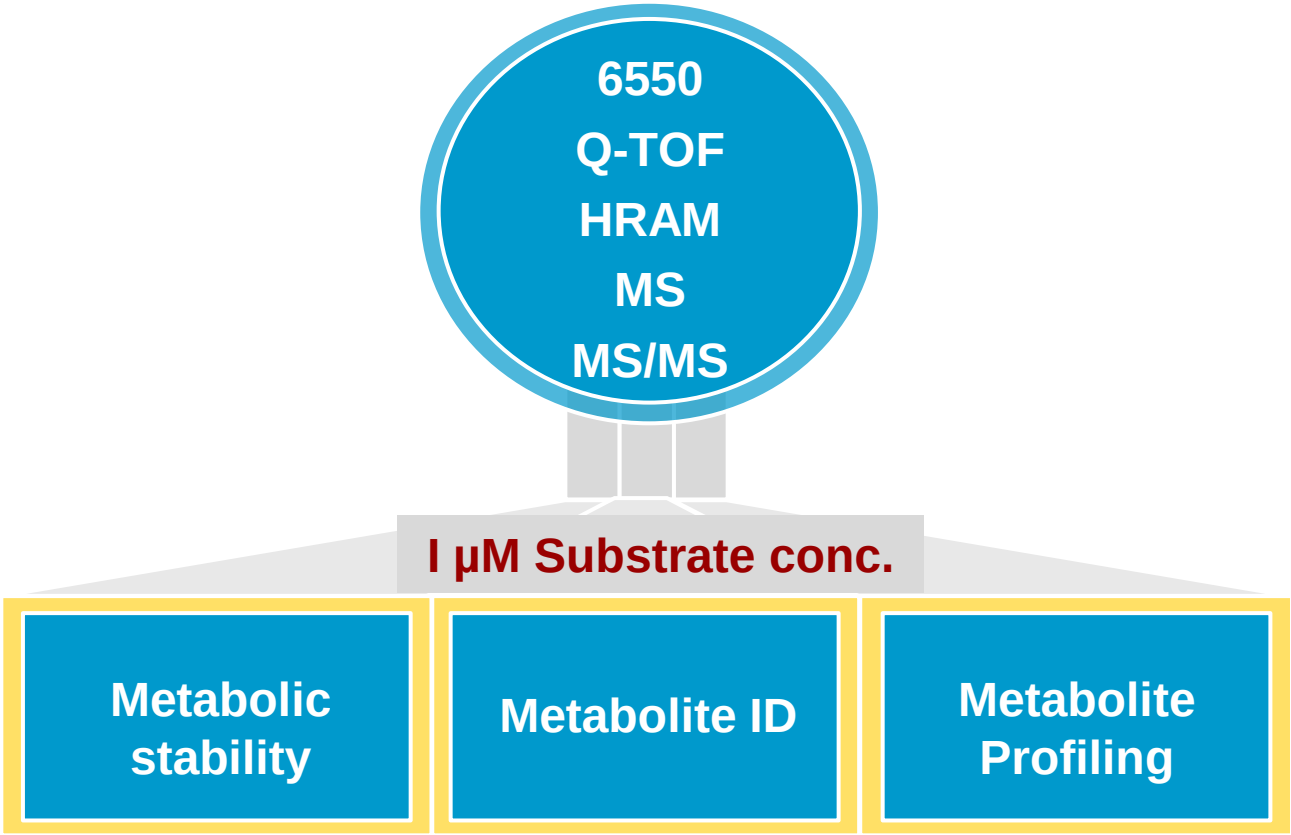
- Centralized data management for Agilent MassHunter

- ✓ Long-term Archival
- ✓ MassHunter Specific Indexing
- ✓ Regulatory Compliance
- ✓ Comprehensive Audit Trails
- ✓ File Revisions
- ✓ Easy to learn & use
- ✓ Extensible to other systems



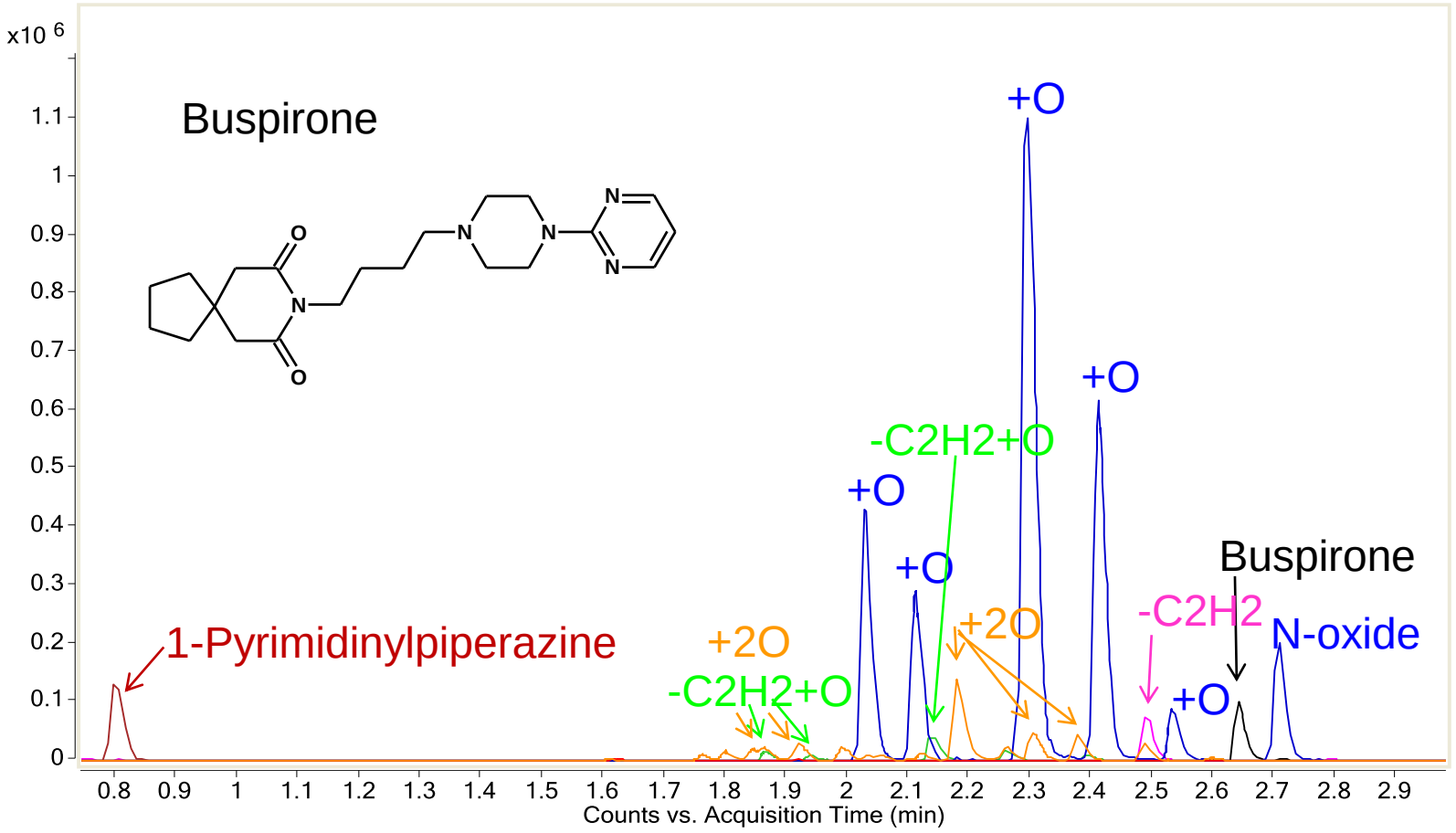
Qual/Quan: on a Single Platform

- more information in a single analysis



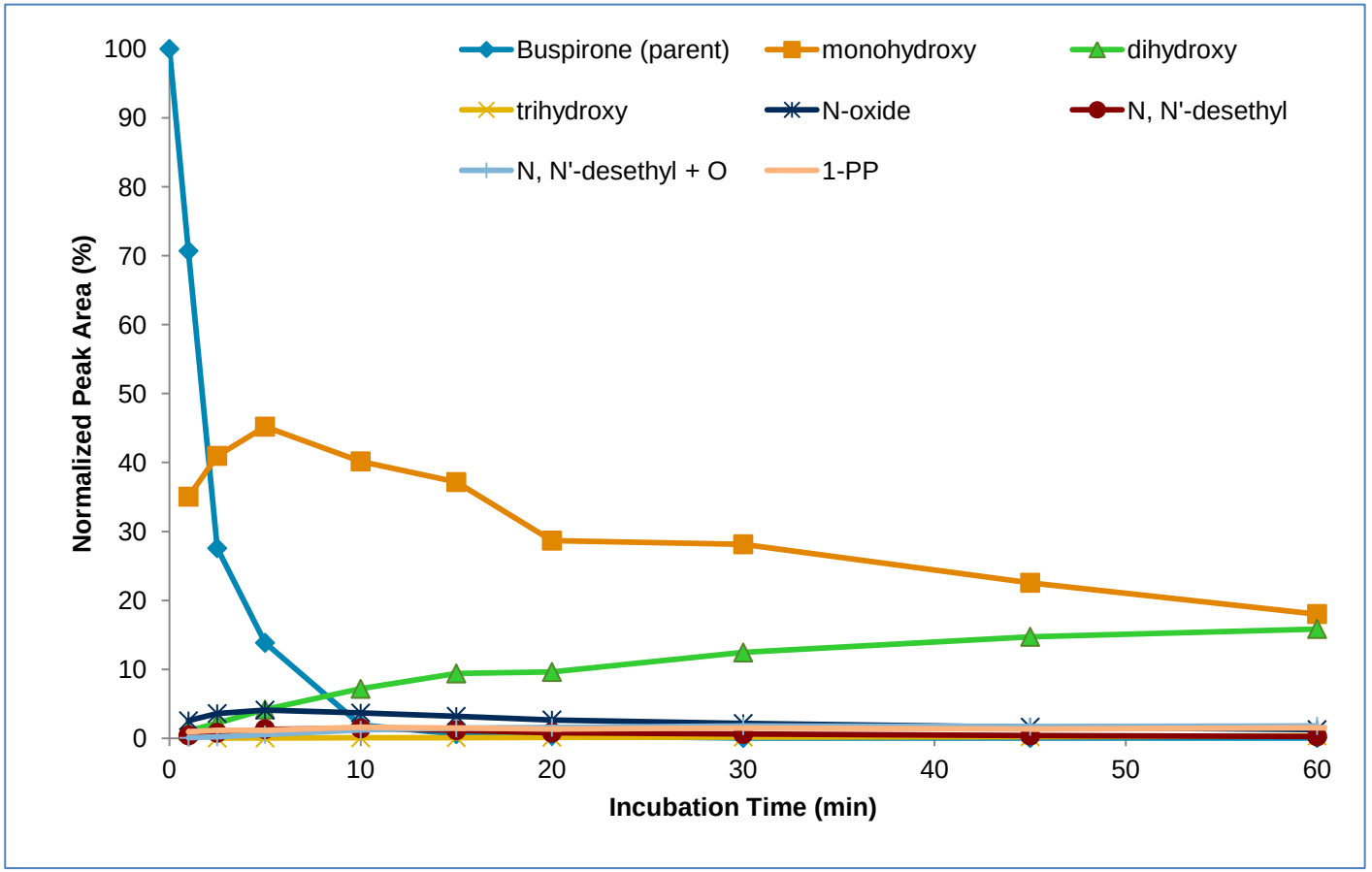
Simultaneous Metabolite Identification

10 min incubation sample



Metabolic Stability and Metabolite Profiles

Buspirone (1.0 µM in RLM)



1-PP: pyrimidinylpiperazine

Summary and Conclusions

For Metabolite ID workflows

MassHunter Qual: A great tool for determining specific biotransformations based on MS/MS data. Uses untargeted approach.

Mass Metasite: A software provided through Molecular Discovery with the ability for structure prediction and correlating predictions to MS/MS data

For Quantitative workflows

MassHunter Quant

Study Manager: fully automate both high throughput ADME screening and BioAnalysis quantitative measurements

LIMS integration and Compliance

For Qual/Quan

Agilent's 6550 LC/Q-TOF has sufficient quantitative performance to allow performing bioanalytical measurements and providing the accurate mass MS data needed for metabolite identification from one injection

Thank you!

Agilent Technologies

Frank Delaglio

Marketing Manager,
Magnetic Resonance Software
Agilent Technologies

Dave Russell

Applications Scientist,
NMR Division
Agilent Technologies

Agilent Technologies

Magnetic Resonance for ADME/T Studies
Making the Complex Simple

Frank Delaglio and Dave Russell

Agilent Technologies

Frank Delaglio

What can NMR do in an ADME laboratory?

The two primary tasks faced in an ADME study are identification and quantification of metabolites.

NMR spectroscopy has been the definitive tool for structural studies over many years. Modern probe technology allows researchers to collect high quality structural data on very small samples, while advances in console design have opened the door to absolute concentration measurements on any sample.

While Mass Spectrometry is the predominant technique used for ADME investigations, NMR has a critical role to play in this environment, too.

Agilent 600 MHz NMR system

Sample Workflow

Agilent NMR systems remove the learning curve

The traditional NMR workflow:

1. Insert the sample
2. Load parameters
3. Adjust tuning manually
4. Adjust lock freq. manually
5. Adjust shims manually
6. Run test spectra
7. Adjust experiment parameters manually
8. Collect data
9. Input sample information
10. Save data
11. Process data
12. Adjust processing parameters
13. Save processing parameters

Operator requires 30 minutes or more for every sample

The VnmrJ workflow:

1. Insert the sample
2. Select experiments to be collected
3. Input sample information
4. Submit sample
   - Auto tune
   - Auto shim
   - Auto lock
   - Auto optimization
   - Auto process
   - Auto save
   - Auto archive
   - Auto email results

Operator requires 3 minutes for a sample, less for repeat measurements.

VnmrJ

Streamlined Workflows for Sample Submission

VnmrJ VERSION 4.0

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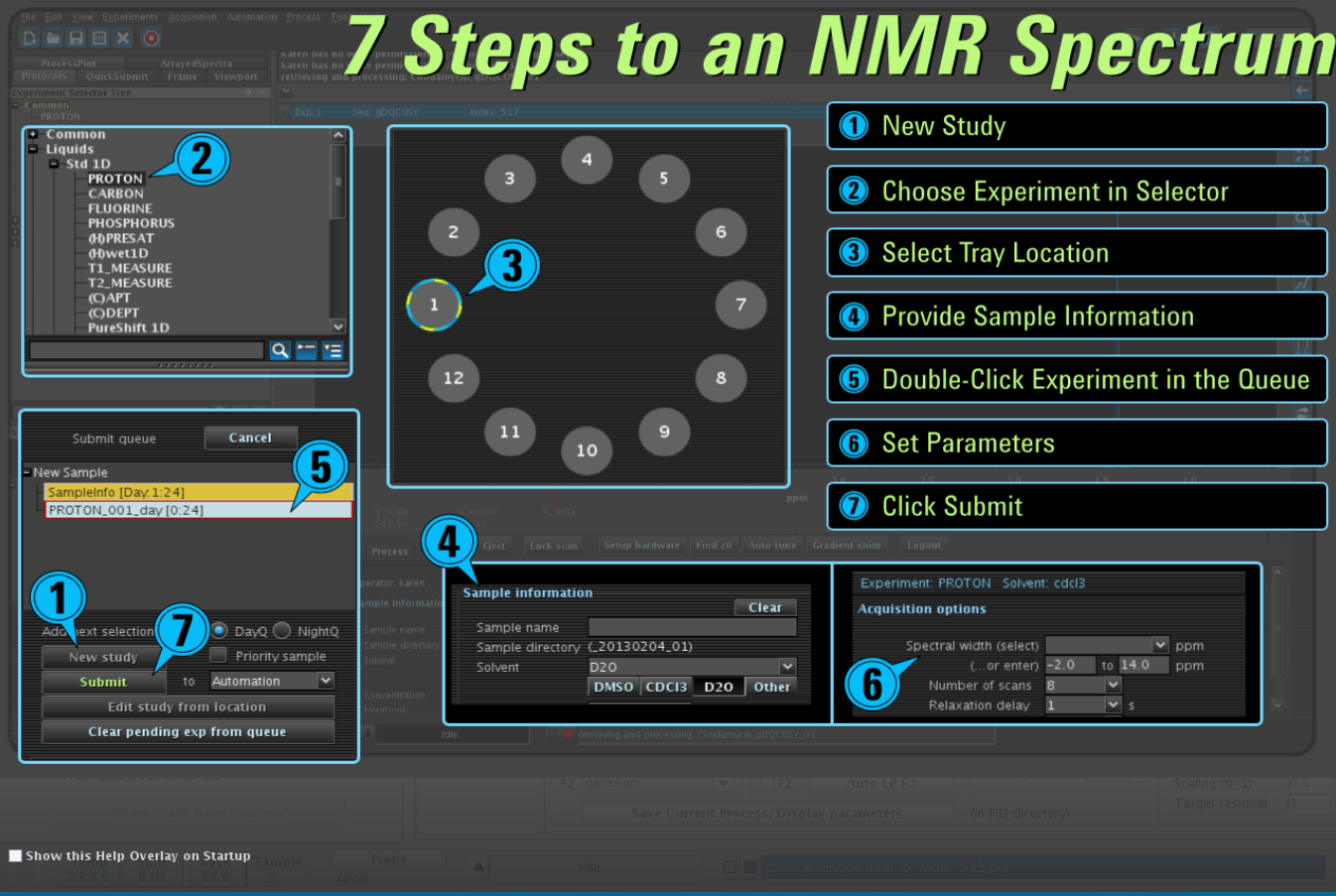

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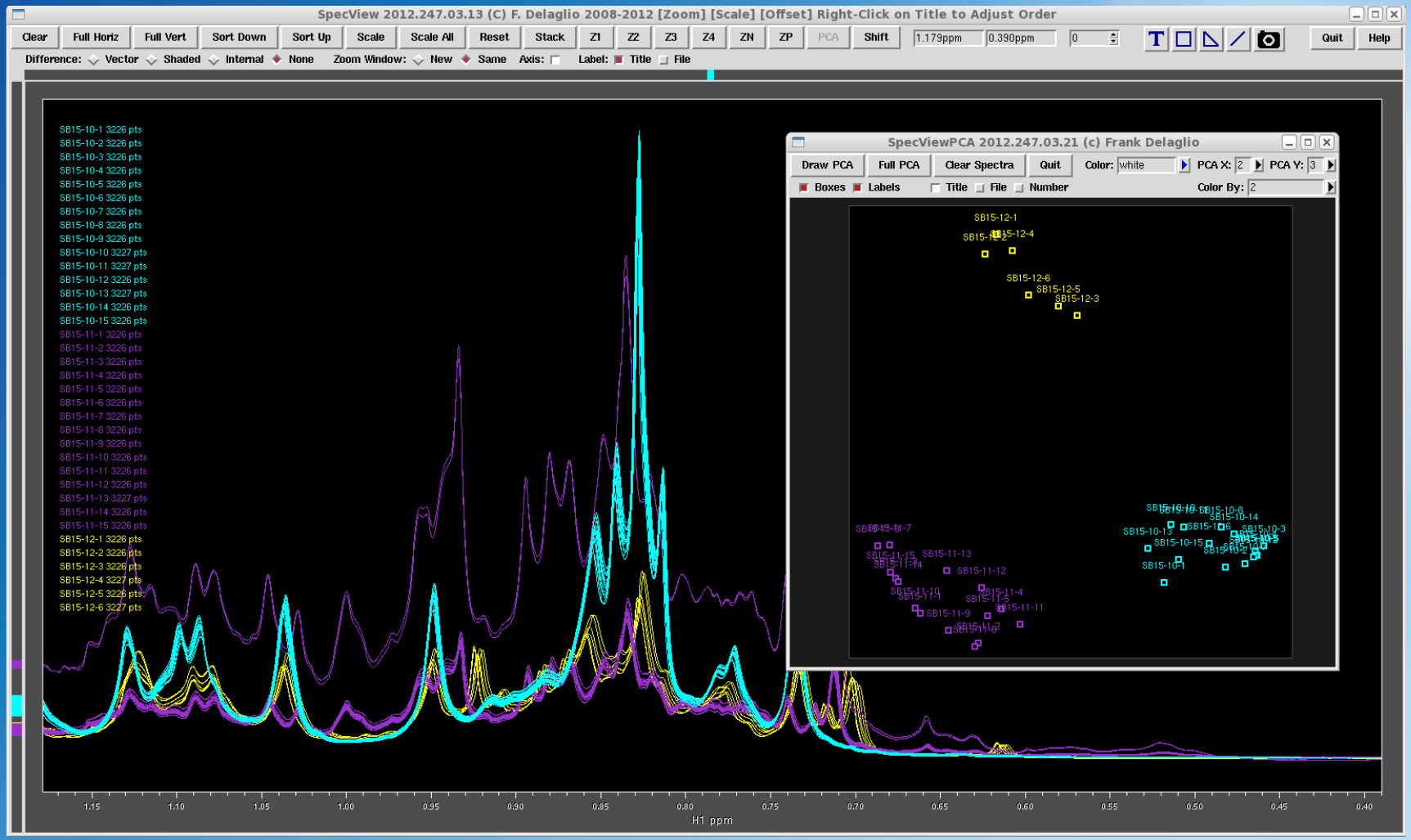
Go to www.spinsights.net to register.

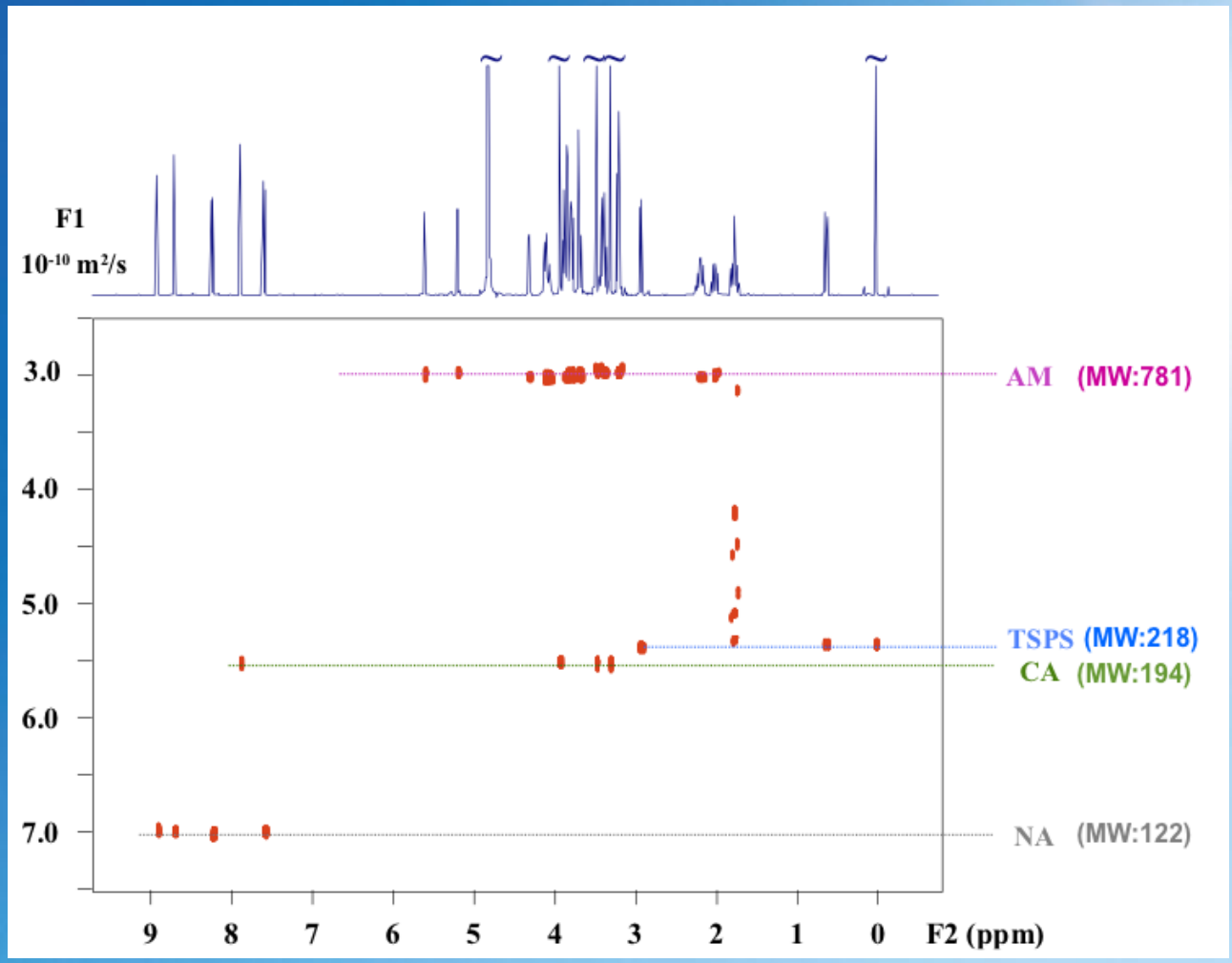
VnmrJ 4.0 – NMRPipe SpecView

Interactive Principal Component Analysis to Characterize Spectral Differences



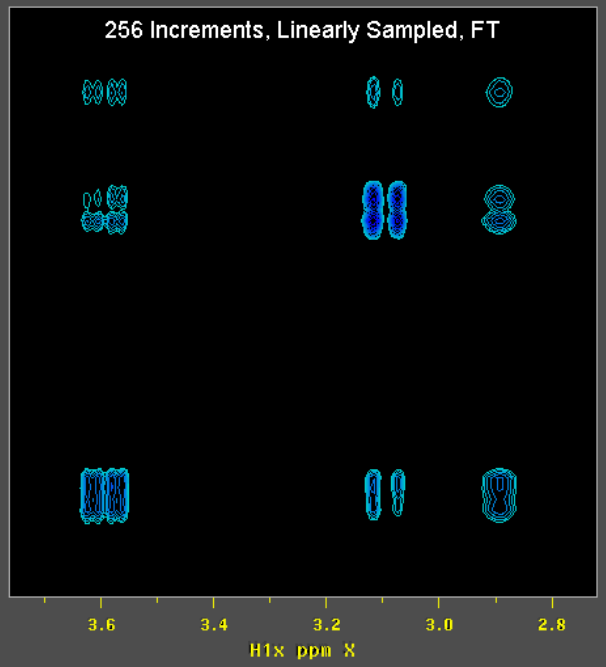
VnmrJ 4.0

Industry-Leading DOSY Solutions

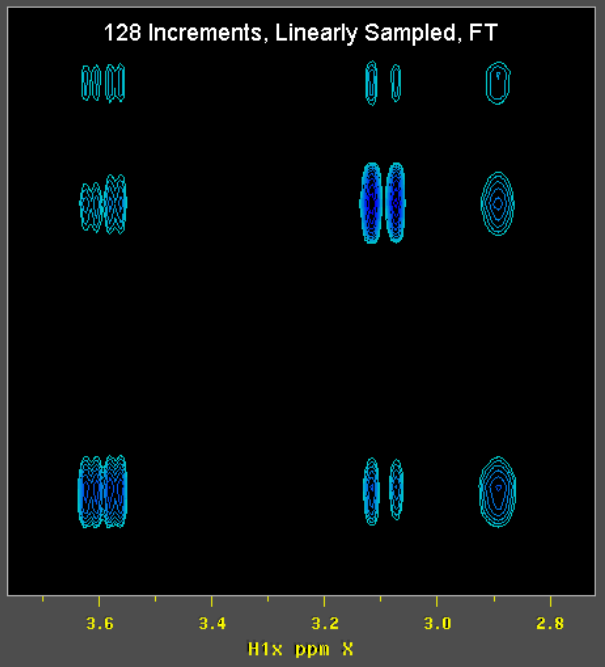


VnmrJ 4.0 allows routine users to apply Non-Uniform Sampling (NUS) to most any experiment, for sharper spectra with less measurement time

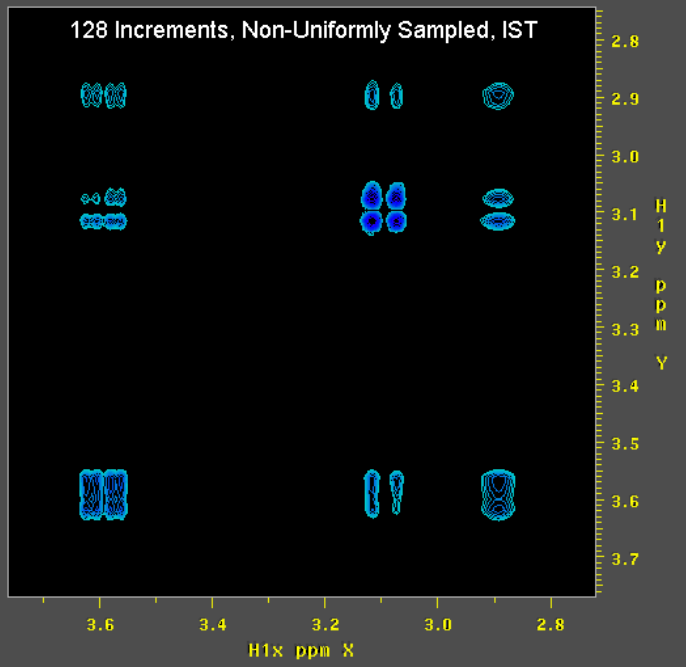
Conventional Linearly-Sampled



Conventional - Half of Original Measurement Time



NUS – Half of Original Measurement Time



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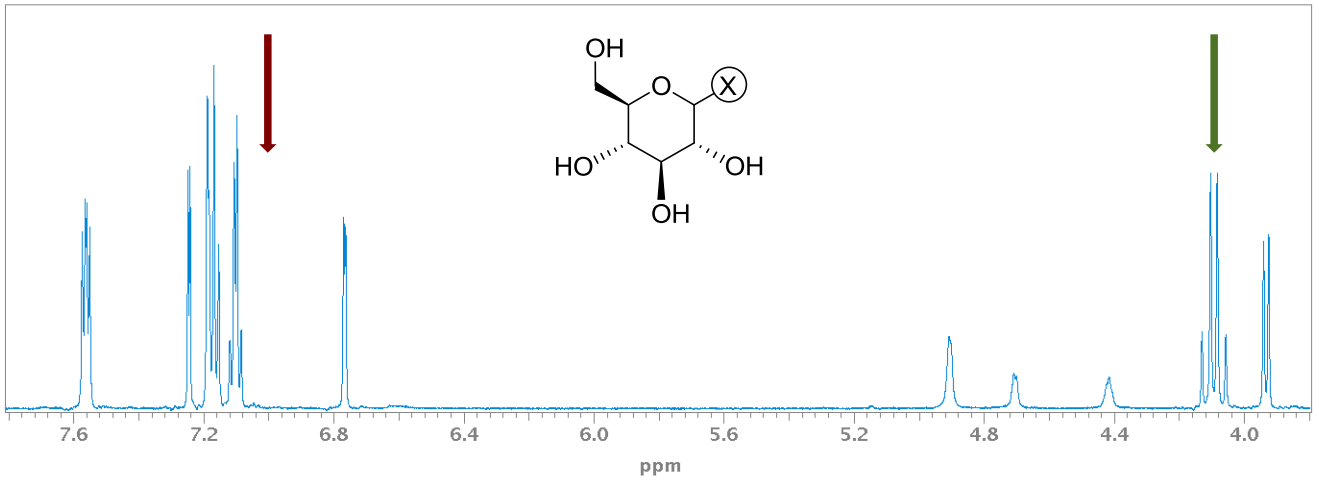
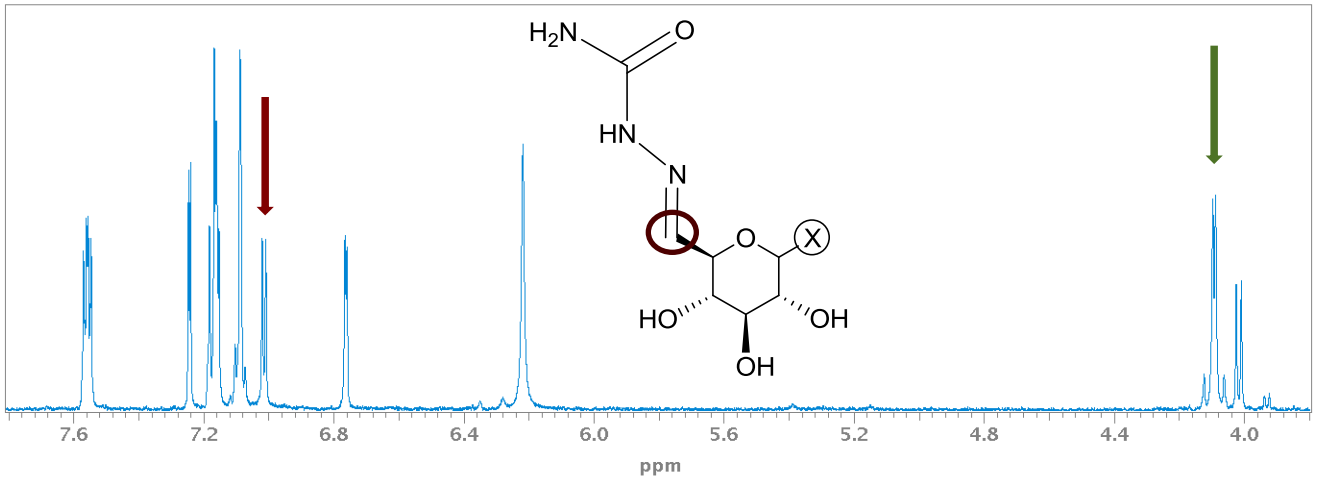
Dave Russell

NMR as a structural tool

Comparison of an SGLT-2 inhibitor and metabolite

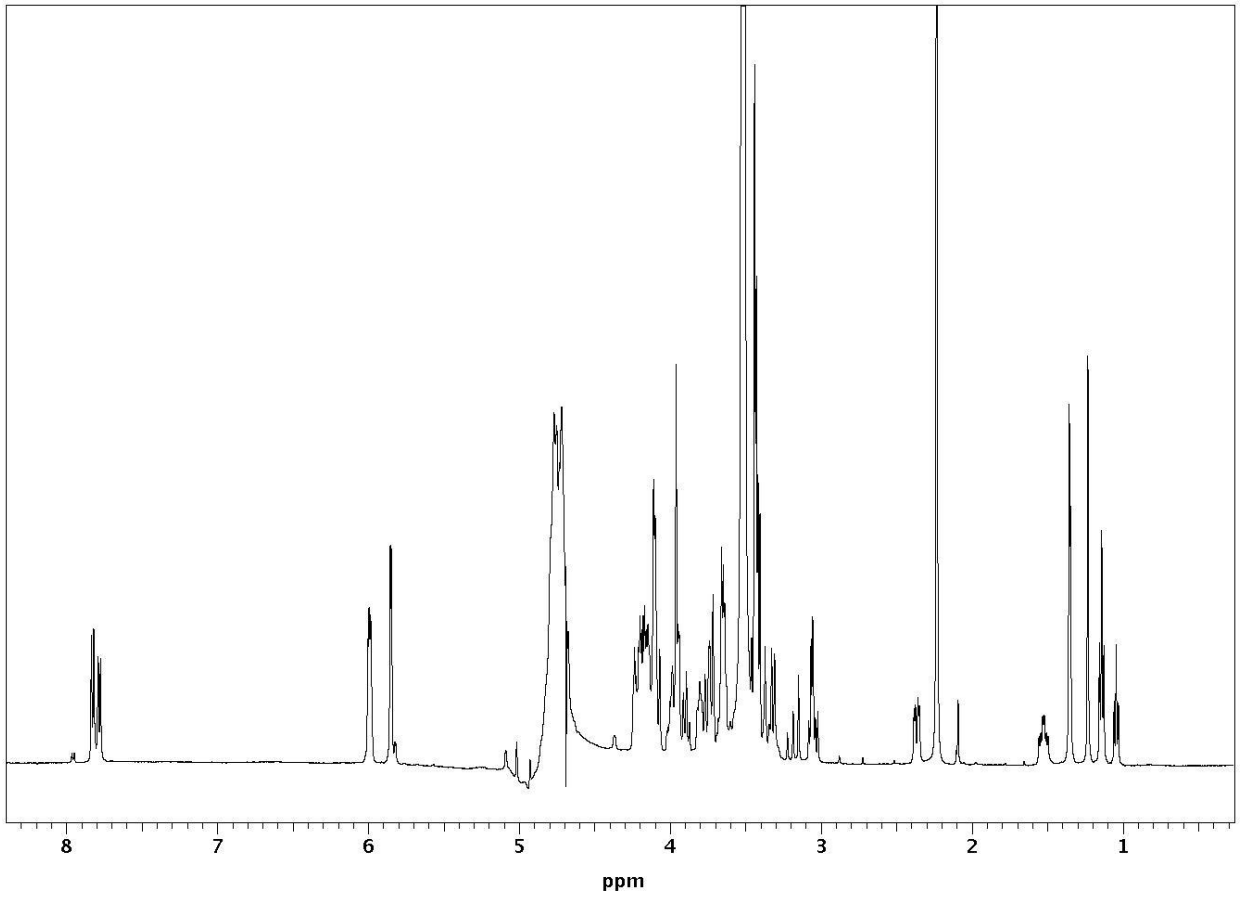




Sample Courtesy: Mark Grillo, Amgen

Automated Solvent Suppression

100% water



Microsample Cold Probe:
Excellent Mass Sensitivity for Small Molecule NMR

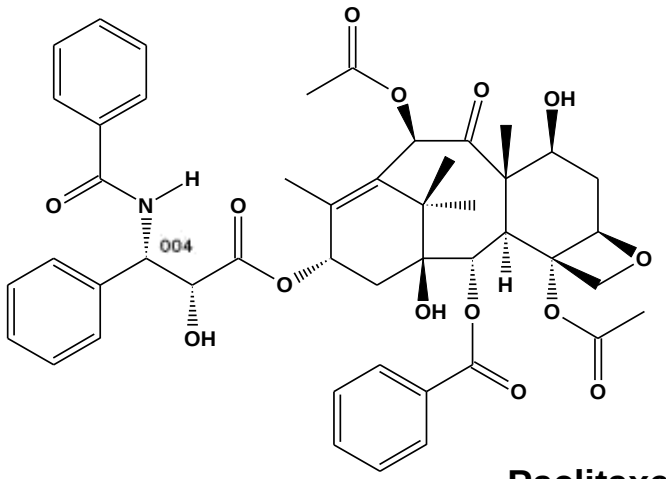
3 µg Paclitaxel

(3 nanomoles)

128 transients / 10 minutes

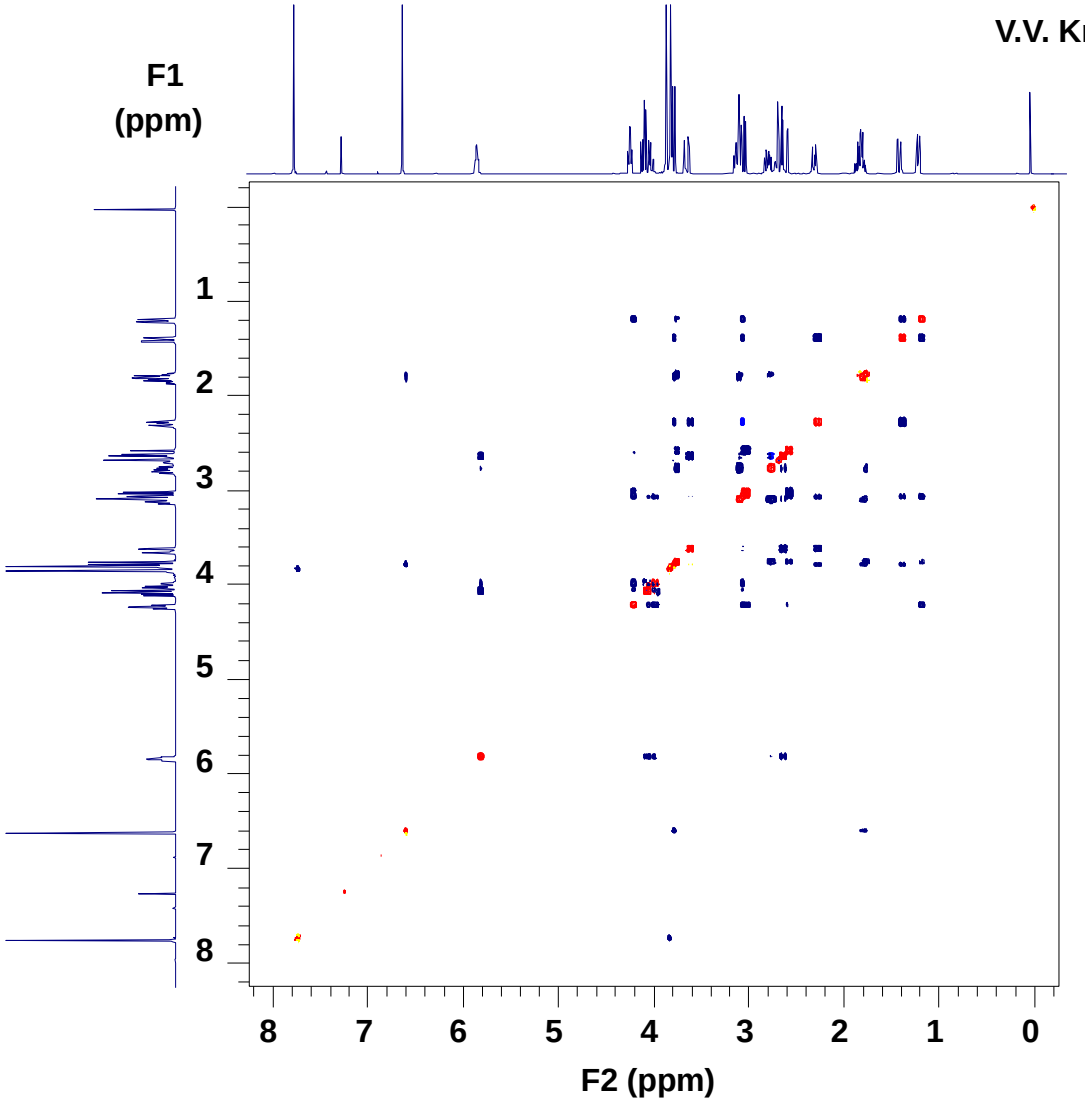
(high-frequency region)

.

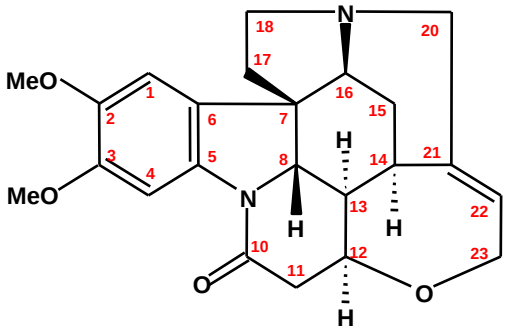


Paclitaxel

Band-selective Homodecoupled 2D NOESY (bashdNOESY)



V.V. Krishnamurthy, *Magn. Reson Chem.* 35, 9 (1997)



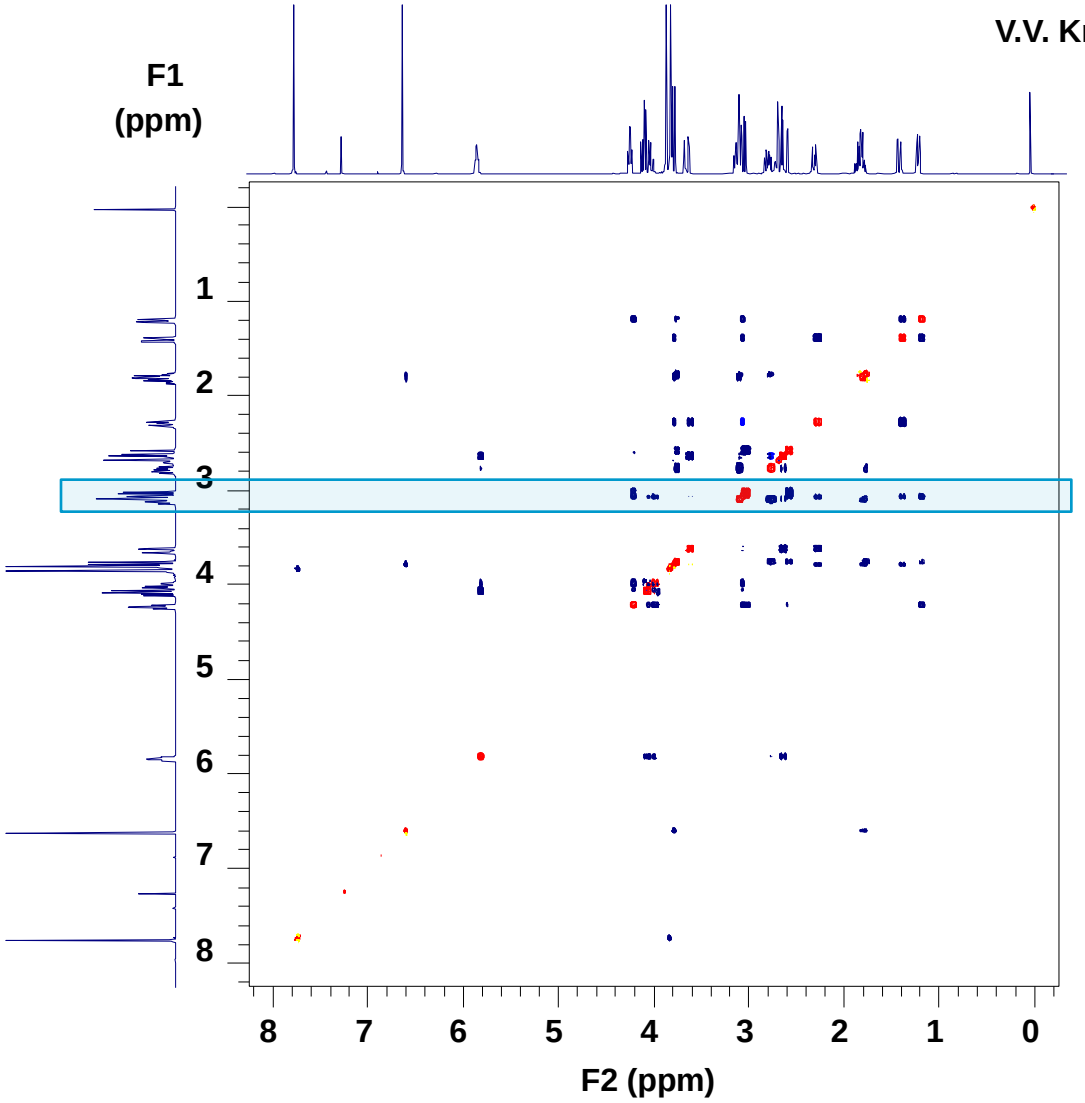
sw=sw1=4006, ni=480, at=0.256

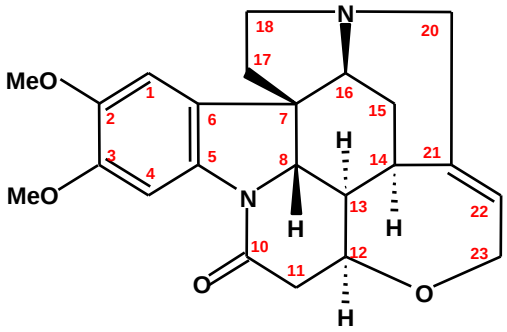
exp. time = 440 min

Agilent Technologies

Band-selective Homodecoupled 2D NOESY (bashdNOESY)

V.V. Krishnamurthy, *Magn. Reson Chem.* 35, 9 (1997)

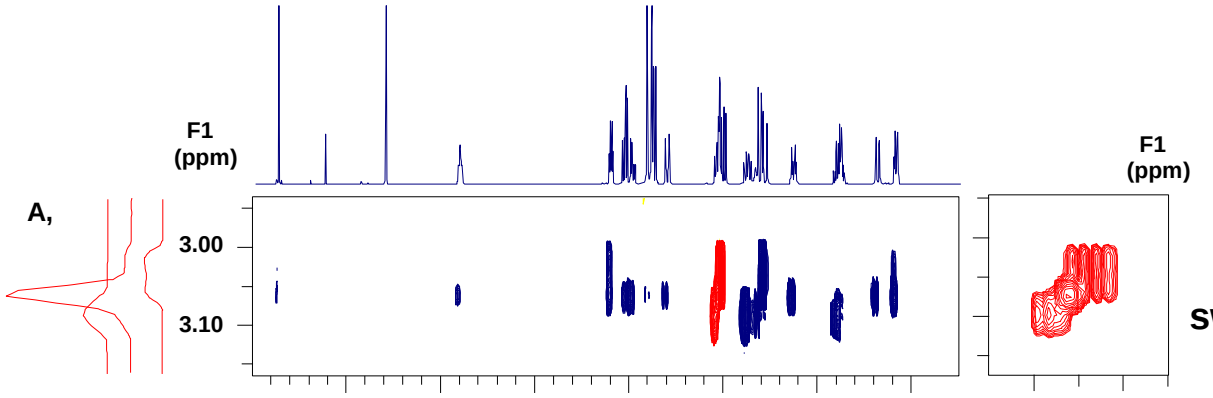




sw=sw1=4006, ni=480, at=0.256

exp. time = 440 min

Band-selective Homodecoupled 2D NOESY (bashdNOESY)

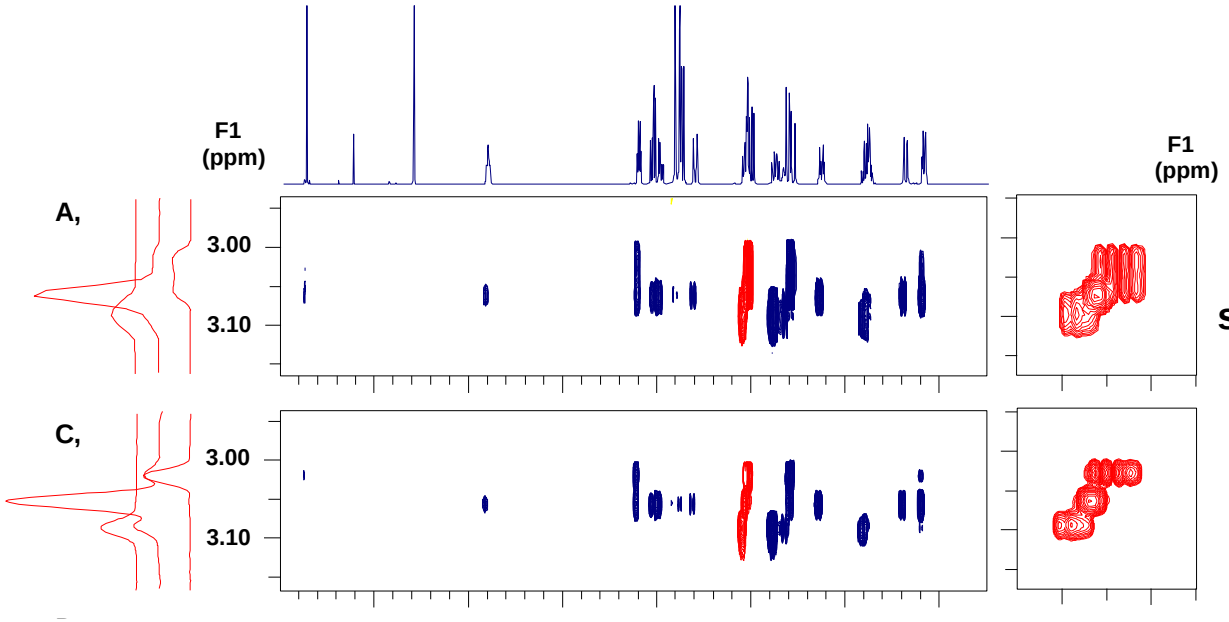


2D NOESY (expansion)

sw1=4006, ni=480, at=0.256

exp. time = 440 min

Band-selective Homodecoupled 2D NOESY (bashdNOESY)



2D NOESY (expansion)

sw1=4006, ni=480, at=0.256

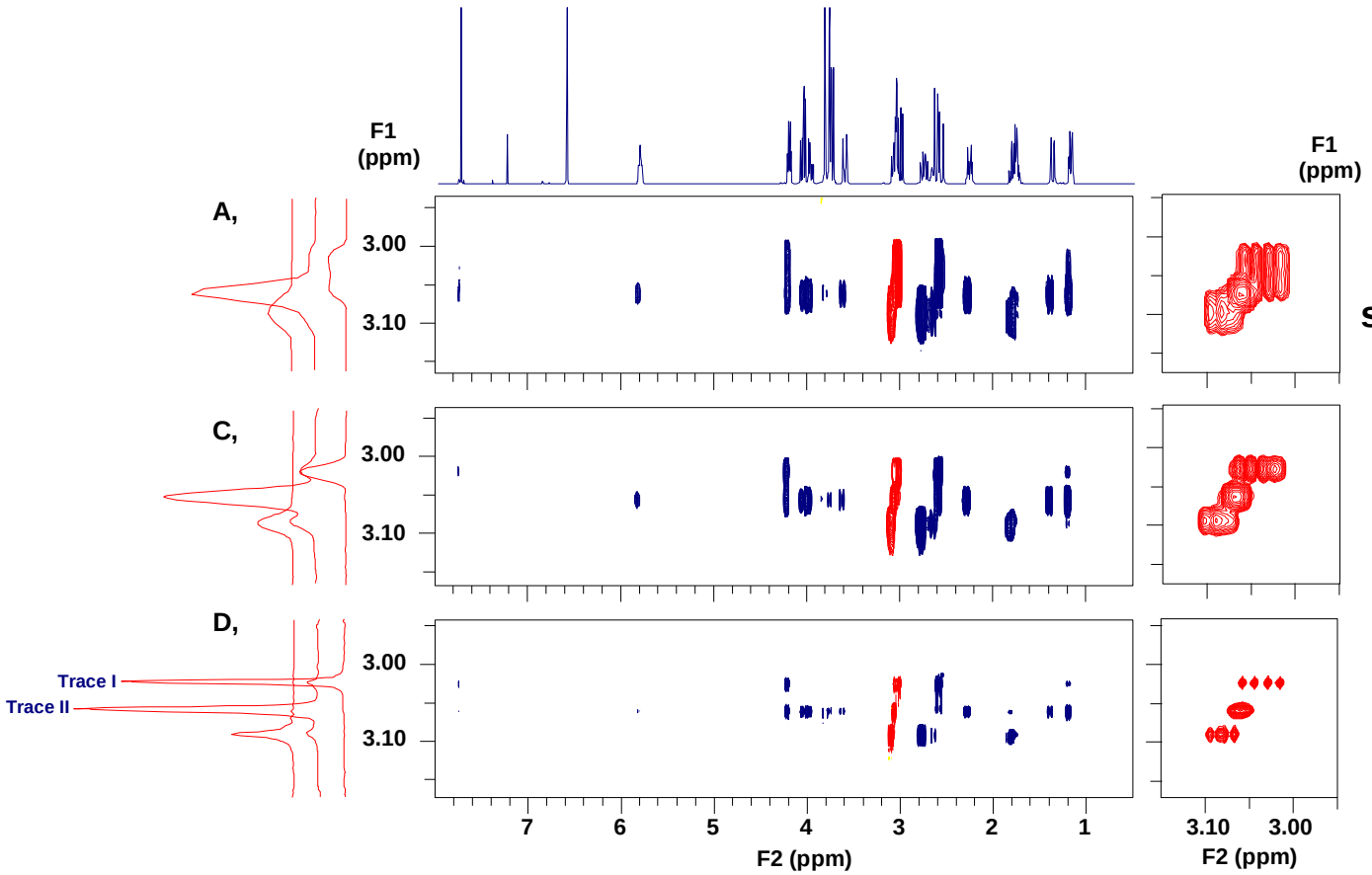
exp. time = 440 min

2D bashdNOESY

sw1=92.5, ni=12, at=0.256

exp. time = 13 min

Band-selective Homodecoupled 2D NOESY (bashdNOESY)



2D NOESY (expansion)

sw1=4006, ni=480, at=0.256

exp. time = 440 min

2D bashdNOESY

sw1=92.5, ni=12, at=0.256

exp. time = 13 min

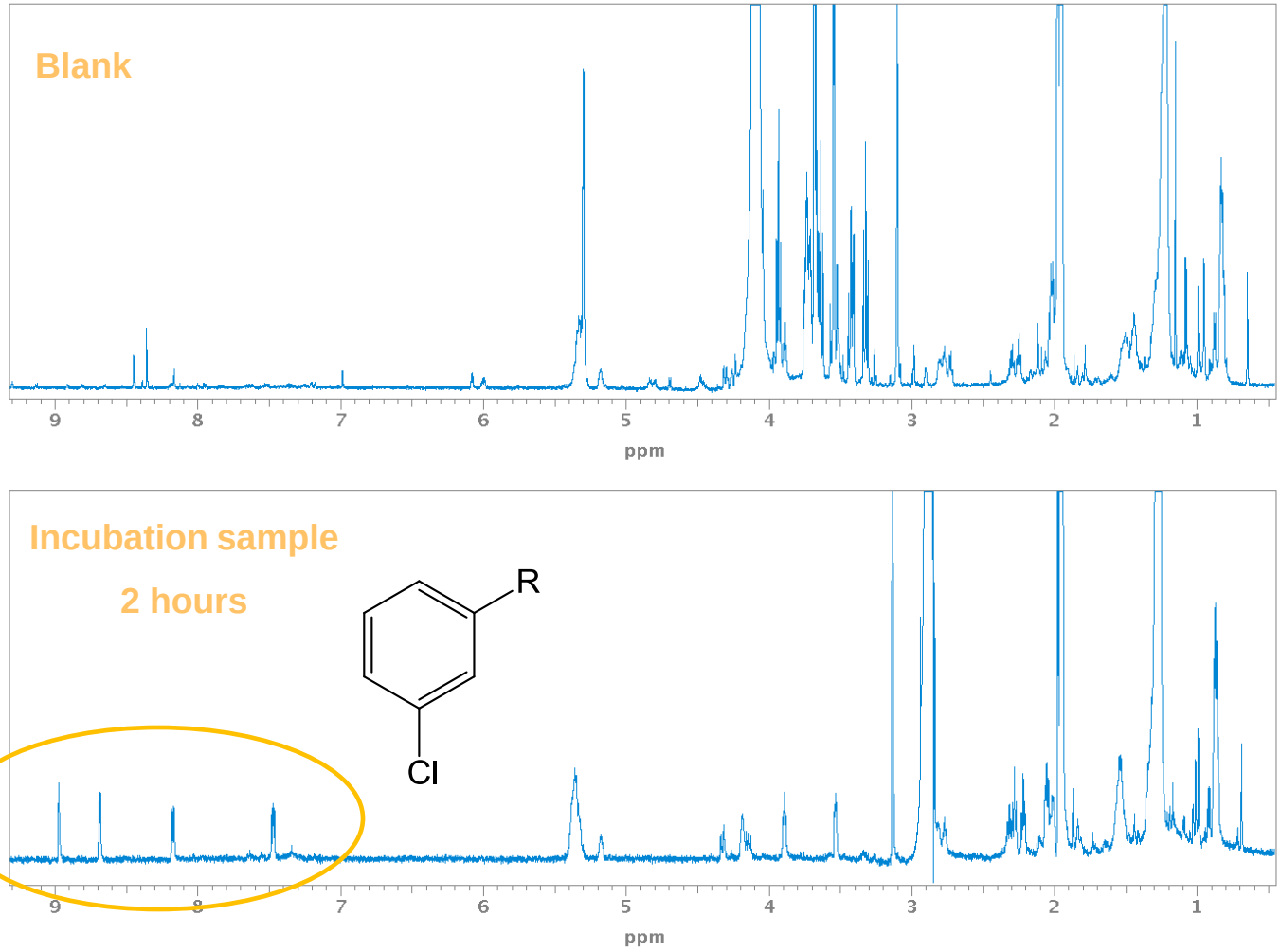
2D bashdNOESY

sw1=92.5, ni=24, at=1-02

exp. time = 30 min

Microsomal Incubation of Bupropion

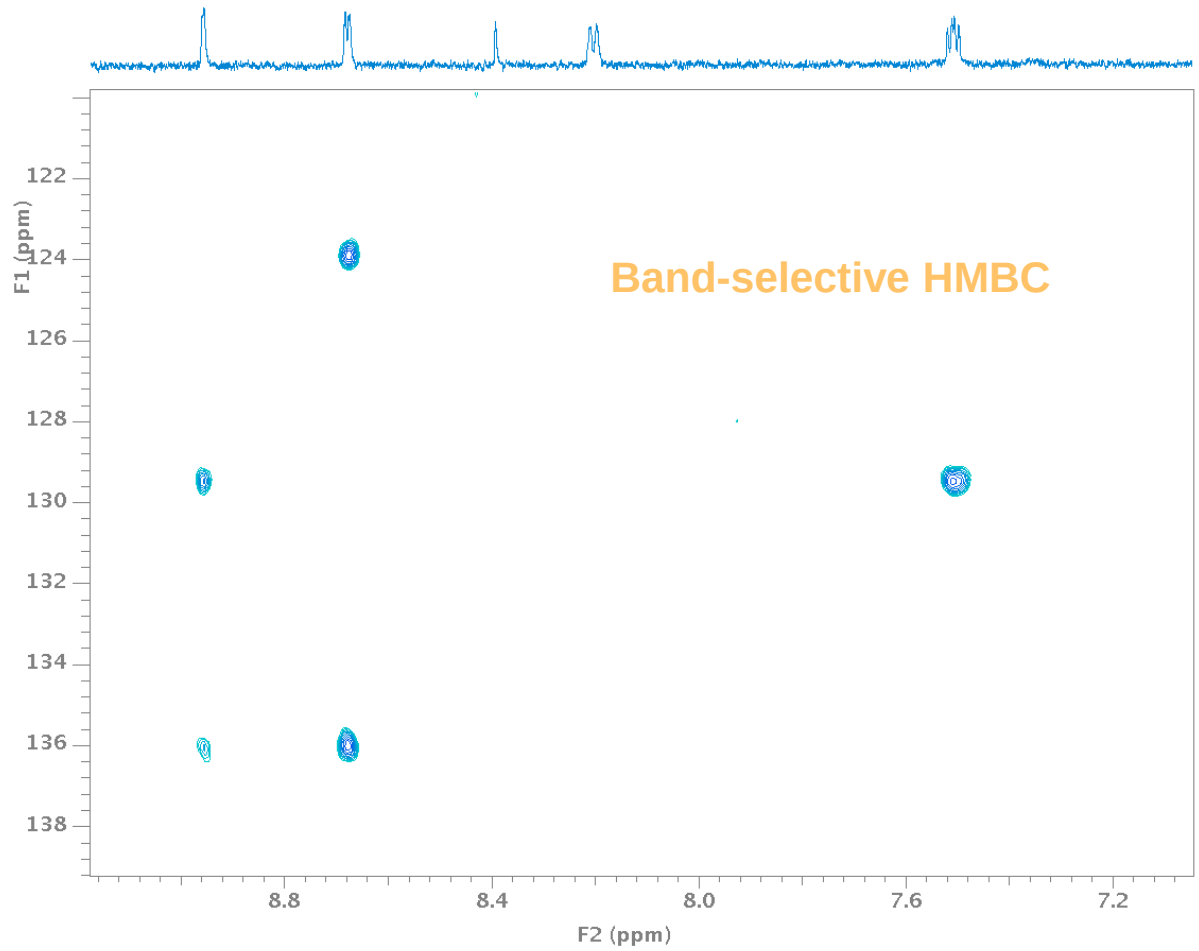
Detecting drug in the protein precipitated sample



Sample Courtesy: Ron Aoyama, Rigel

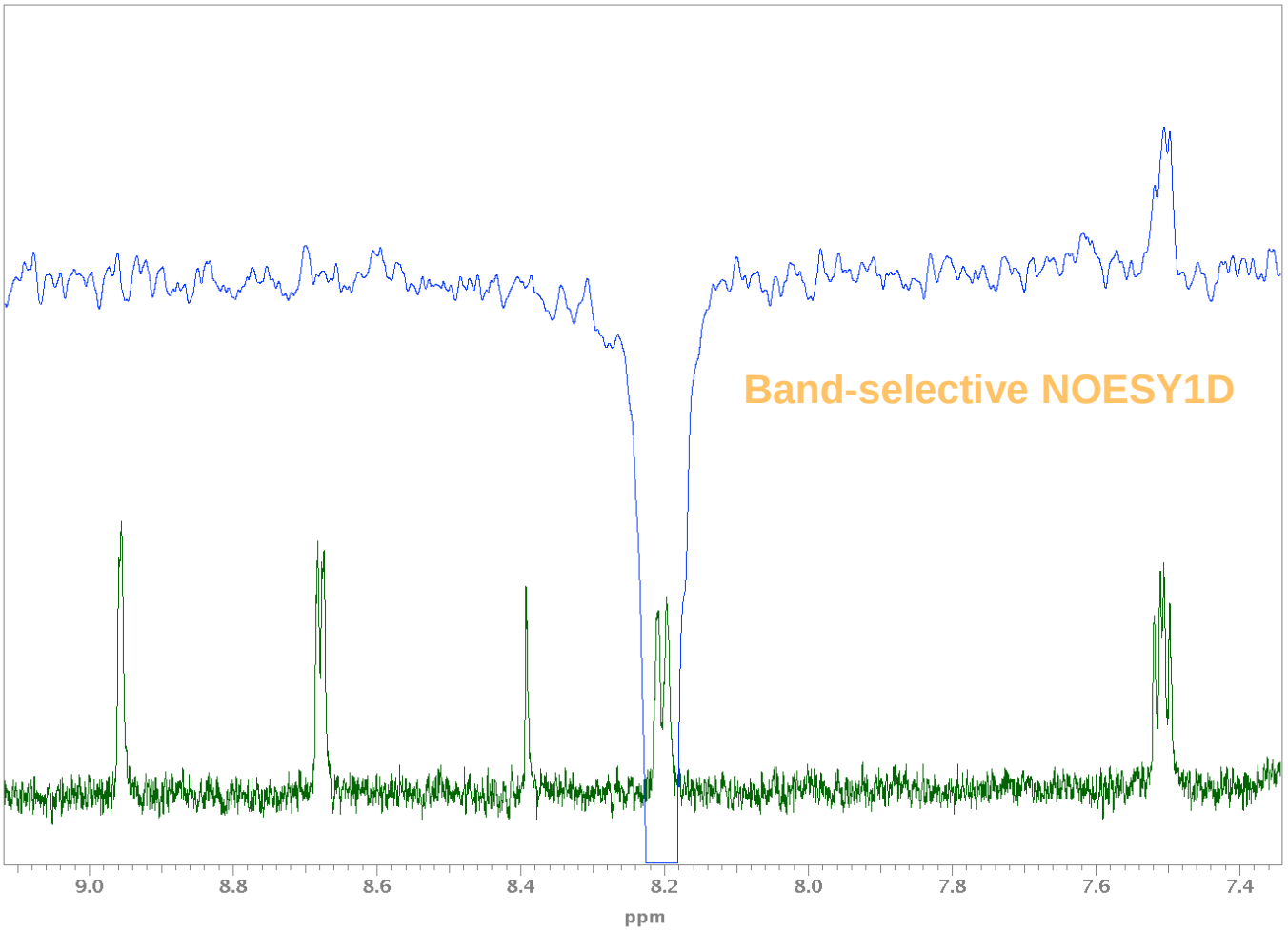
Microsomal Incubation of Bupropion

Detecting drug in the protein precipitated sample



Microsomal Incubation of Bupropion

Detecting drug in the protein precipitated sample



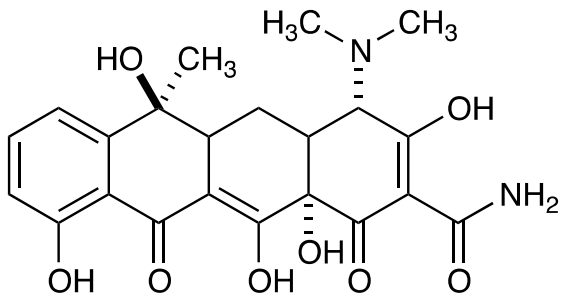
Quantitative NMR Spectroscopy - qNMR

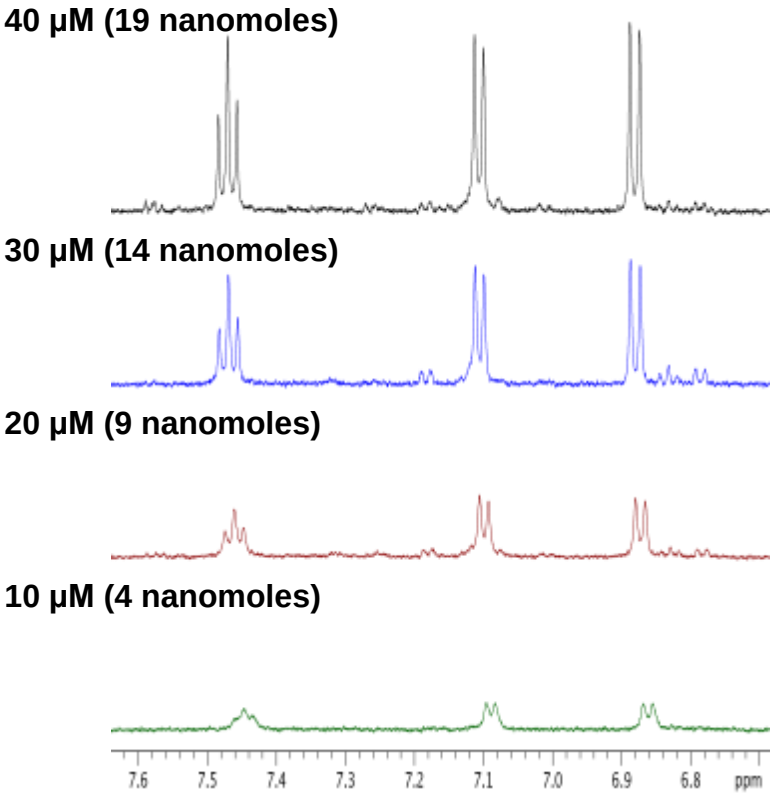
Why is NMR a valuable tool for quantitation?

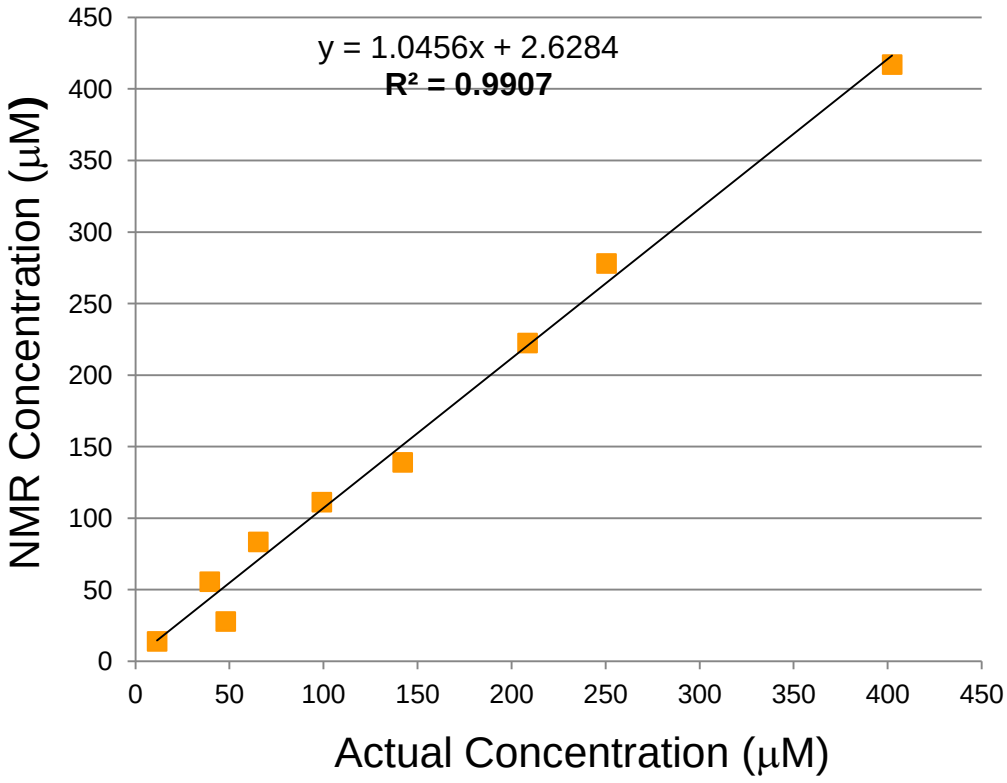
In contrast to almost all other common quantitative techniques, the NMR experiment is fundamentally quantitative. The response factor for any given nuclei is 1.000, regardless of molecular structure or environment.

Historically, the limiting factor for using NMR for routine quantitative studies has been poor reproducibility in the console hardware. Compensating for this required an internal standard or injection of an artificial reference signal. Both of these solutions are suboptimal.

Quantitation of Tetracycline in D_2O





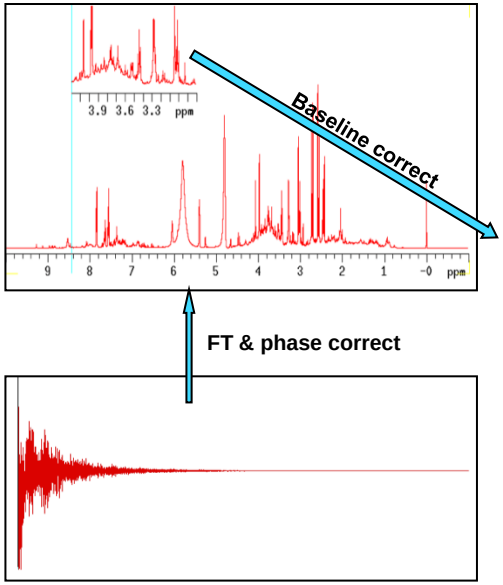


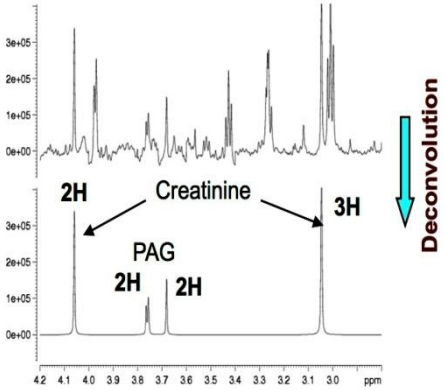
LOQ of ~5 µM or 2 nanomoles

Current NMR Analysis Workflow

There are various drawbacks to interpretation based on a processed spectrum.

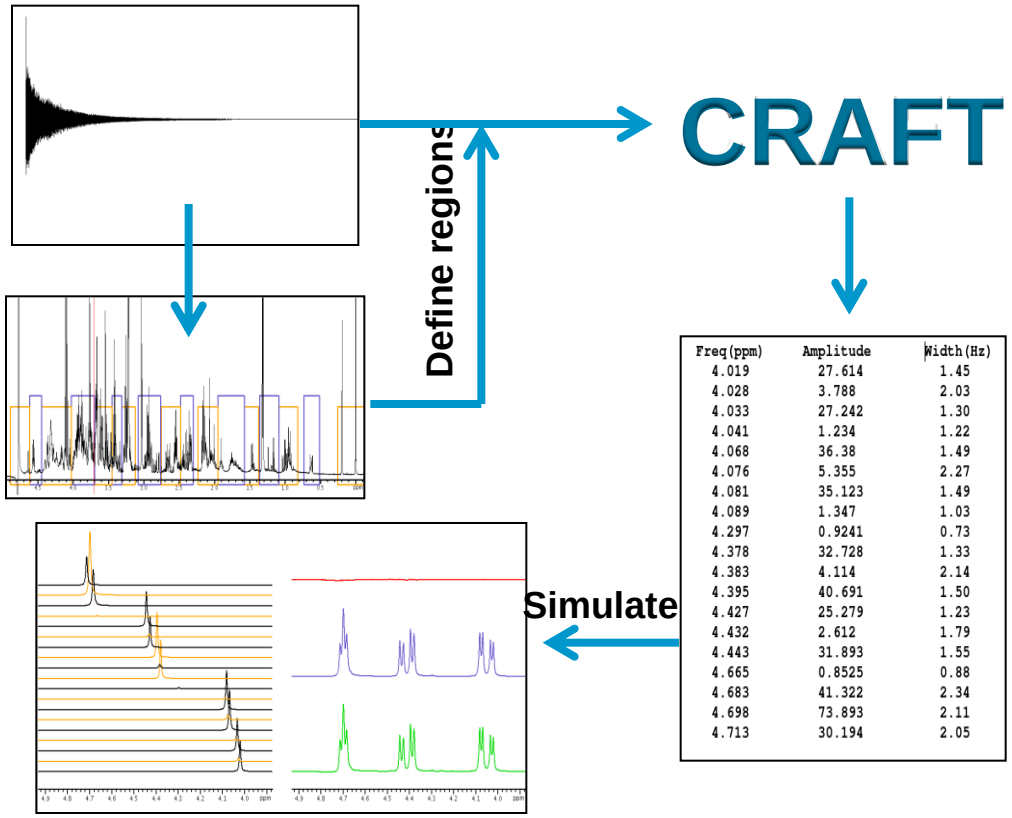
Significant issues can be introduced by the Fourier Transform, including baseline issues and phase distortions.





Manual processing. Manual data reduction. Manual *everything*!

CRAFT – Deconstructing a Spectrum

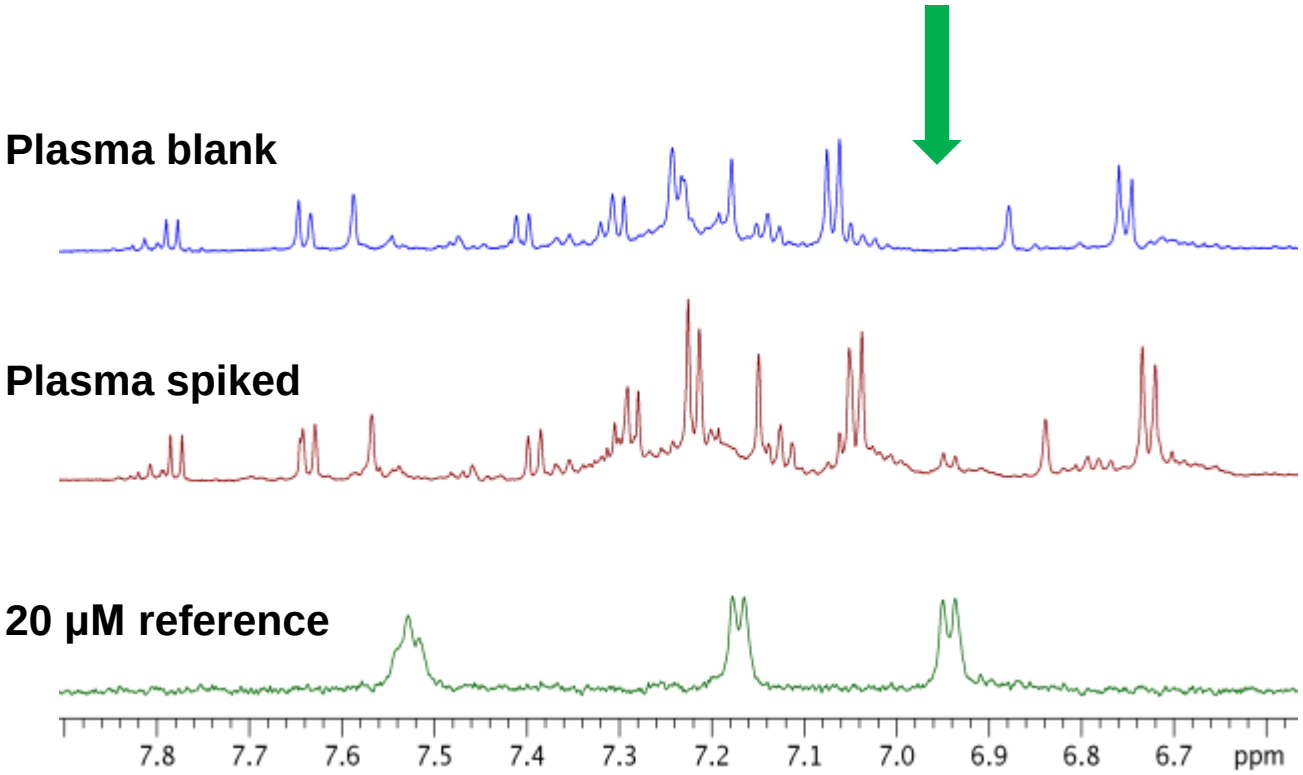


Once data have been captured in the spreadsheet, analysis is essentially complete.

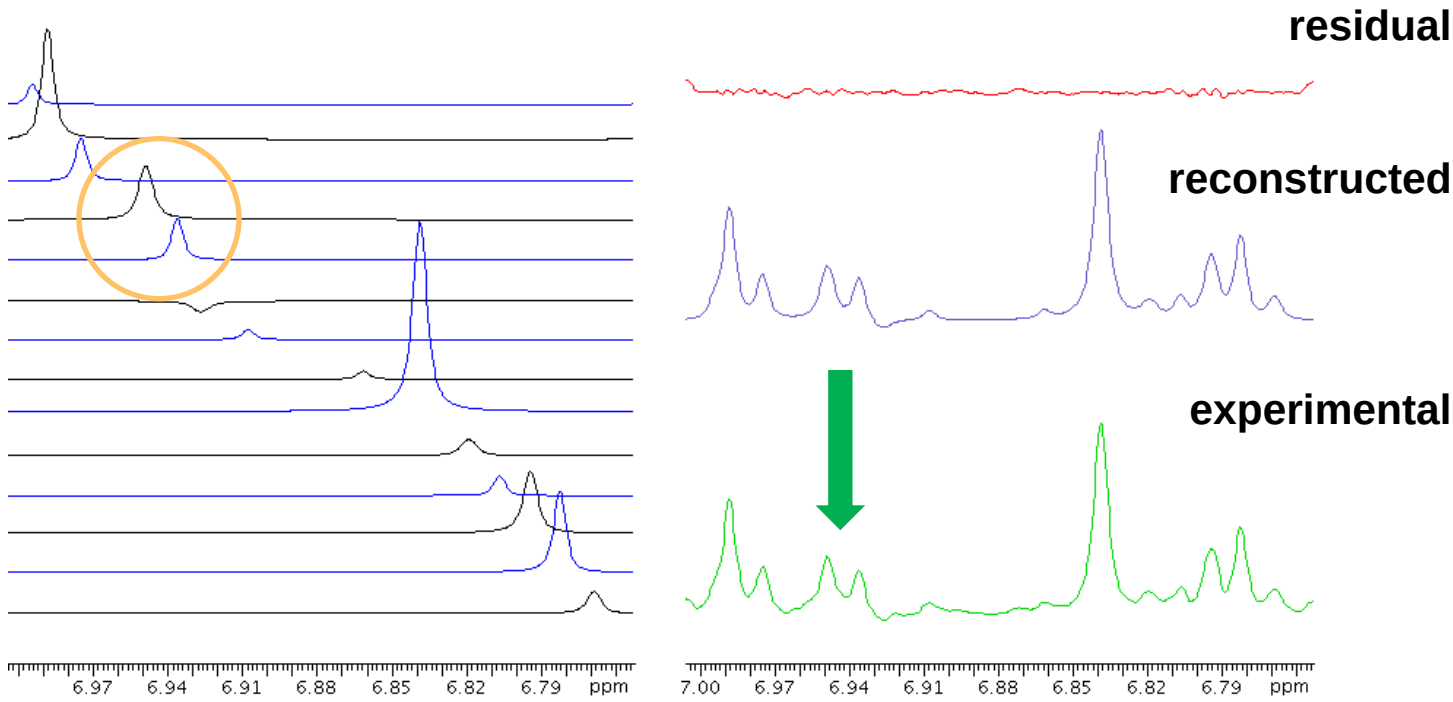
Most users want to see how well the data reduction step worked. CRAFT includes a complete set of tools to allow visual inspection of the results.

Spectra of Tetracycline in Plasma

Tetracycline is known to bind to endogenous proteins in plasma. NMR can be used to directly measure the concentration of free tetracycline by simple integration of a drug peak.



Deconvolution of Tetracycline Resonances



Tetracycline is known to bind to endogenous proteins in plasma. NMR can be used to directly measure the concentration of free tetracycline by simple integration of a drug signal.

Summary

- **Agilent NMR systems are easy to use.** By providing an array of automated tools and utilities, VnmrJ allows you to focus on learning about your samples rather than learning how to use the software.

- **Sophisticated NMR techniques, such as band-selective experiments, are available to any researcher.** A powerful and complete library of over 360 experiments can be used by anyone with just a few mouse clicks.

- **You can use NMR to measure the absolute concentration of practically any sample.** Agilent's NMR systems provide the stability and linearity needed to measure absolute concentration without the need for internal standards or artificial reference signals.

- **CRAFT provides automated Spectrum-to-Spreadsheet deconvolution.** Deconvolution of NMR spectra has reached reached the 21st century.

Thank you!

Agilent Technologies

info@bioanalysis-zone.com

Look for more webinars brought to you from *Bioanalysis* and a recording on today's event on

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