Efficiency in Drug Discovery:
Rapid Identification of Drug-like Molecules in Drug Metabolism and Pharmacokinetics (DMPK)

Mehran Moghaddam, PhD, MBA
CEO | OROX BioSciences, Inc.
San Diego, CA, USA

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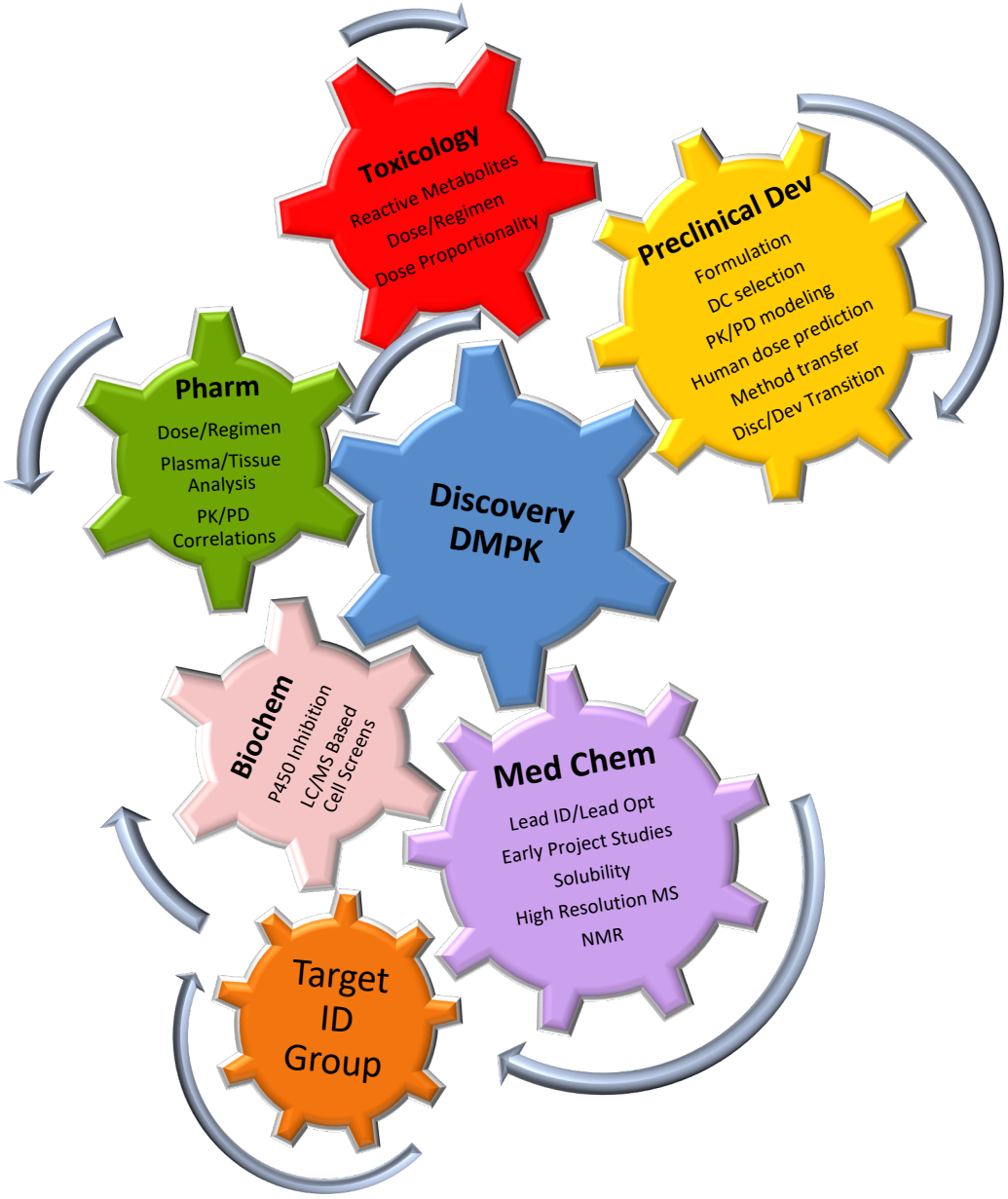
About OROX BioSciences, Inc.

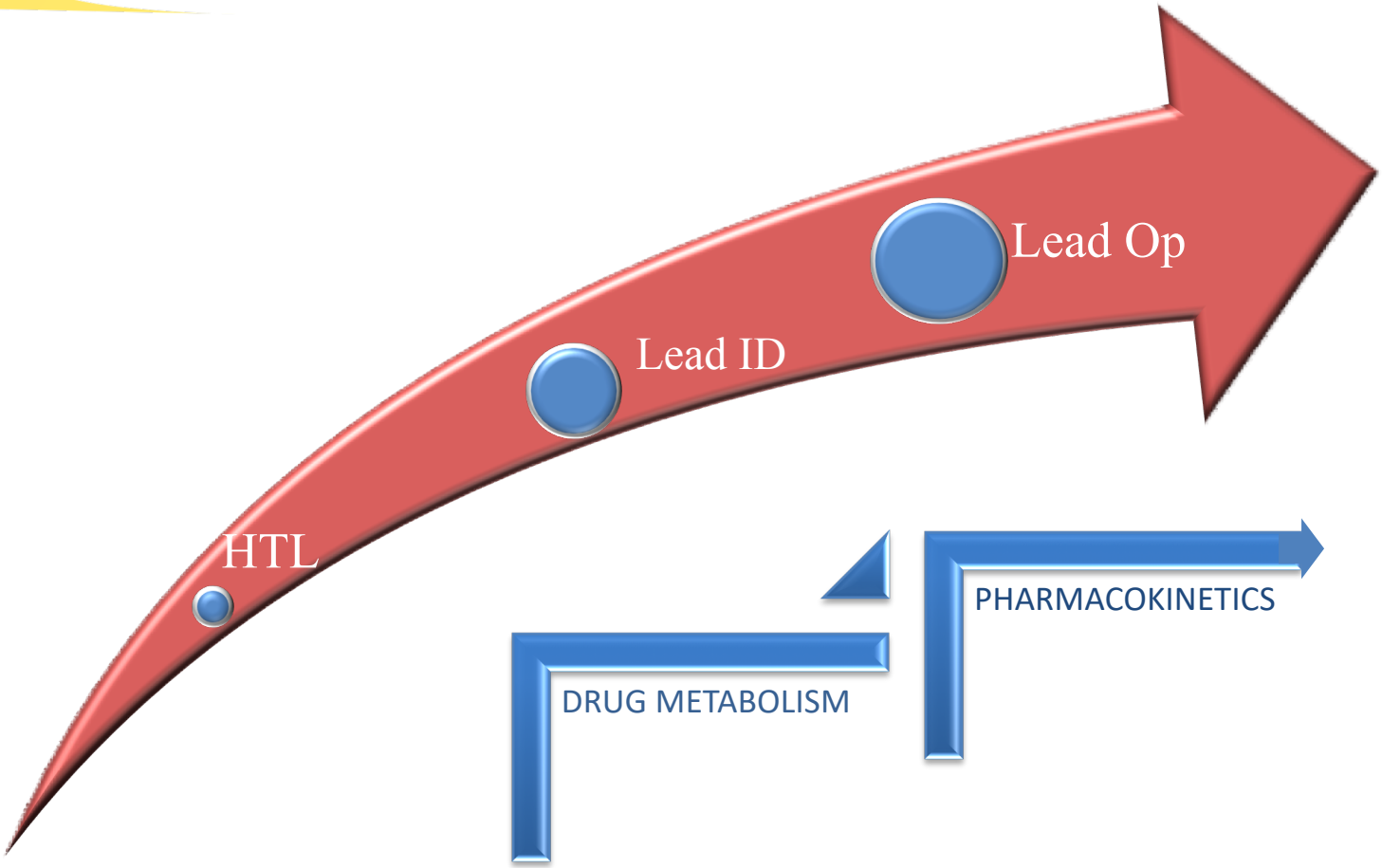
OROX BioSciences, Inc. is a biotechnology company dedicated to:

1. Discovery of small molecule drugs for treatment of fibrotic diseases
2. Development of efficient DMPK screening methods

Central Role of DMPK in Drug Discovery-Development

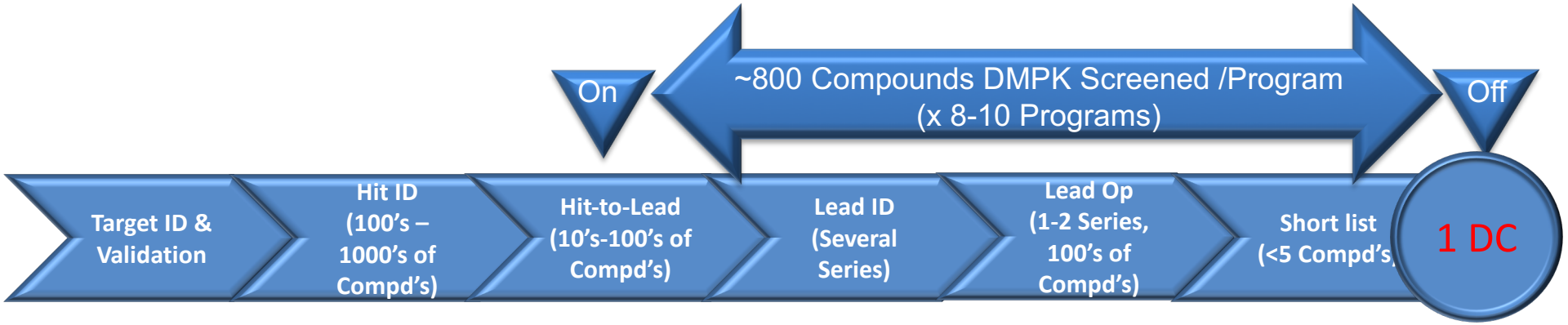


Discovery Value Chain



Thousands of Compounds Enter DMPK Screens Per Year

What Compounds Do We Advance into Pharmacology?



1. Ideas
2. Literature
3. Collaborations
4. Development organization

Biochemical triage:

1. ID CC's with desired % Inhibition
2. Generate IC50 and enzyme kinetic data to confirm hits

Chemical triage:

1. Group into series
2. Investigate ease of synth
3. Make a few close analogs to rule out exceptions
4. Investigate patent space
5. Collect some **DMPK** and biological data

Ramp up **DMPK** and biological data collection to narrow down to a few series

Select 1-2 series and optimize in **DMPK**, Pharm, ETox

Full characterization in **DMPK**, Pharm, ETox

The Challenge

- Takes 24-36 months to screen and isolate a shortlist of compounds
- Pharma spends ~$1B/year in DMPK screening
- Can we make drug discovery process faster and more efficient?
- What are the obstacles?
- Can we remove any of these obstacles?

Significance of Efficient DMPK Testing

- Faster rate of **drug-like** molecule design and synthesis = Successful drug discovery
- Superior starting points = superior drug candidates
- Optimizing against the therapeutic targets more straightforward than optimizing DMPK
- Discovery stage projects focus on target potency at the expense of good DMPK properties
- It is beneficial to assay for drug-likeness early and independently of target activity: TEST ADME FIRST, even preemptively (Hann and Keseru, *Nature Rev. Drug Disc.* **2012**, *11*, 355-365)

All VALID POINTS, BUT

CHALLENGING AND IMPRACTICAL

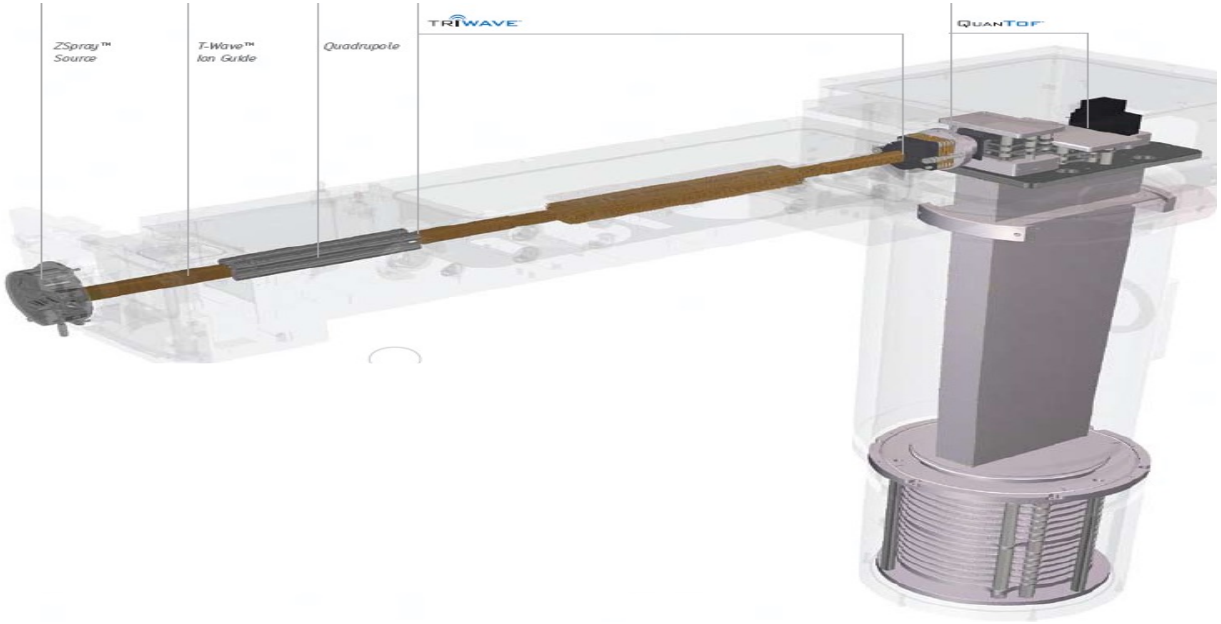
WITH CURRENT ADME TOOLS!

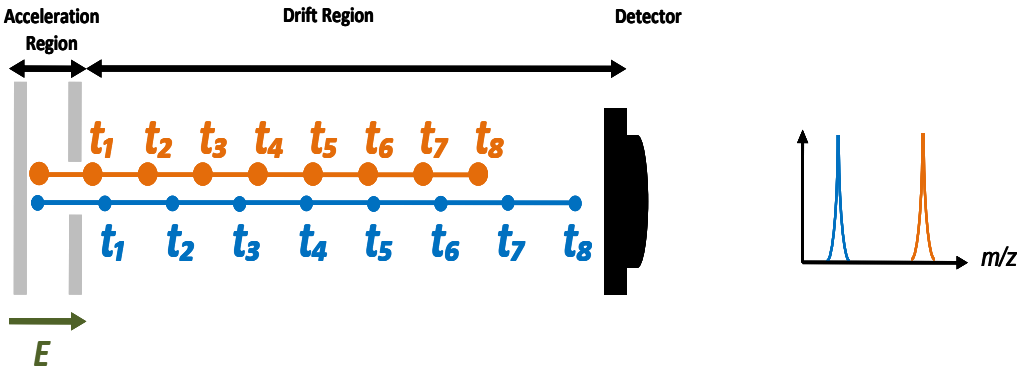
Implementation of a novel ultra fast metabolic stability analysis method using exact mass TOF-MS

Joseph D. Manna, Samantha J. Richardson, Mehran F. Moghaddam

***Bioanalysis,* 2017, 9(4): 359-368**

Principles of the Quad-Time of Flight





- **High Mass Resolution (m/Δm) is achieved (<1ppm or ± 0.0002 Da), due to time-of-flight as a mass filter.**

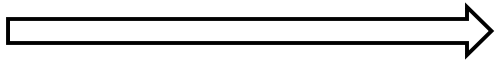
Benefits of Exact Mass

Clear Mass Assignments

Compound A

m/z: 520.2

+ 32



Metabolite

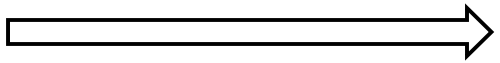
m/z : 552.1

Dioxidation? Or
Sulfhydryl?

Compound A

m/z: 520.2034

+ 32



Metabolite

m/z: 552.1755 Sulfhydryl

m/z : 552.1932 Di-Oxidation

Introducing Efficiency Through Q-TOF LCMS (UFAST-MS)

Rapid Screening for Metabolic Stability

- High throughput liver S9 incubations (60 min)
- 384-well high throughput method was developed on a TECAN
- 96 compounds in quadruplicate across 2 species (human and rat)
- At 0 and 60 min, Incubations quenched by ACN:MeOH and centrifuged
- Supernatants diluted with 1:1 with water and injected onto LC-QTOF-MS

(Richardson, et al. Drug Metabolism Letter. 2016, 10:83-90)

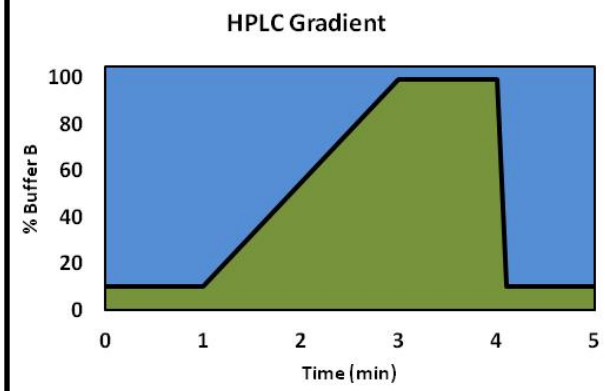
- Waters Xevo-G2XS Q-TOF-MS with an Waters I-class LC system
- Imtakt Unison C18 LC column (20mm x 3mm, 3µm particle size)
- Mobile phases - A: Water (0.1% Formic acid), B: Acetonitrile (0.1% Formic acid)
- Waters software package: UNIFI® Scientific Information System

Traditional Verses Ballistic LC Gradient

(A) Represents the traditional HPLC gradient run for typical metabolic stability assays

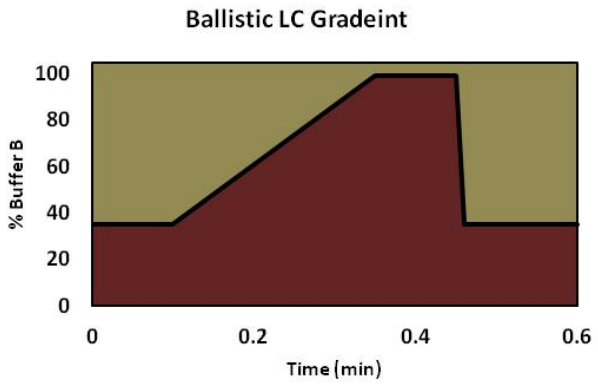
(B) Represents the sub 1 min ballistic LC gradient developed for U-FAST-MS

A



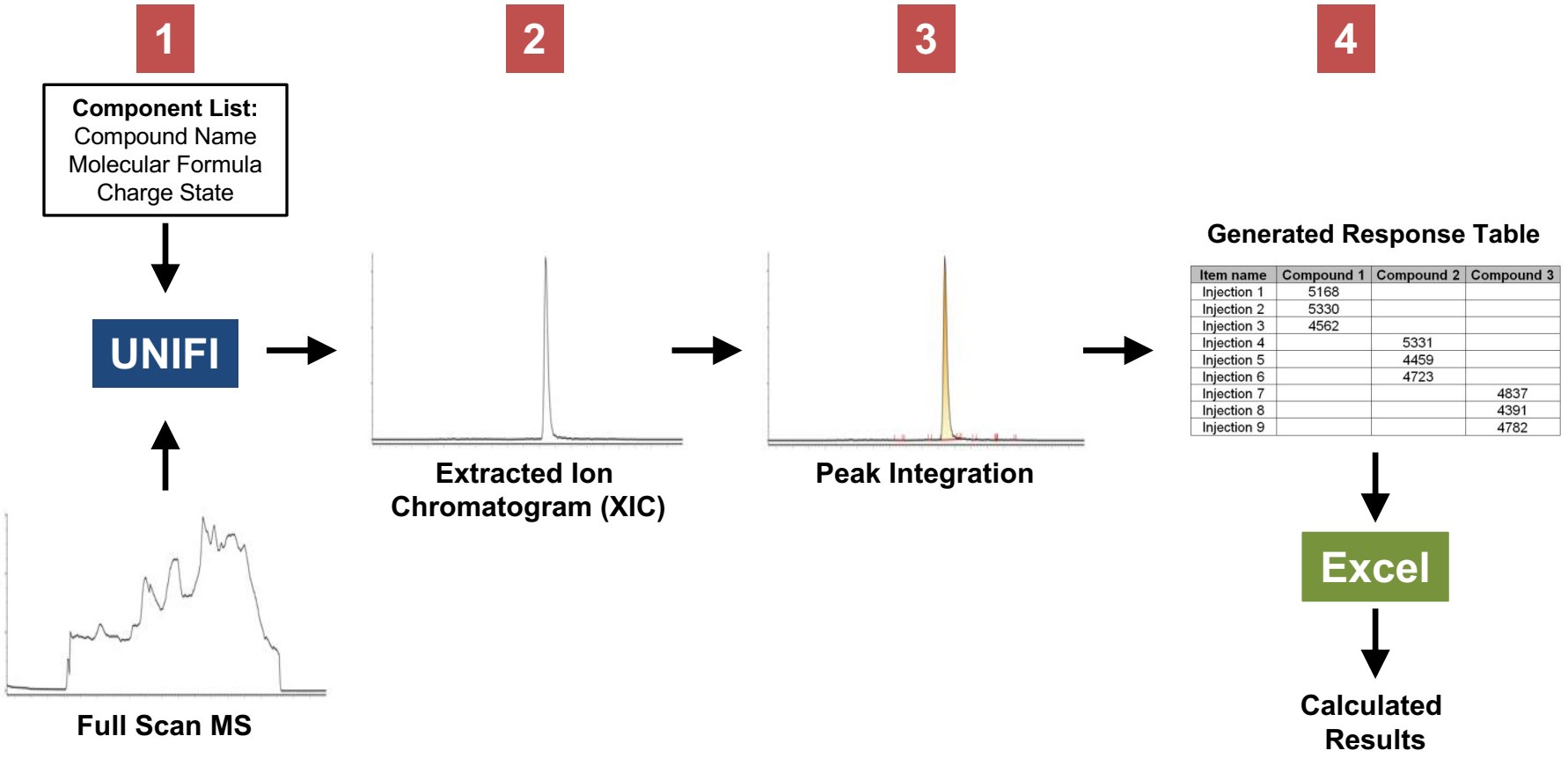
Tradition HPLC Gradient	
Time (min)	*% B*
0.00	10
1.00	10
3.00	99
4.00	99
4.10	10
5.00	10

B



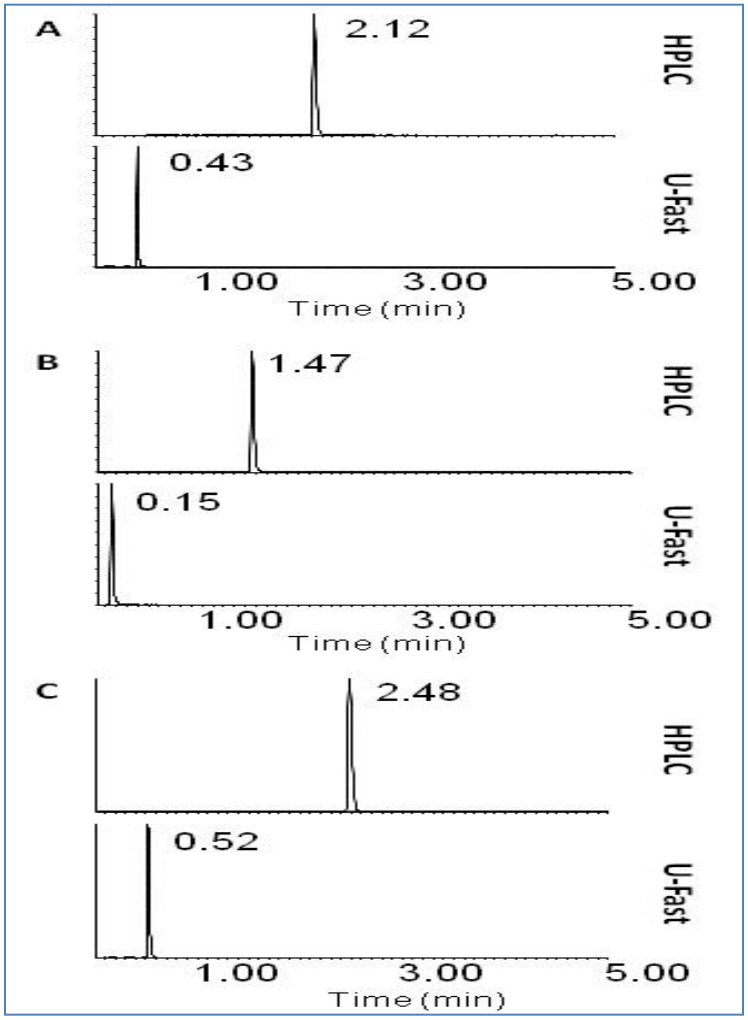
UFast-MS Gradient	
Time (min)	*% B*
0.00	35
0.10	35
0.35	99
0.45	99
0.46	35
0.60	35

UNIFI Workflow for Data Acquisition and Processing



Comparison of HPLC Verses Ballistic Traces

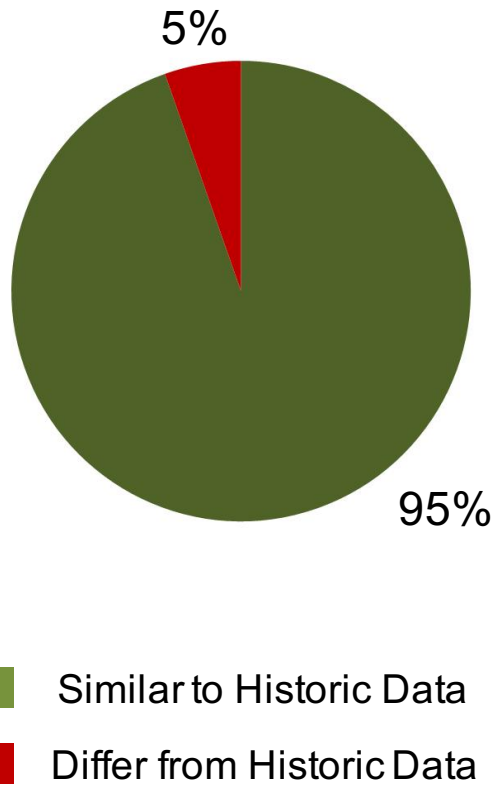
(A) 7-EC and 2 test articles (B and C) show comparable quality of separation with superior speed for UFast-MS compared to HPLC



Comparison of Stability Data

Traditional vs. UFAST-MS

	Historic Stability Data	U-Fast-MS Stability Data
Compound	% Remaining ± StDev	% Remaining ± StDev
7-Ethoxycoumarin (7-EC)	59 ± 8	58 ± 7
Celgene Compound 1	93 ± 1	95 ± 3
Celgene Compound 2	84 ± 13	95 ± 9
Celgene Compound 3	86 ± 3	90 ± 7
Celgene Compound 4	78 ± 1	82 ± 3
Celgene Compound 5	91 ± 2	96 ± 2
Celgene Compound 6	2 ± 0	6 ± 1
Celgene Compound 7	95 ± 9	85 ± 2
Celgene Compound 8	37 ± 2	41 ± 1
Celgene Compound 9	49 ± 2	50 ± 3
Celgene Compound 10	6 ± 0	13 ± 0



Subsequently, a large data confirmed this finding

Summary of UFAST-MS Metabolic Stability Workflow

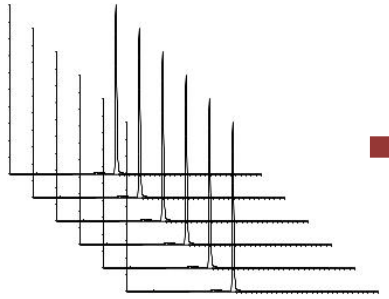
384 well High Throughput S9 Stability Assay
(2 species in quadruplicate)

Waters Xevo-G2XS Q-TOF

Compound ID	Molecular Formula
Compound 1	MF 1
Compound 2	MF 2
Compound 3	MF 3
Compound 4	MF 4

Compound names and molecular formulas are loaded into the software

UNIFI Software
(Data Acquisition, Mining and Processing)



UNIFI scans all data for uploaded compound masses (m/z) and calculates Peak Area for all identified components

% Remaining, Standard Deviation and %CV

Impact

PREVIOUSLY OPTIMIZED PROCESS:	UFAST-MS PROCESS:
~190 Compounds/Week	~190 Compounds/Week
- 3 FTE's	- 1 FTE
- Significant LC/MS Method Development	- No LC/MS Method Development
- 2 Mass Spec's Occupied for 2 Working Days	- 1 Mass Spec Occupied for 1 Working Day
- 6 Working Days	- 4 Working Days

~10,000 compounds studied/year with comparable quality in both methods, but

UFAST-MS Required:
30% the workforce,
25% of Mass Spec and Chemical Resources, and
Had 30% Faster Turnaround Time

SAME DATA, LESS RESOURCES, FASTER

Future Efforts
(ADME-F)

DMPK-screen of 1000 Compounds

Based on POC data (more data pending)

Significant Factors	DMPK Screening Models		
	Pharma	Biotech	OROX
Time to Shortlist (months)	**24-36**	**24-36**	**<12**
Expenditure/Program ($MM)	4.2	2.8	1.1
Total Burden of Synthesis on Med Chem (g)	13	12	5

Conclusions

- Successful collaboration between internal DMPK, IT, and Waters Corp led to U-FAST-MS
- U-FAST-MS is a novel HT-bioanalytical workflow for metabolic stability assay
- Accomplished by assembling existing methods in a novel manner and implementation of custom-designed data processing
- Eliminated the need for LC-MS method development and optimization
- Rapid analysis of 96 compounds in quadruplicate across 2 species in <24h
- Increased capacity and reduced analysis time by several fold, while maintaining quality
- This is a significant improvement on previous methods
- ADME-F methodology is under development at OROX BioScicenes

Acknowledgments

THANK YOU