Making the Transition From a Quantitation Lab to a Quant*Information* Lab

Chris Holliman

Global Small-Molecule Discovery Bioanalytical

Phamacokinetics, Dynamics & Metabolism

Acknowledgements

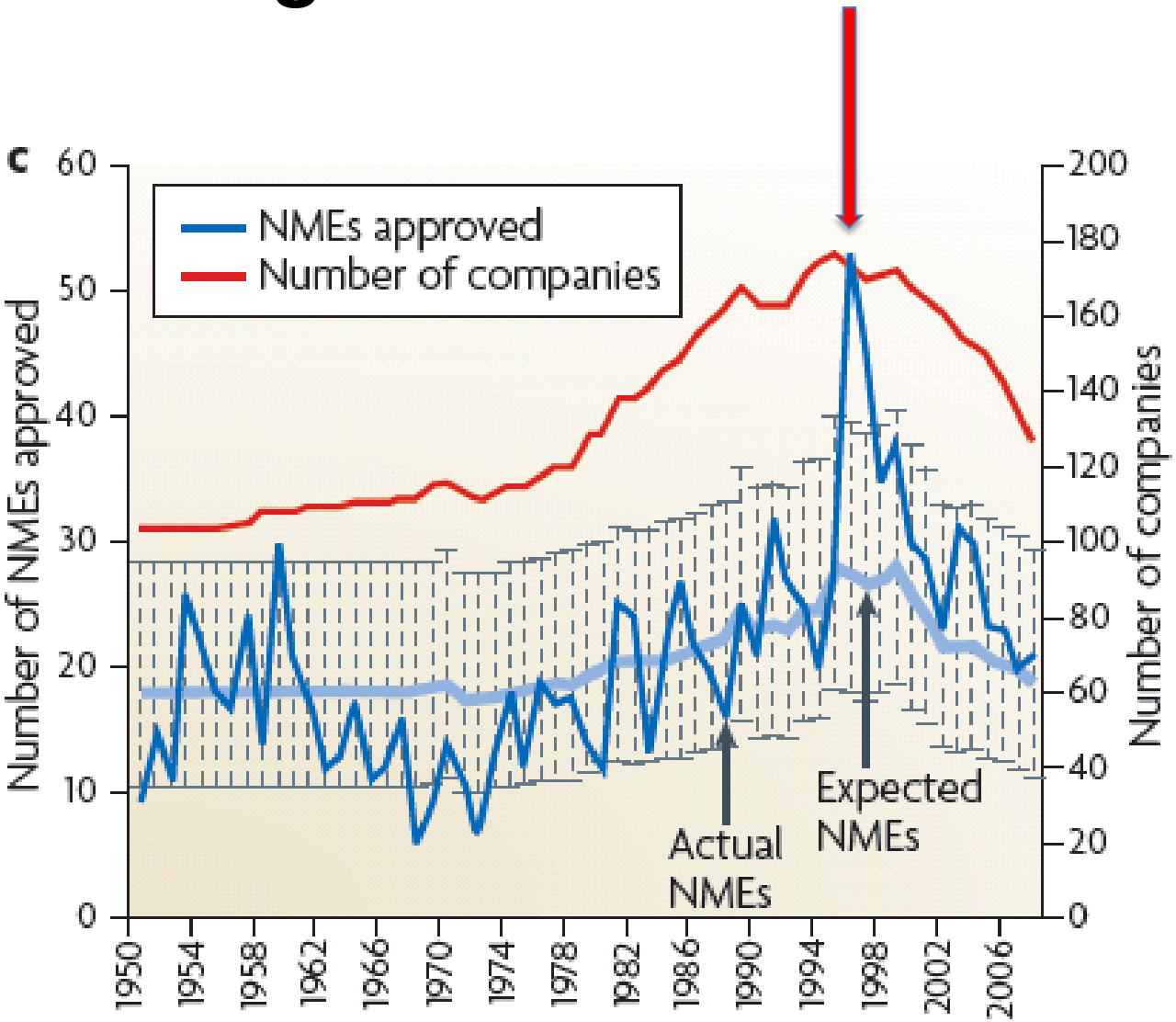
- Andre Negahban
- Jason Baricklow
- Hongying Gao
- The Team at Pfizer

- Nick Molinaro
- Rich Klein
- Pat Bennett
- The Team at Thermo

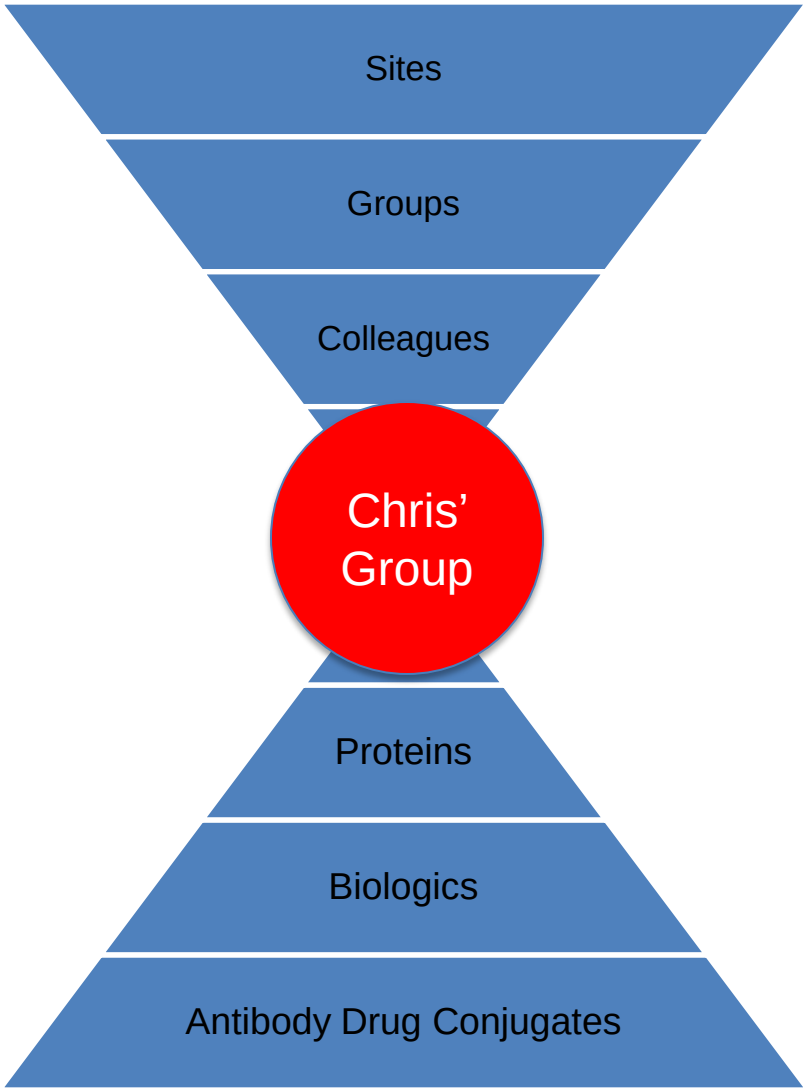
Our Group and Deliverables

- Small molecule quantitation
- Discovery, Non-Regulated
- Biological matricies
- Protein Precipitation
- Triple-Quadrupole Detection
- Multiple Reaction Monitoring

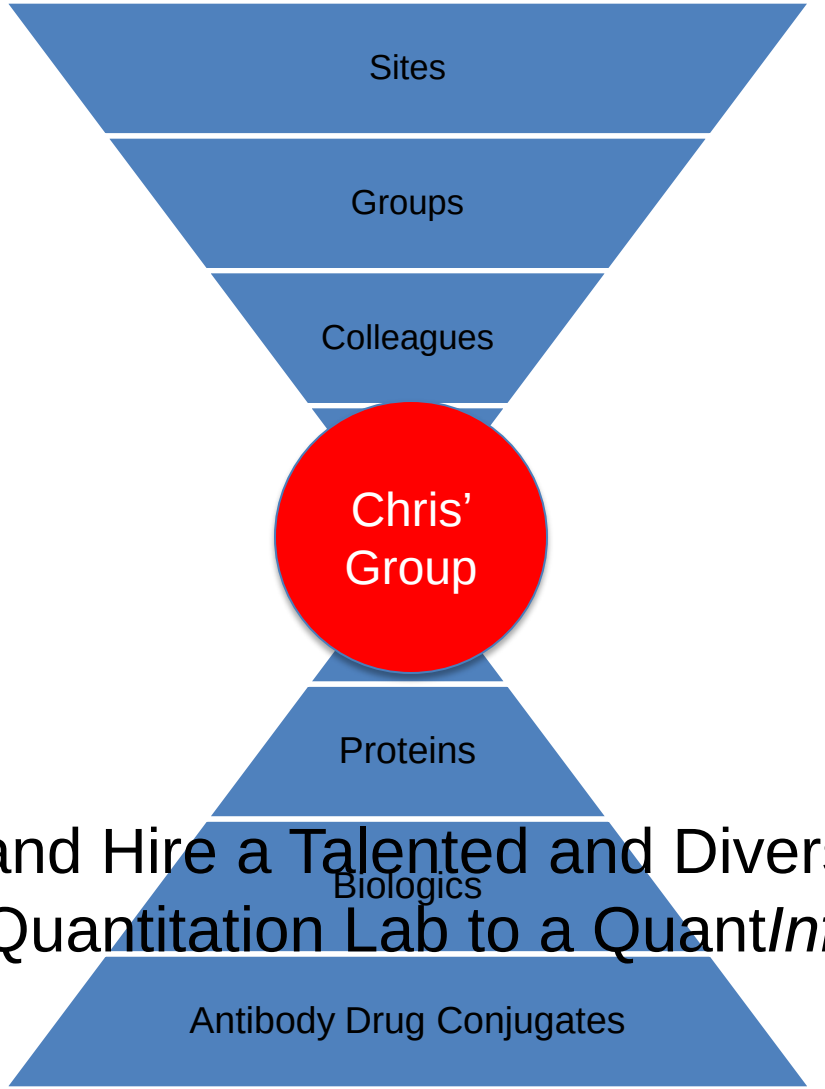
Hey, It's Tough Out There



We’re In a Pinch



How Do We Survive in the Pinch?



Maintain Develop and Hire a Talented and Diverse Workforce

Transition From a Quantitation Lab to a Quant*Information* Lab

High Resolution High Mass Accuracy Quantitation

Comprehensive comparison of liquid chromatography selectivity as provided by two types of liquid chromatography detectors (high resolution mass spectrometry and tandem mass spectrometry): "Where is the crossover point?"

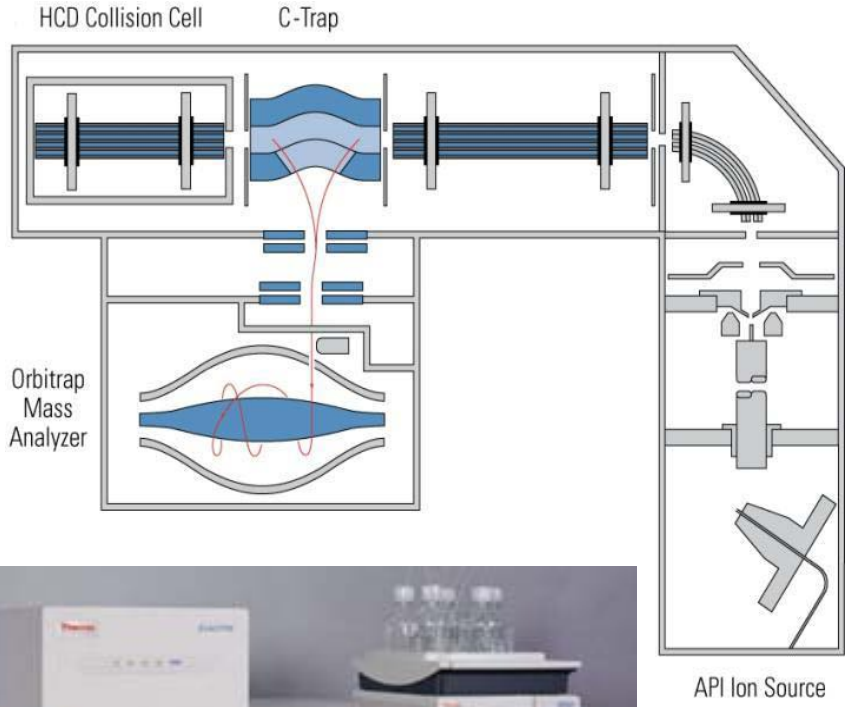
A. Kaufmann, P Butcher, K. Maden, S. Walker, M. Widmer
Official Food Control Authority, Switerzerland
Analytica Chimica Acta 673 (2010) 60–72

Optimization of Exactive Orbitrap™ acquisition parameters for quantitative bioanalysis

Richard L Wong, Baomin Xin & Timothy Olah
Bristol-Myers Squibb
Bioanalysis ,April 2011, Vol. 3, No. 8, Pages 863-871

The Candidate: Exactive™ Overview

- Exactive Hardware Overview
  - No Data Dependent Scanning
  - Quantification & Qualitative
  - High Resolution and Accurate Mass
  - Full Scan MS
  - Positive / Negative Switching
  - Optional HCD Fragmentation

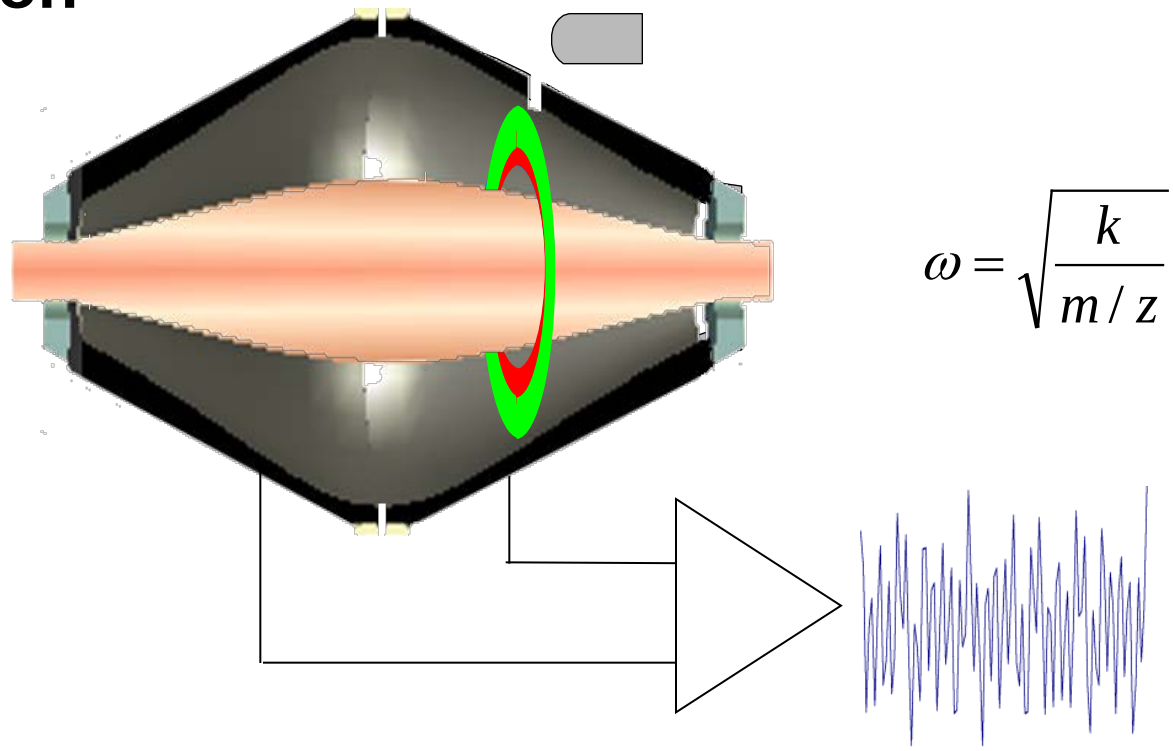


- Resolution
  100,000 at 1 scan per second
  10,000 at 10 scans per second

- Mass accuracy
  <3 ppm for 48 Hours

- Mass Range
  m/z 50 - 4000

Orbitrap Detection



$$\omega = \sqrt{\frac{k}{m/z}}$$

•Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

What we look for in a candidate

We Perform Small Molecule Quantitation in a Disovery Environment

- **Quantitation Fundementals**
  - Accuracy
  - Precision
  - Sensitivity
  - Selectivity
  - Ruggedness
- **The Ability to “Crank”**
- **The Ability to Solve Tough Bioanalytical Issues (Diversity of Capabilities)**

The Interview

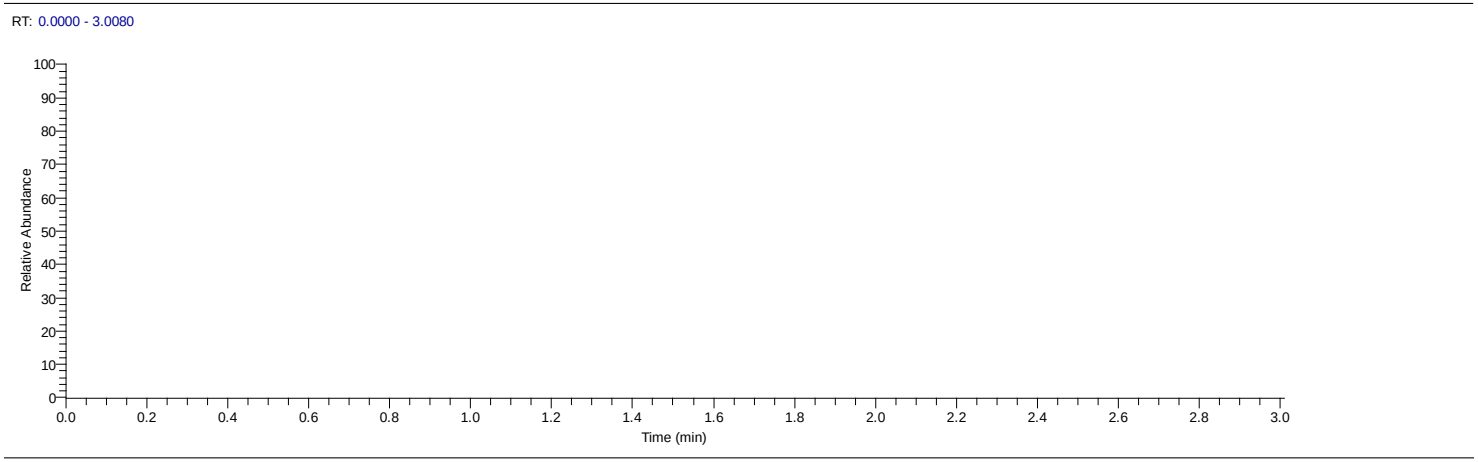
- **Can the Exactive Perform Small Molecule Quantitation Day In/Day Out in Our Work Environment?**

- **How Generic is the Methodology? Do You Even Have to Know Mass Spec or Do You Just Need to Know the Molecular Formula to Quantitate?**

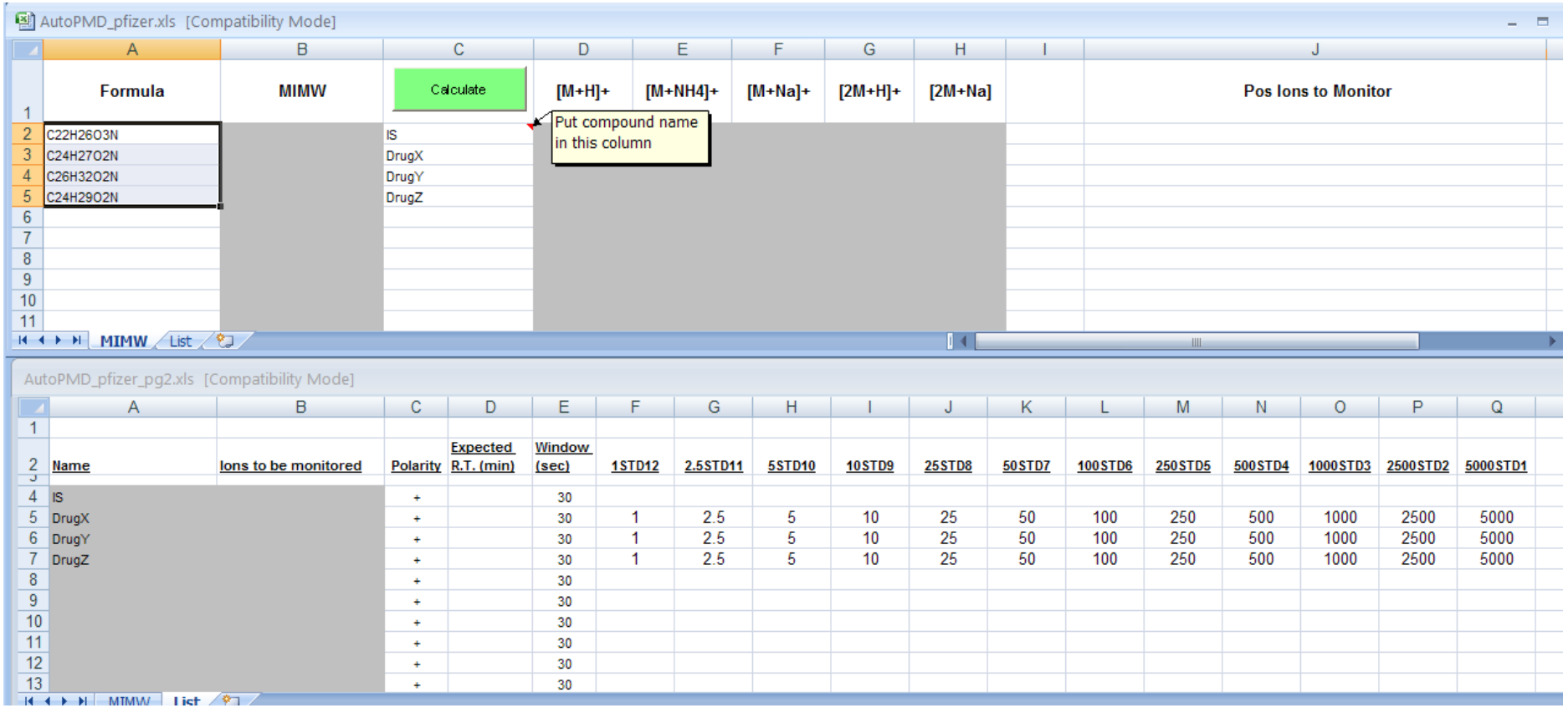
The Interview

- **Accela UPLC and Exactive Mass Spectrometer**
  - Skilled Bioanalyst with no Orbitrap Experience
  - Generic Chromatographic 3-minute Gradient
  - Method Building = Input of Exact Mass
  - Diverse Portfolio Chemical Space
    - 3 Therapeutic Areas, ~20 Compounds, 25 Studies
  - Studies are analyzed on both Triple-Quad and The Exactive.
  - 30 Days

The completely blinded approach



Processing Method Spreadsheet



AutoPMD_pfizer.xls [Compatibility Mode]

	A	B	C	D	E	F	G	H	I	J
1	Formula	MIMW	Calculate	[M+H]+	[M+NH4]+	[M+Na]+	[2M+H]+	[2M+Na]		Pos Ions to Monitor
2	C22H26O3N		IS							
3	C24H27O2N		DrugX							
4	C26H32O2N		DrugY							
5	C24H29O2N		DrugZ							
6										
7										
8										
9										
10										
11										

Put compound name in this column

MIMW | List

AutoPMD_pfizer_pg2.xls [Compatibility Mode]

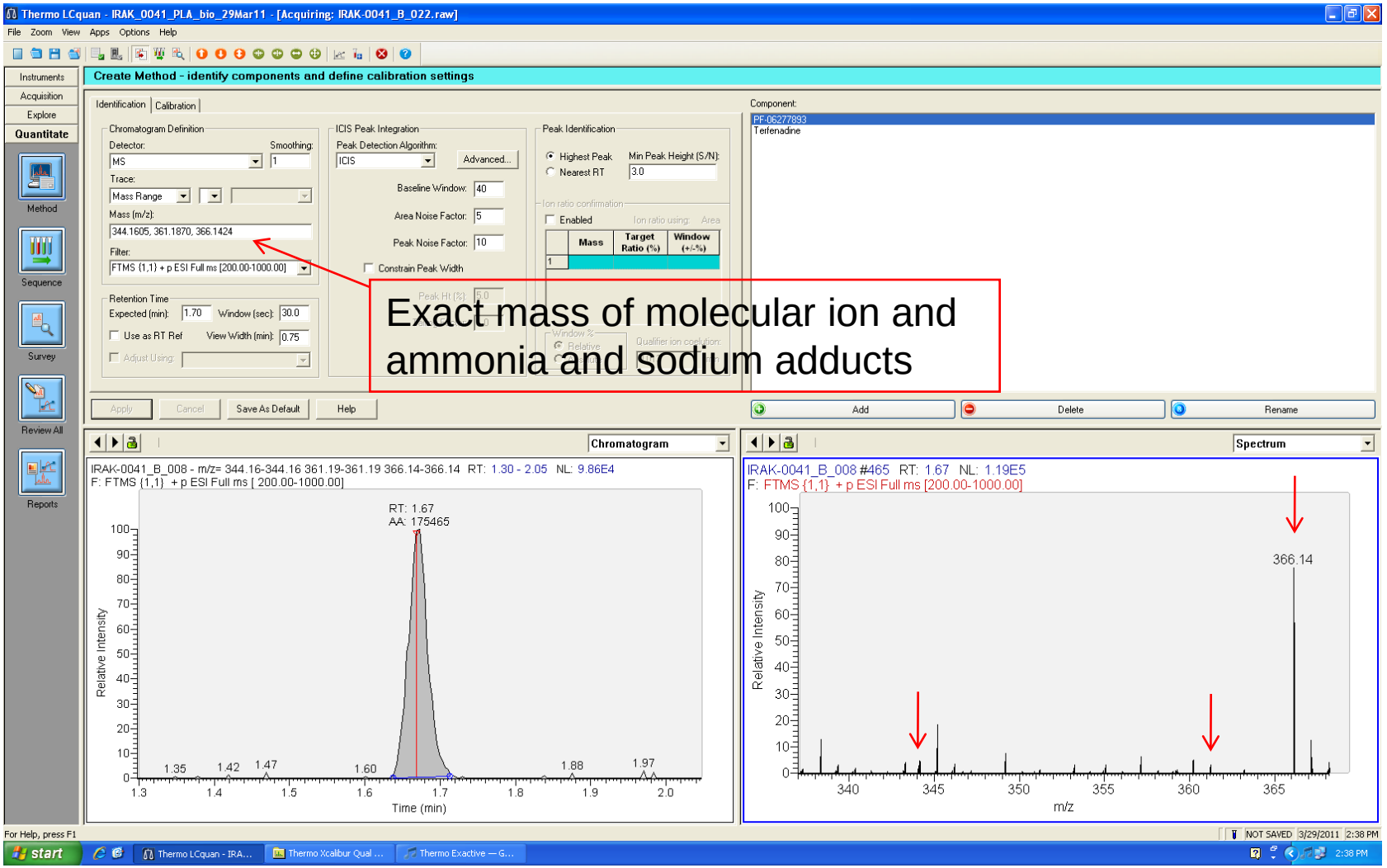
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1																	
2	Name	Ions to be monitored	Polarity	Expected R.T. (min)	Window (sec)	1STD12	2.5STD11	5STD10	10STD9	25STD8	50STD7	100STD6	250STD5	500STD4	1000STD3	2500STD2	5000STD1
4	IS		+		30												
5	DrugX		+		30	1	2.5	5	10	25	50	100	250	500	1000	2500	5000
6	DrugY		+		30	1	2.5	5	10	25	50	100	250	500	1000	2500	5000
7	DrugZ		+		30	1	2.5	5	10	25	50	100	250	500	1000	2500	5000
8			+		30												
9			+		30												
10			+		30												
11			+		30												
12			+		30												
13			+		30												

MIMW | List

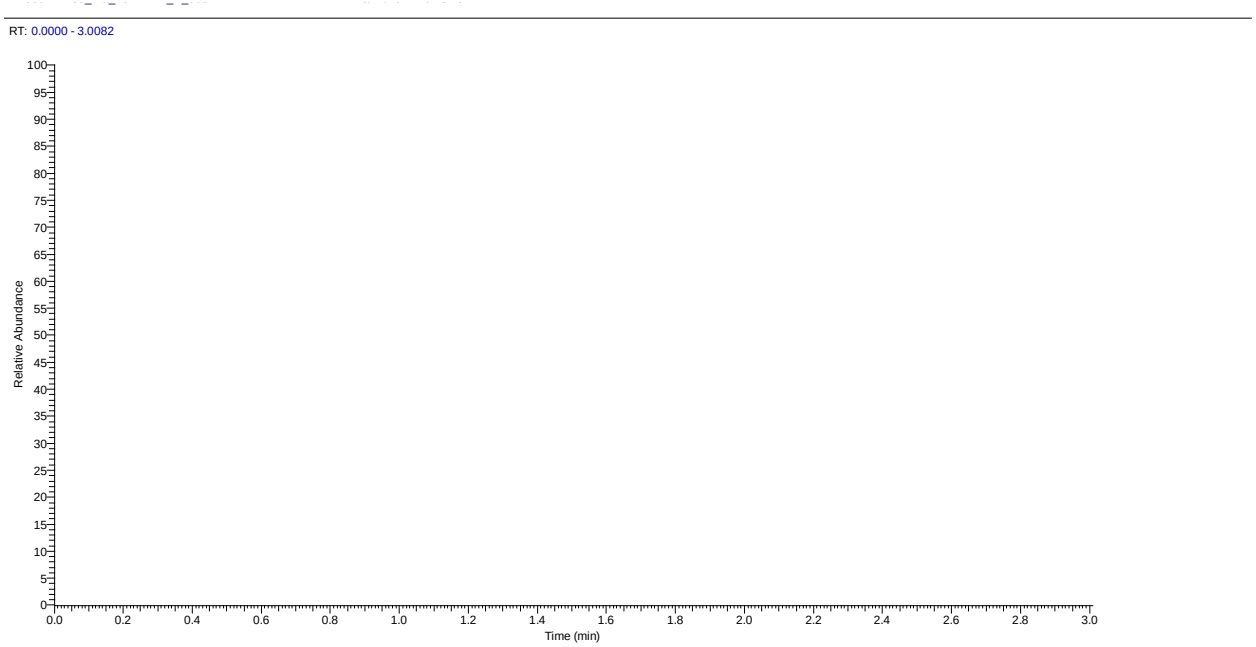
- Enter Chemical Formula's and compound Names, sheet 1
- Hit the Calculate button to calculate exact masses
- Copy Standard concentrations to all compounds, sheet 2
- Run the auto.pmd executable to create the processing method

Pfizer

Updating Method – Adduct Selection



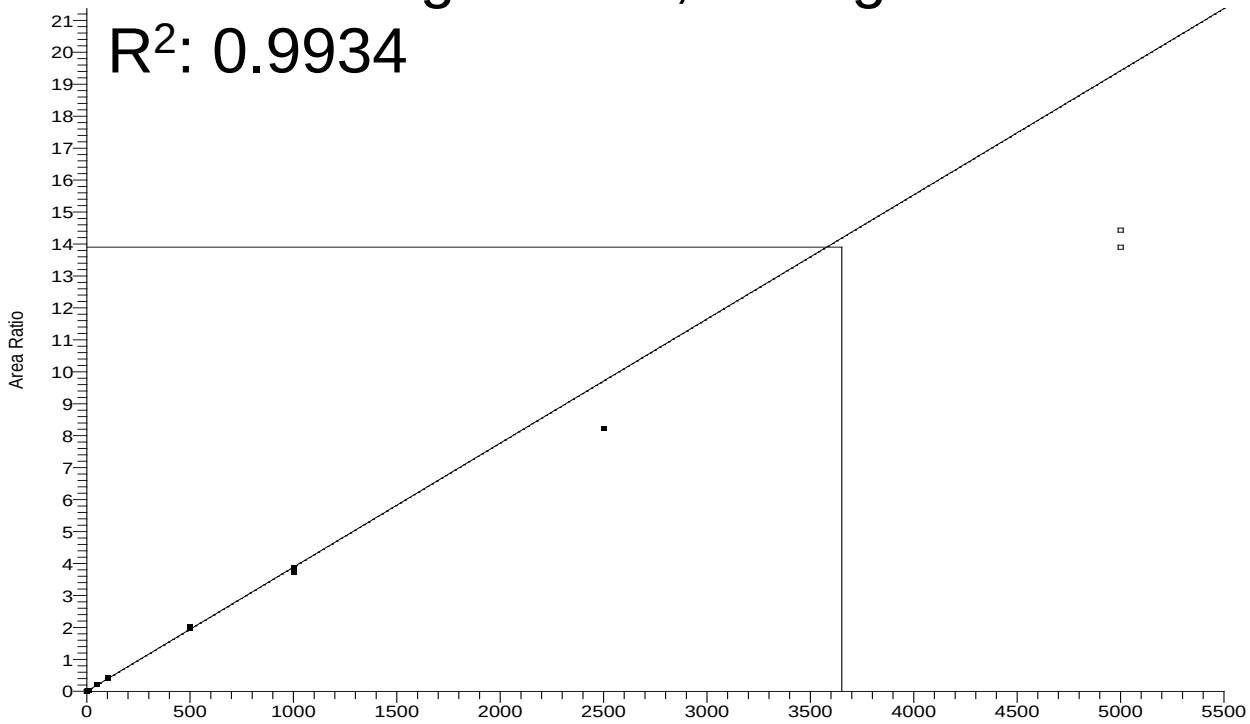
Our Experience: Sensitivity



Calibration Curve on Exactive

Linear Range: 2.5-2,500 ng/mL

R^2: 0.9934



Our experience leads us to anticipate that a new compound will typically Give us linearity of at least 3-orders of magnitude and will be on the order of what we see on our previous generation triple quads.

Our Experience: Ruggedness Day 2

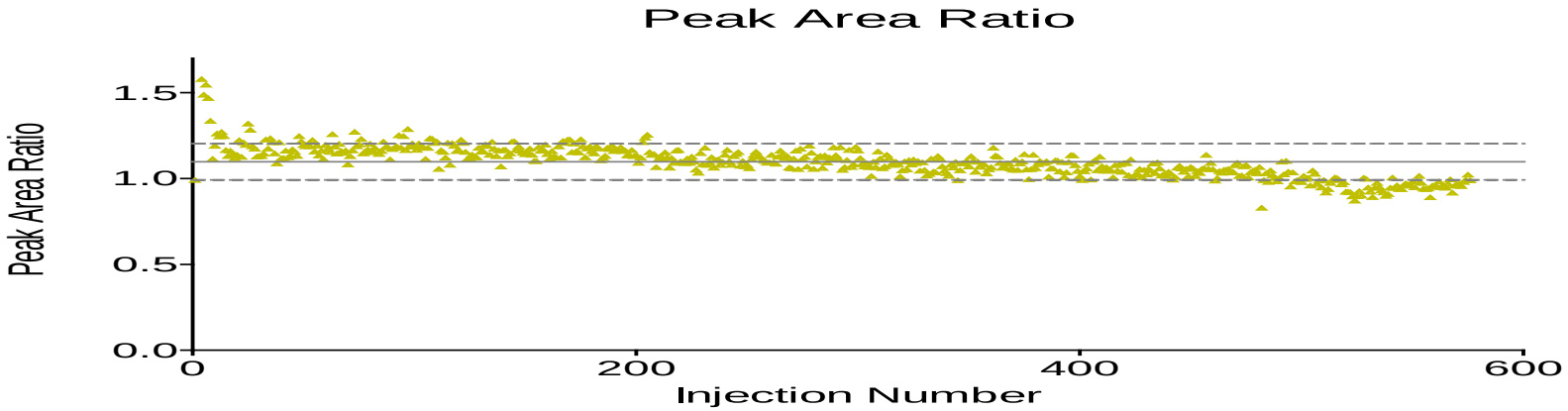


Plate	Mean	Std Dev	%CV
1	1.21	0.150	12%
2	1.16	0.039	3.3%
3	1.12	0.040	3.6%
4	1.08	0.041	3.8%
5	1.05	0.034	3.2%
6	0.967	0.045	4.6%
Overall	1.10	0.106	10%

Our Experience: Ruggedness

- **Two days of “Ruggedness” testing returned a mean value of 1.10 for the drug/Internal Standard Ratio and a plate-to-plate mean (n=12) %CV of 10%**

- **The instrument was re-calibrated and the transfer tube exchanged, every 2-3 days as a matter of routine maintenance 15-30 min.**

Quant*Information*

- **Not *currently* our strength**
  - Metabolite Scouting
  - Biomarker
  - Peptide and Protein Digests
- **All investigations were performed at a "proof of concept" level and all generated positive and encouraging results.**

Hiring Decision

- **Sorry we found someone else**
- **Evaluation of the Q-Exactive**
  - Three Therapeutic Areas
  - 8 of our most difficult Triple-Quad/Exactive Compounds

Your Hired

	LLOQ (ng/mL					

What's Next? Quant*Information*

We're not the First Industry to Contract

1982
1985
1997
2002
2005