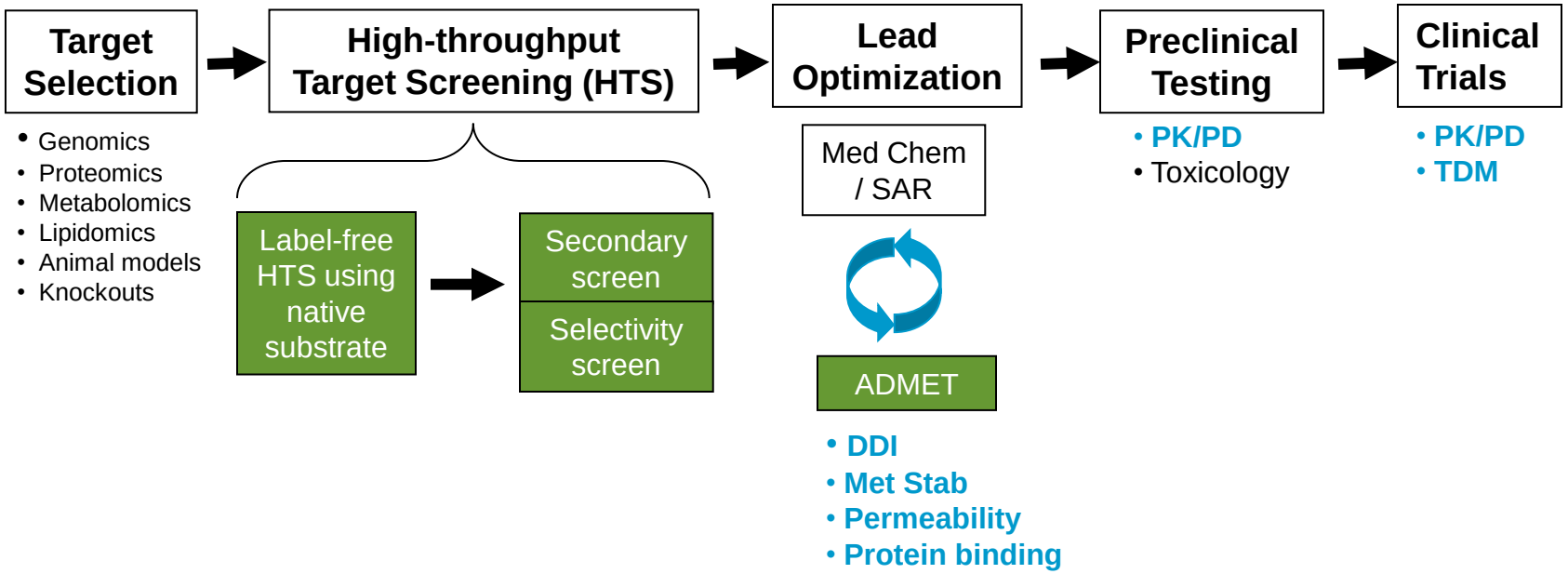
RapidFire®
High-throughput MS technology

Enhancing Drug Discovery
Turning Mass Specs into
Plate Readers

Agilent Technologies

Drug Discovery with RapidFire

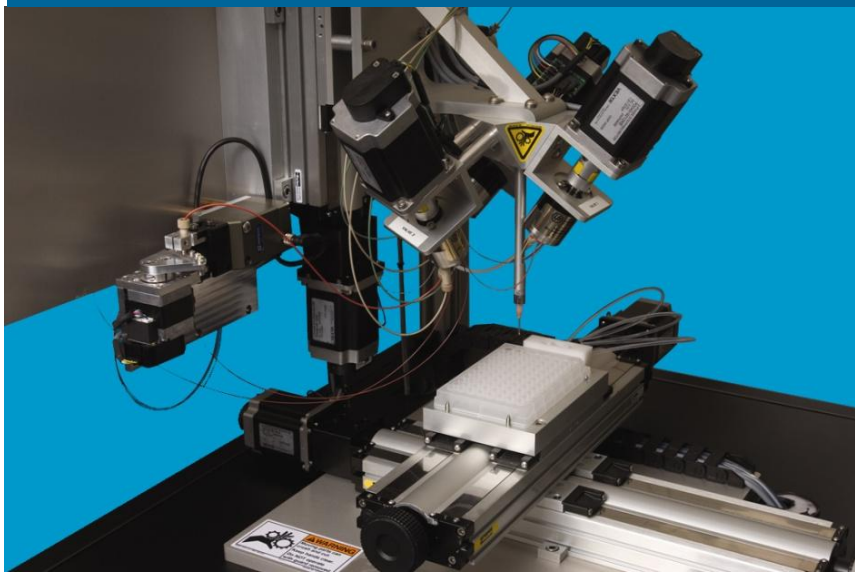


- Identify the best hits: Eliminate HTS compromises
- Select the best leads: Data-driven lead selection
- Eliminate compromises when designing experiments

RapidFire® Mass Spectrometry

Fast autosampler & SPE system

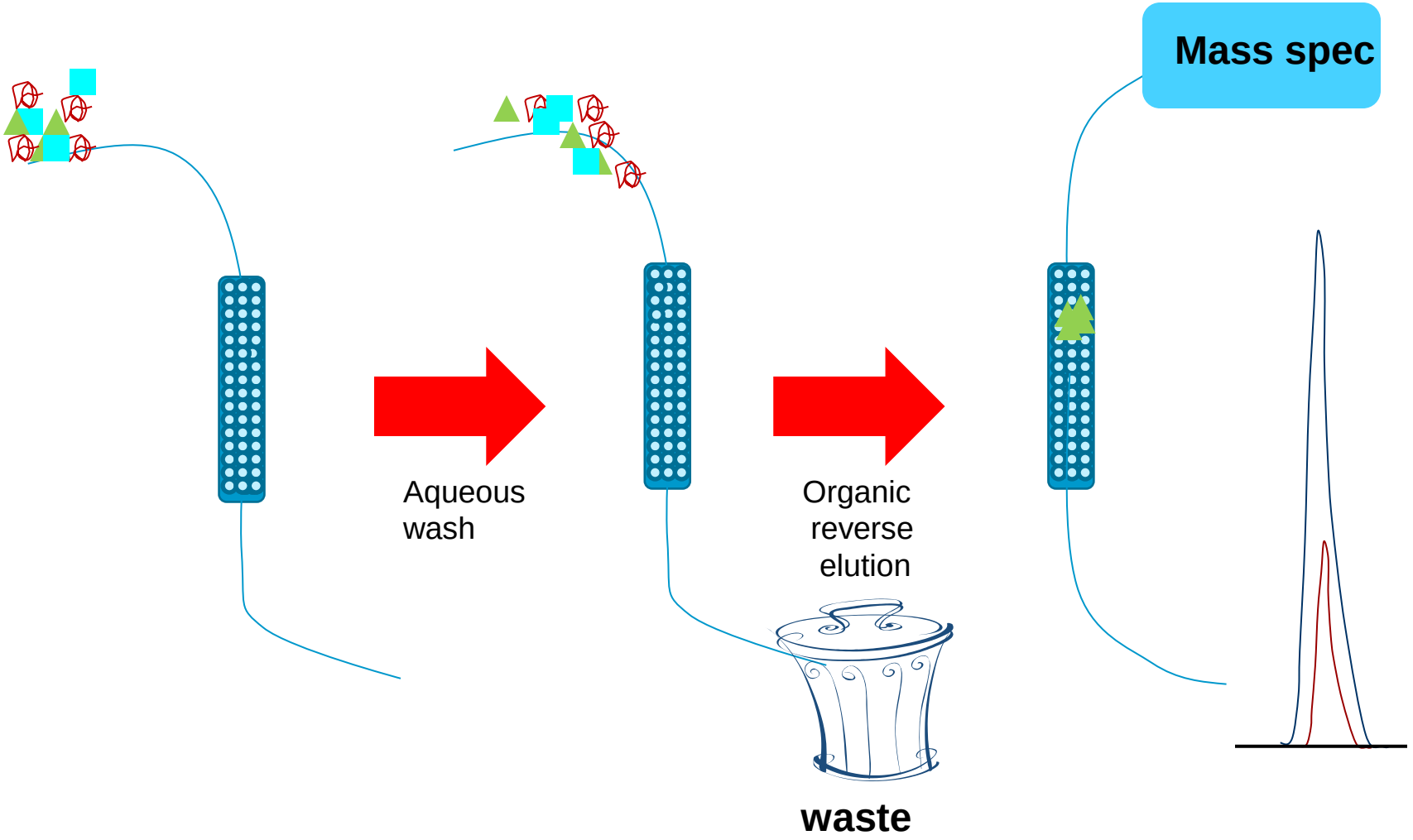
- Replaces LC in LC/MS
- Integrated, automated, micro-scale solid-phase extraction
- Integrates with standard ESI MS instruments
- **cycle time: 5–10s/sample**



Compatible with biological matrices

- Microsomal preparations
- Cell culture supernatants
- Tissue extracts
- Plasma

SPE-MS/MS Analysis

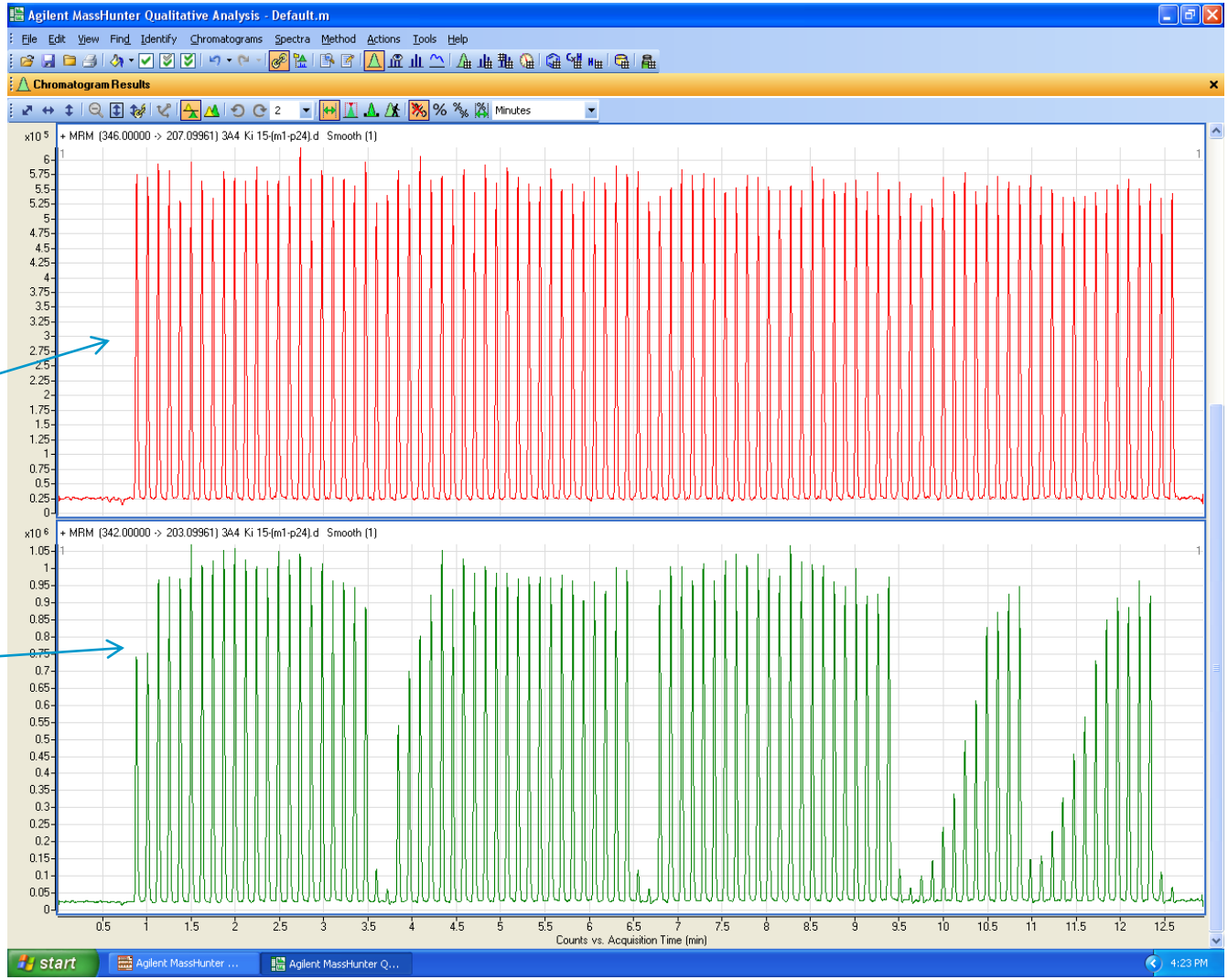


RapidFire Data

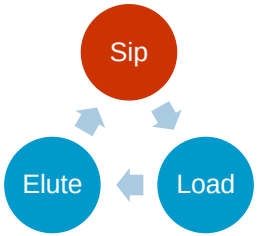
96-well plate analyzed in <12 minutes

IS data

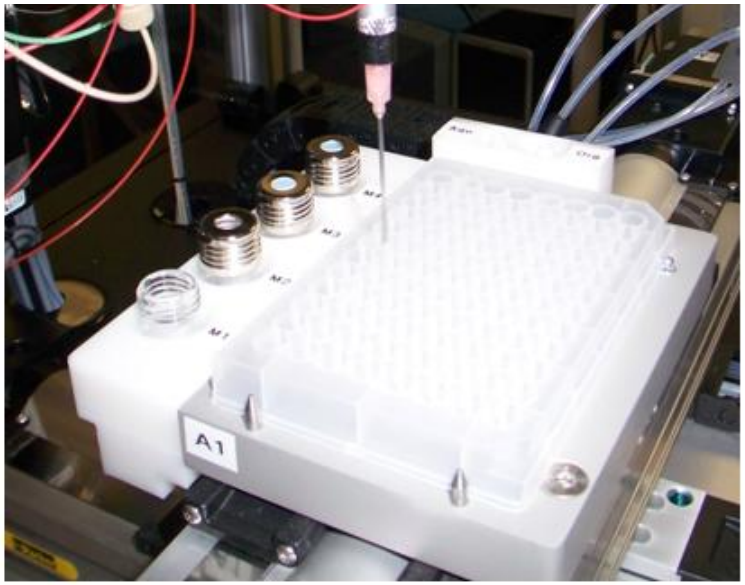
Sample data

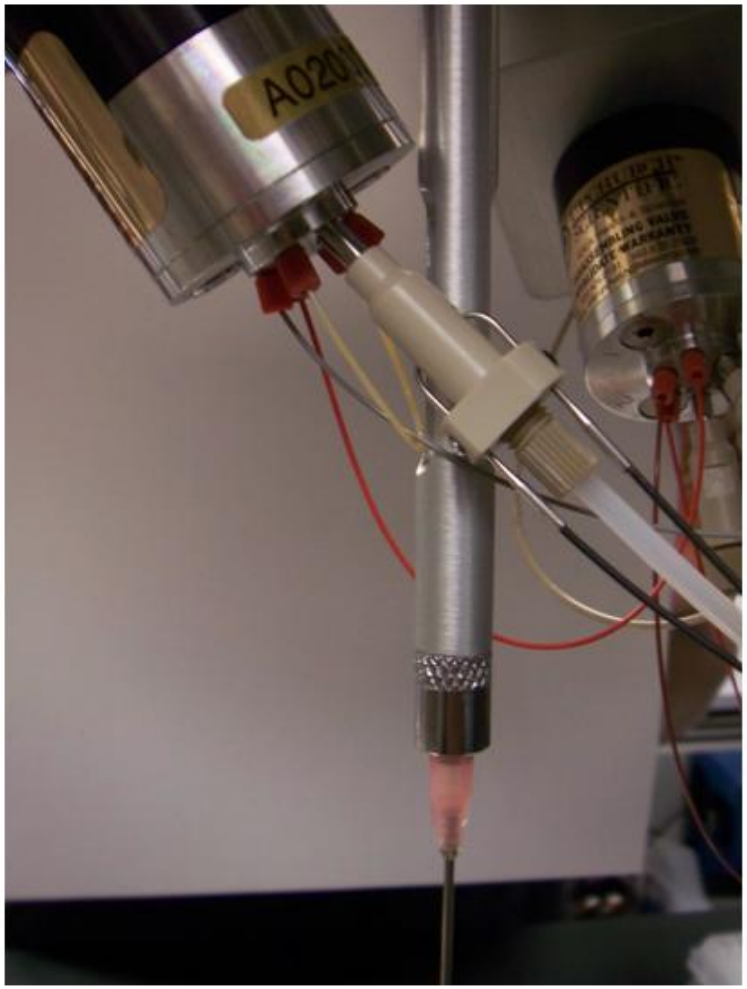


RapidFire Cycle – Sip/Equilibrate

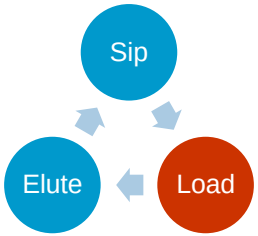


- Standard 96 or 384-well plates
- Sample interface (sipper)
  - Washed with aqueous and organic solvents
- Optical sip sensor
  - No wells missed (even if half full)

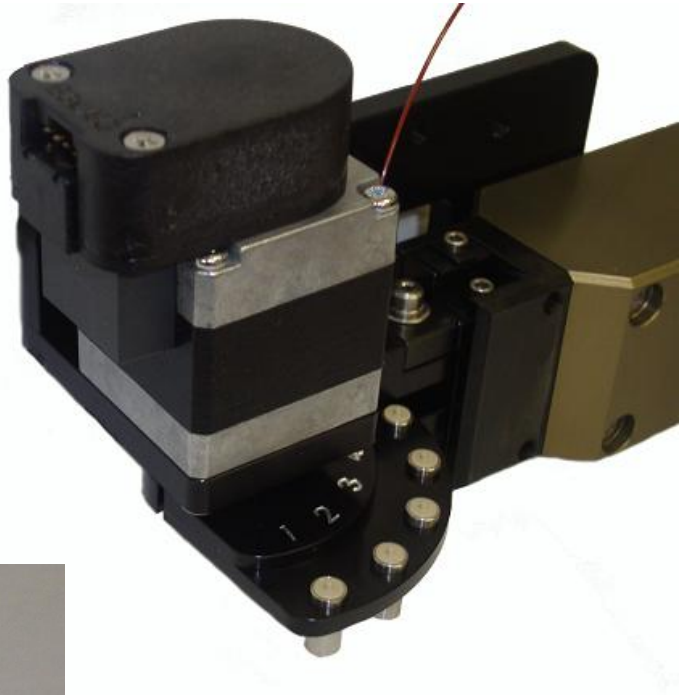


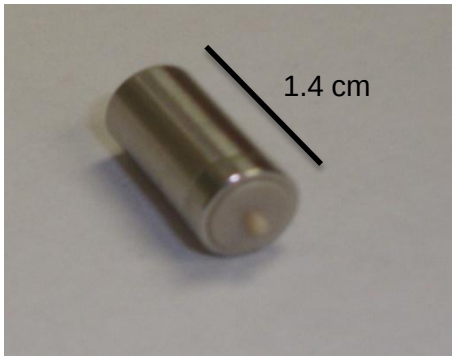


RapidFire Cycle – Load/Wash

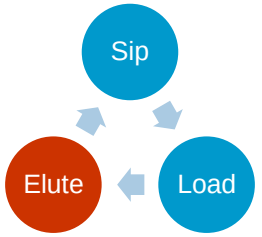


- 10-µL injection loop onto 4-µL RapidFire cartridge
- No offline sample preparation
  - SPE purification by proprietary cartridge (variety of packing beds) of quenched assays
- Cartridge changer (6 slots)
  - Automatic or programmed switch
  - 24-hour unattended runs
  - Facilitates assay development
  - Packing Material
    - C4, C8, C18
    - Phenyl, Cyano
    - HILIC
    - Client specified

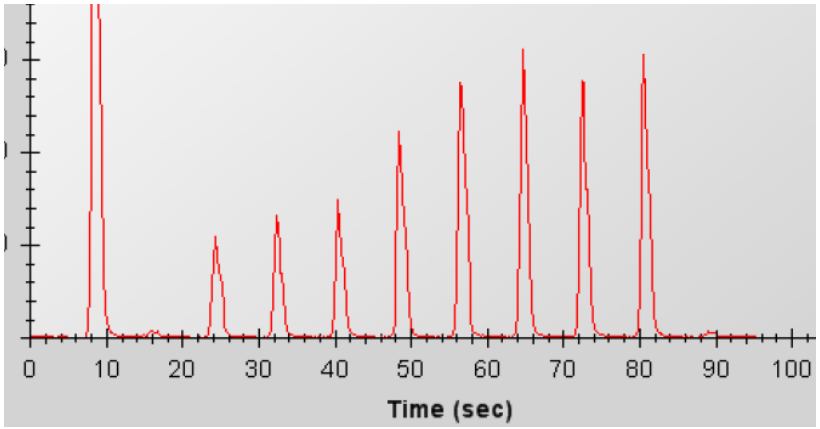




RapidFire Cycle – Elute/Clean



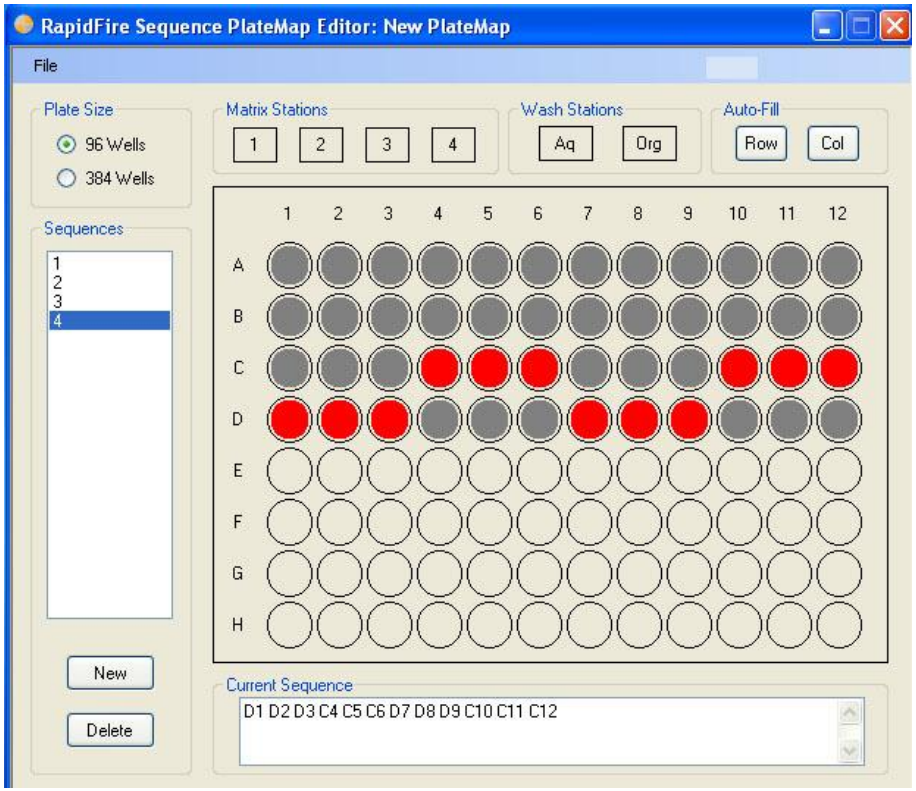
- Compatible with any ESI or APCI flow injection analysis MS
- MS at 5-10 seconds/sampl
  - Micro-scale fluidic path
  - Baseline resolution
  - PEEK minimizes carryover



RapidFire - Automation & Efficiency

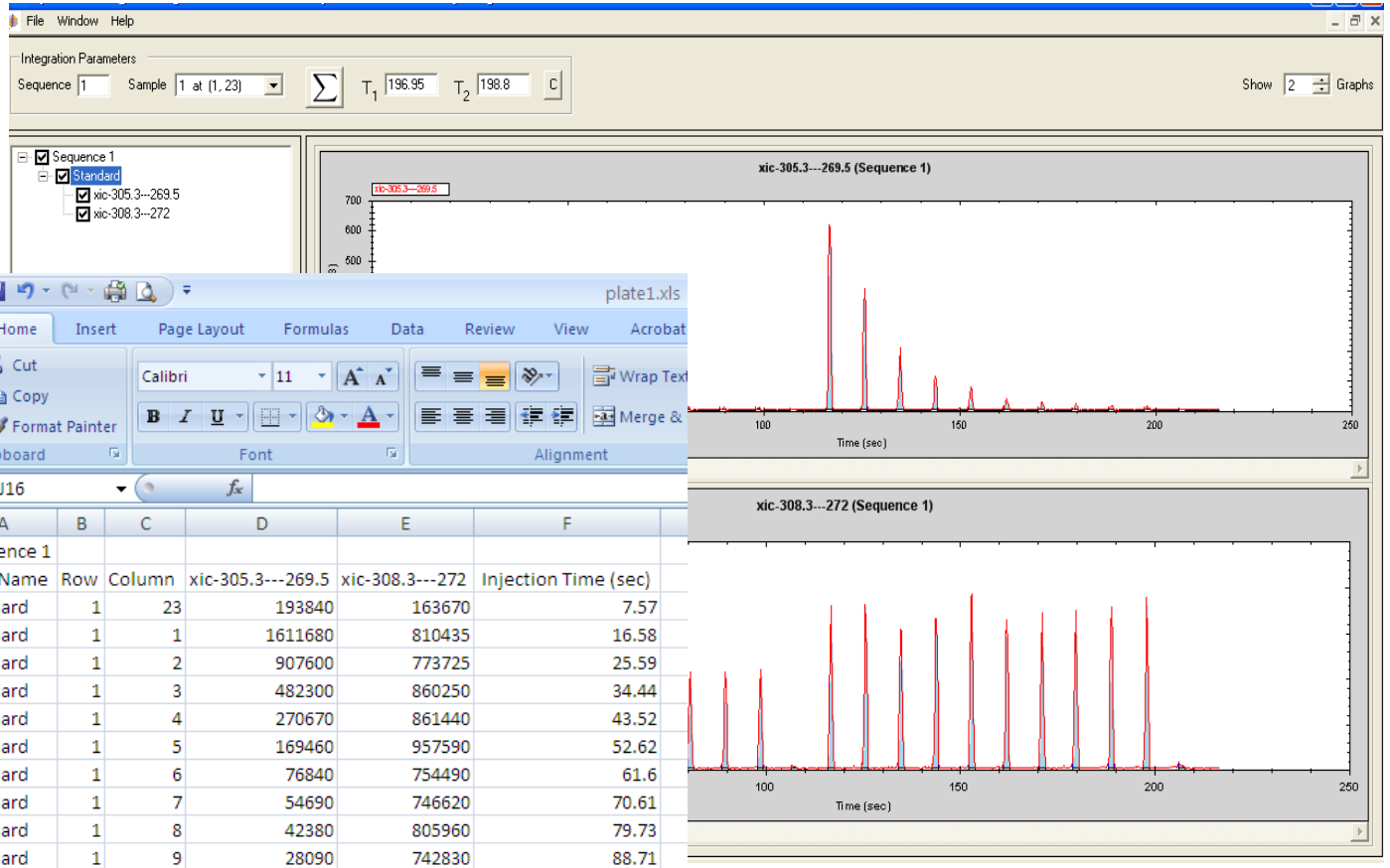
Acquisition Software Features

- Sequence-based protocol
  - injections from plates in any well order
- Multiple MS methods per plate
- Enter all pre-developed parameters at once
  - for full stack of plates (7000 samples/run)
- Barcode scanner
- 24-hour unattended operation
- Active monitoring
  - Error → flag upon suspension



RapidFire Integrator - Data Analysis & Output

- Proprietary, intuitive data analysis software
  - Outputs results as text files compatible with standard databases



	A	B	C	D	E	F
1	Sequence 1					
2	PlateName	Row	Column	xic-305.3---269.5	xic-308.3---272	Injection Time (sec)
3	Standard	1	23	193840	163670	7.57
4	Standard	1	1	1611680	810435	16.58
5	Standard	1	2	907600	773725	25.59
6	Standard	1	3	482300	860250	34.44
7	Standard	1	4	270670	861440	43.52
8	Standard	1	5	169460	957590	52.62
9	Standard	1	6	76840	754490	61.6
10	Standard	1	7	54690	746620	70.61
11	Standard	1	8	42380	805960	79.73
12	Standard	1	9	28090	742830	88.71
13	Standard	1	10	32710	836000	97.74
14	Standard	1	24	10840	27140	106.71

RapidFire Integrator Software

Internal Standard (d4-1'OH-midazolam)

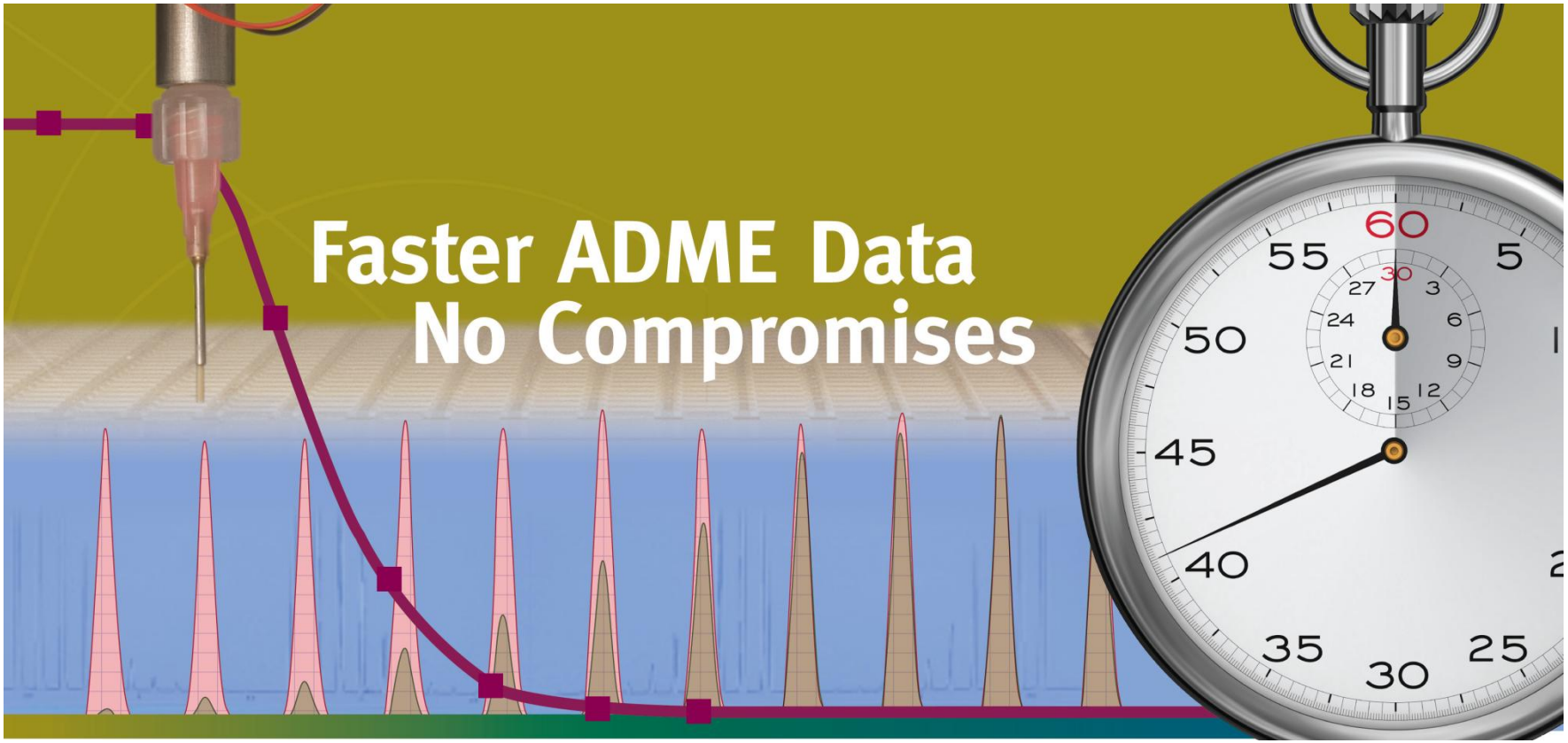
	1	2	3	4	5	6	7	8	9	10	11	12
A	13515322	14269894	14332623	14471745	15116982	15301635	14690330	14579803	14465625	15011230	15237832	15546360
B	17434008	16604680	16948062	16097807	16634851	16445362	16467090	16236566	15934448	16043856	16149485	16732055
C	18620363	17813741	17284582	16651378	16716736	17219301	17699784	17024495	16106934	16662407	17022034	17631943
D	18853237	18639570	17545882	16707887	16859350	17082515	17135769	16917660	16866547	16762991	16662805	16932106
E	19095034	19155820	18632830	17190636	16915409	17698485	18031132	17904120	17498786	16646769	17301091	18276269
F	19501938	18858411	17530737	17476044	17285001	17765209	18191777	17539311	18035244	17721958	17420392	18124271
G	19617689	19641455	20695544	18748491	17433257	17236209	18041601	20432135	18674406	18662003	17706549	18696053
H	19645157	19177960	20062090	19103985	18990975	17804109	18038389	19058414	19193675	19711093	18461084	17856780

Product (1'OH-midazolam)

	1	2	3	4	5	6	7	8	9	10	11	12
A	81217	100602	88629	94434	113207	115549	83772	87924	97878	244550	235432	262948
B	152871	118289	139825	182411	176312	168628	104282	104738	108970	661471	664829	633719
C	246216	215908	197602	291126	302567	285434	149992	150230	142836	1236854	1168309	1328238
D	544895	555992	471635	426800	398887	403895	227402	206803	200213	1849887	1893075	2030754
E	1541495	1436062	1357041	507776	540945	564612	453009	332389	361539	2609866	2769033	3056845
F	2261622	2187441	1995732	715854	714291	764022	583844	527665	515286	3324764	3182154	3653594
G	2834427	2233412	2213744	932589	818246	821289	694814	514781	630866	4214068	3234244	3427977
H	2323314	2461589	2282049	910427	836034	920960	666413	632365	550910	3351768	3794636	3518838

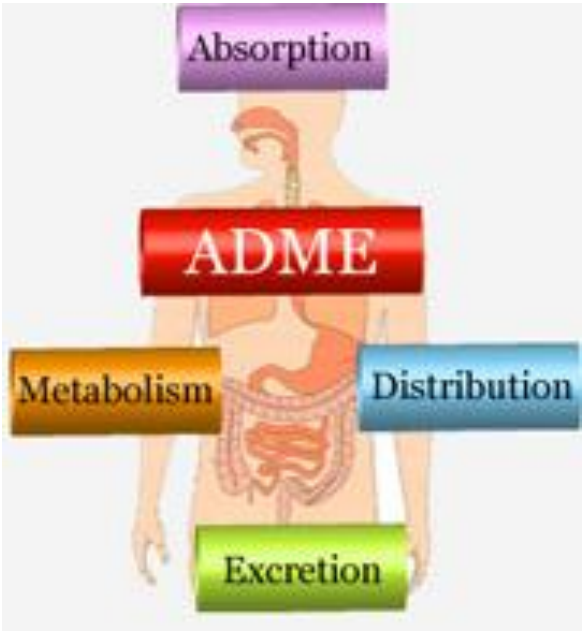
Turning a mass spectrometer into a plate reader

RapidFire applications for *in vitro* ADME
(absorption-distribution-metabolism-excretion)



RapidFire for *in vitro* ADME assays

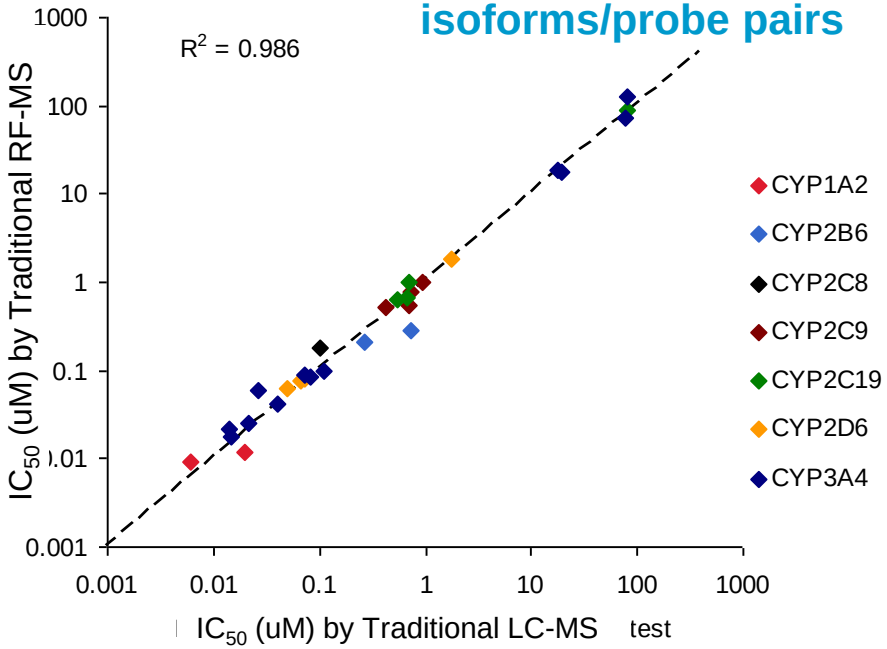
Strong results correlation with traditional technologies



- CYP inhibition (drug-drug interaction)
  - Reversible and Time-dependent
- Metabolic stability
  - HLM or S9
- Permeability
  - cell lines (Caco-2) and/or PAMPA
  - P-glycoprotein inhibition
- Plasma protein binding
  - HSA, brain, microsomal binding

Summary of Analytical Methods Comparison

Direct CYP Inhibition: Comparable results obtained >20-fold faster analysis 8 different isoforms/probe pairs



Caco-2 Permeability: Comparable results obtained nearly 10x faster with RF-MS

	LC-MS	RF-MS
Analysis Time	20 hours	2.5 hours
Linearity	R > 0.99	R > 0.98
LLOQ	5 nM	5 nM
Carry-over	< 0.3%	< 0.3%
Generic method applicability	Generic LC method	Generic SPE method

BD

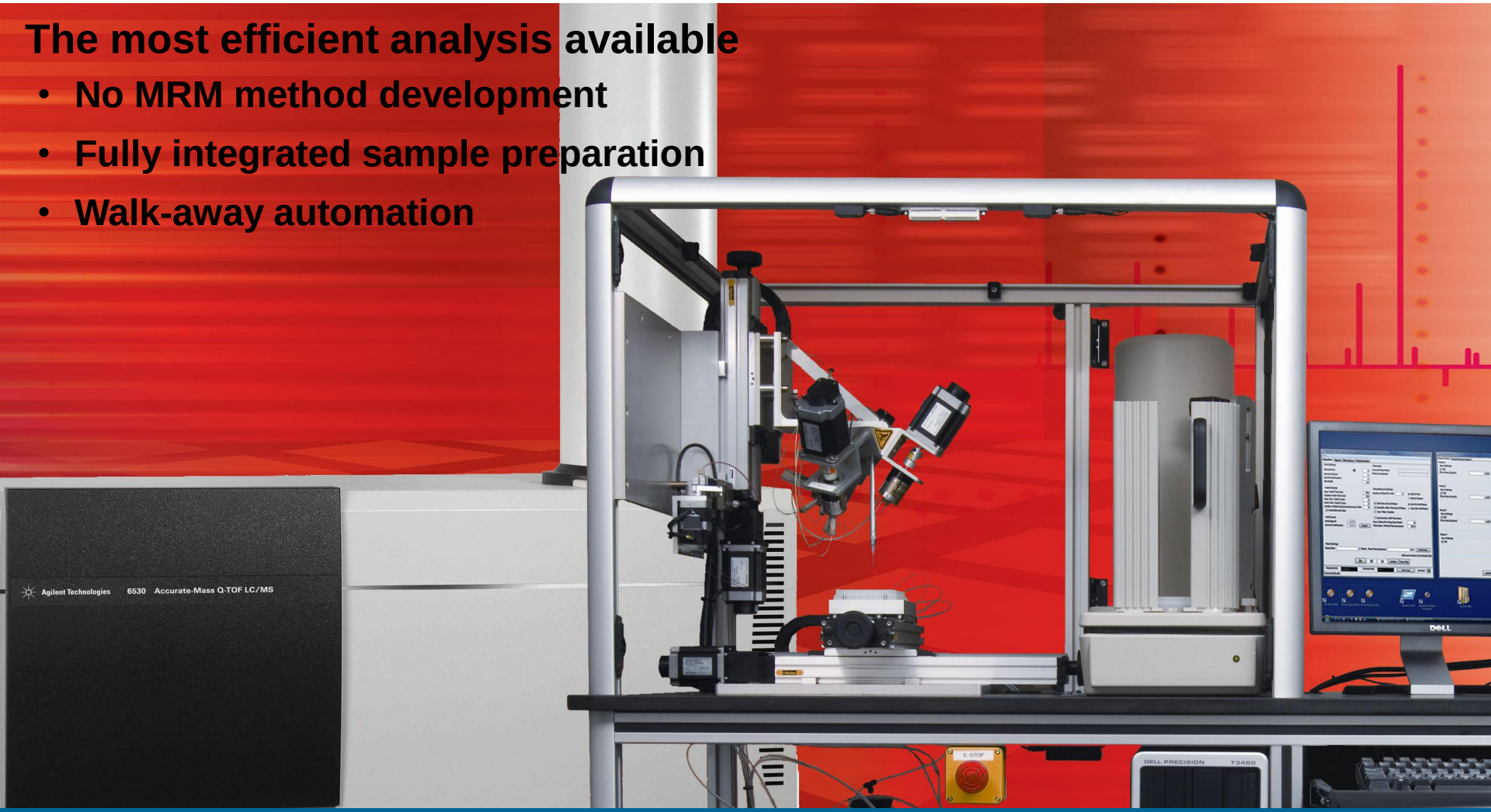
Bristol-Myers Squibb

RapidFire 360 High Resolution System

Eliminates bottlenecks in high-throughput MS

The most efficient analysis available

- **No MRM method development**
- **Fully integrated sample preparation**
- **Walk-away automation**



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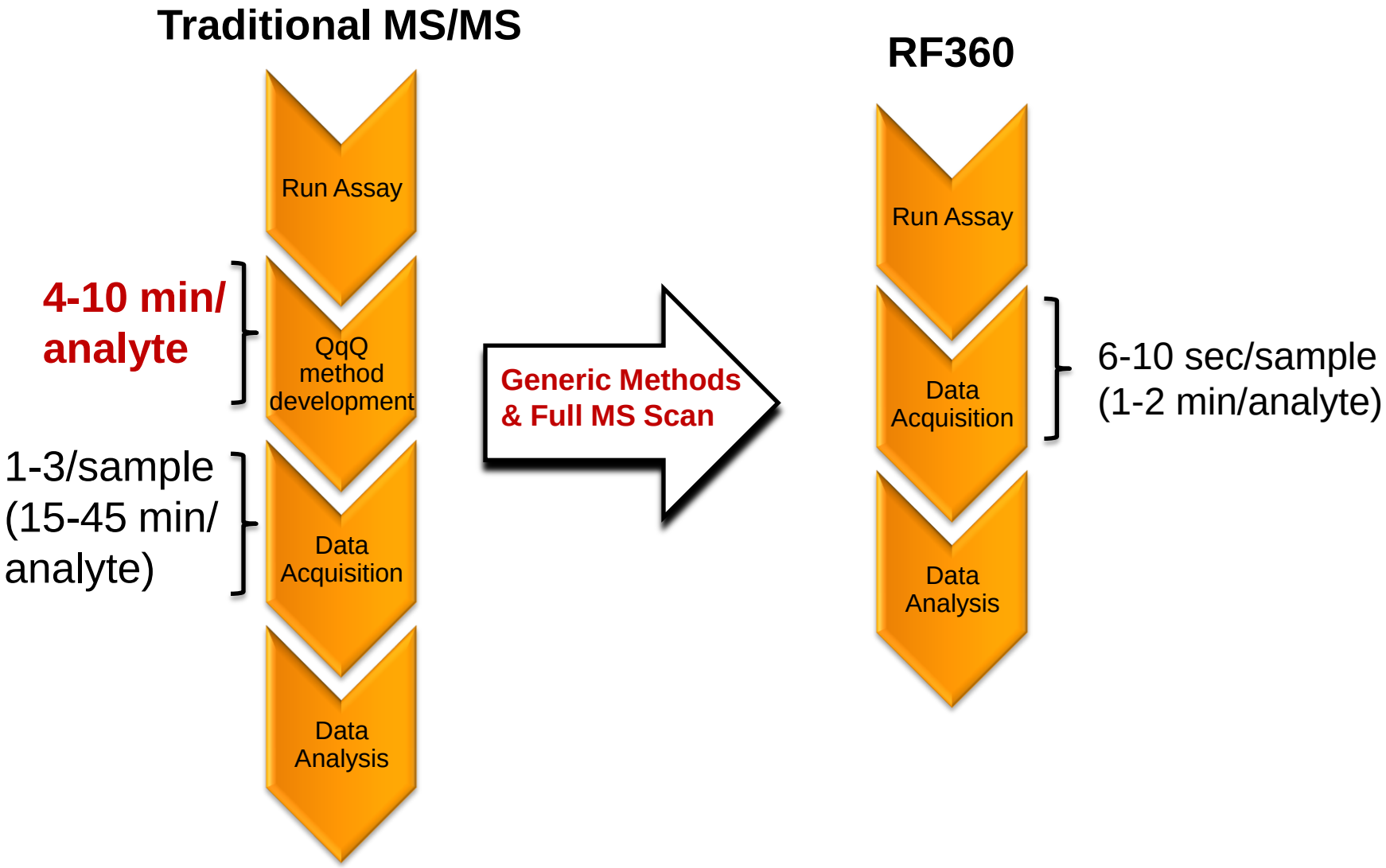
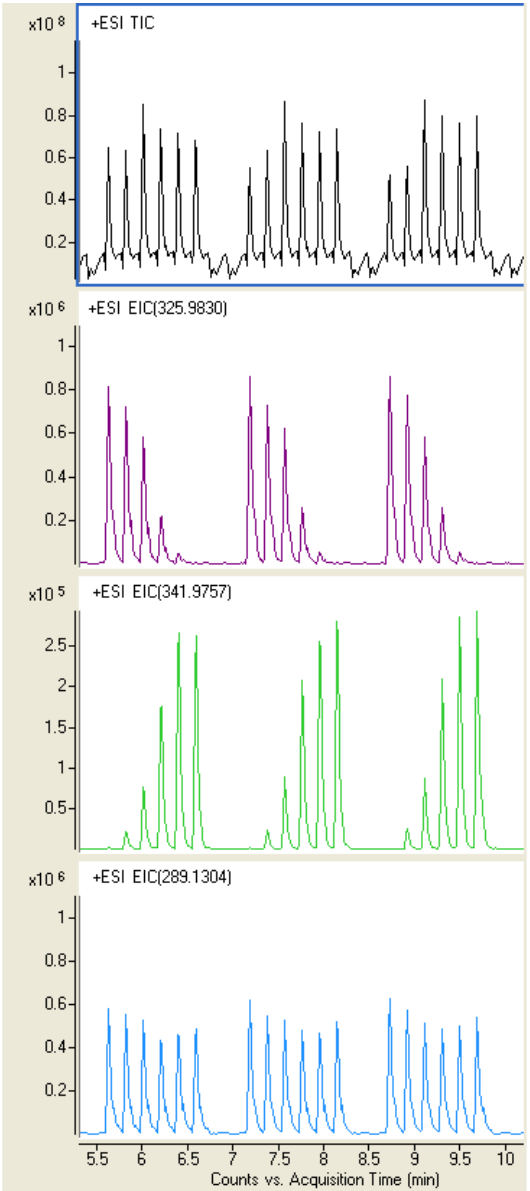
RF360 Removes the MRM Bottleneck



RF 360 Data
Microsomal Stability Assay with Midazolam

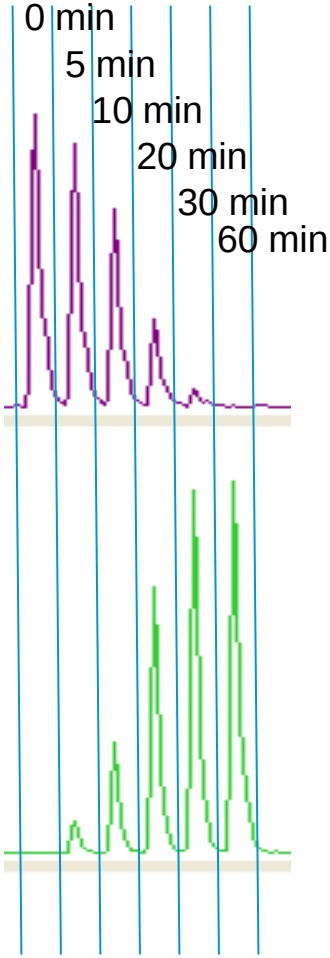


TIC

Parent
(Midazolam)

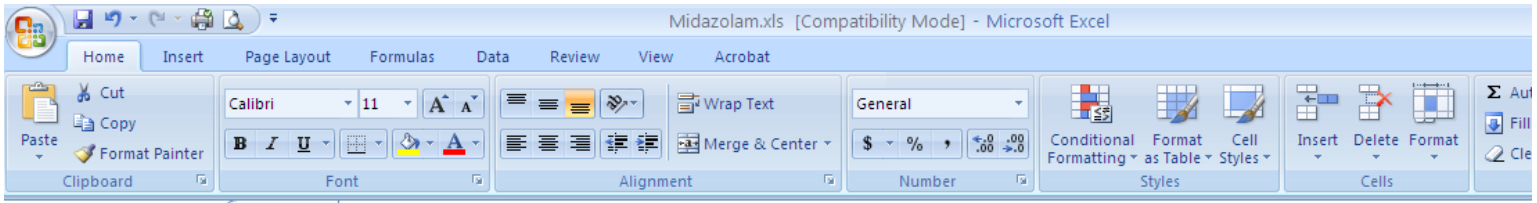
Metabolite
(Hydroxymidazolam)

Internal Standard
(Bupivicaine)



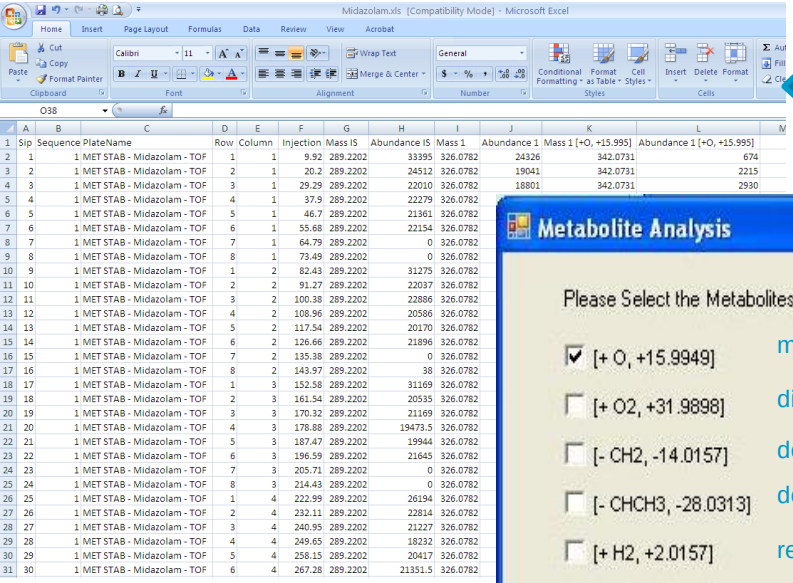
Extraction and peak integration of analytes from TIC is fully automated

Metabolite Analysis Data

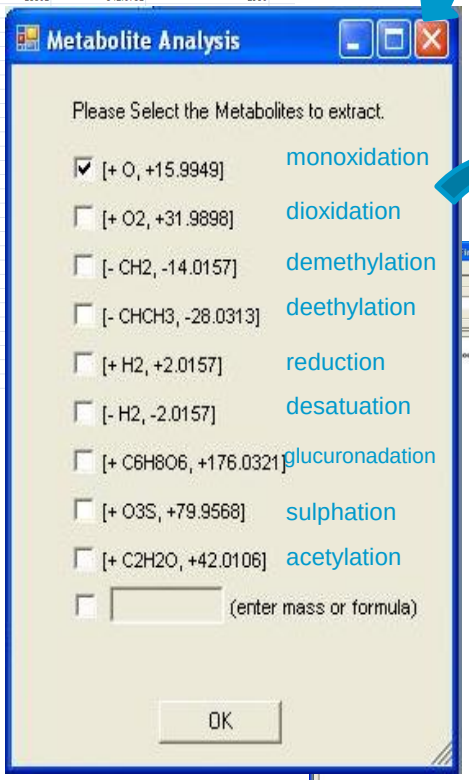


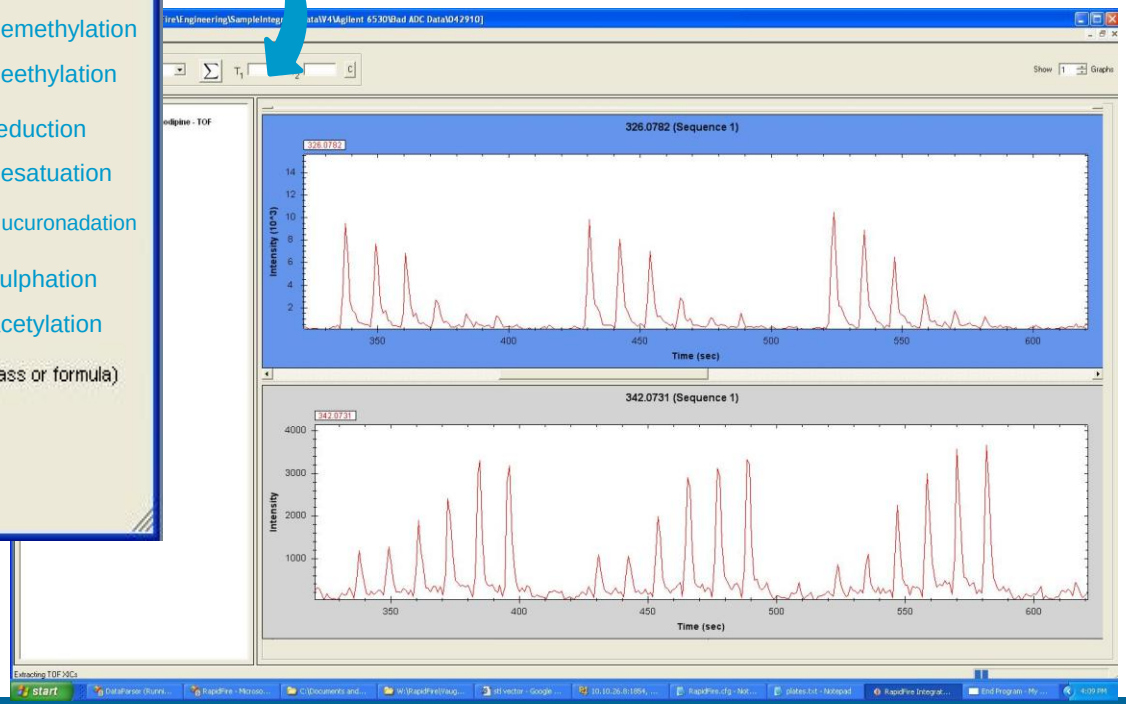
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Sip	Sequence	PlateName	Row	Column	Injection	Mass IS	Abundance IS	Mass 1	Abundance 1	Mass 1 [+O, +15.995]	Abundance 1 [+O, +15.995]	
2	1	1	MET STAB - Midazolam - TOF	1	1	9.92	289.2202	33395	326.0782	24326	342.0731	674	
3	2	1	MET STAB - Midazolam - TOF	2	1	20.2	289.2202	24512	326.0782	19041	342.0731	2215	
4	3	1	MET STAB - Midazolam - TOF	3	1	29.29	289.2202	22010	326.0782	18801	342.0731	2930	
5	4	1	MET STAB - Midazolam - TOF	4	1	37.9	289.2202	22279	326.0782	15342	342.0731	1376	
6	5	1	MET STAB - Midazolam - TOF	5	1	46.7	289.2202	21361	326.0782	15610	342.0731	1836	
7	6	1	MET STAB - Midazolam - TOF	6	1	55.68	289.2202	22154	326.0782	16192	342.0731	1668	
8	7	1	MET STAB - Midazolam - TOF	7	1	64.79	289.2202	0	326.0782	0	342.0731	0	
9	8	1	MET STAB - Midazolam - TOF	8	1	73.49	289.2202	0	326.0782	84	342.0731	0	
10	9	1	MET STAB - Midazolam - TOF	1	2	82.43	289.2202	31275	326.0782	23765	342.0731	667	
11	10	1	MET STAB - Midazolam - TOF	2	2	91.27	289.2202	22037	326.0782	17683	342.0731	1601	
12	11	1	MET STAB - Midazolam - TOF	3	2	100.38	289.2202	22886	326.0782	18244	342.0731	1928	
13	12	1	MET STAB - Midazolam - TOF	4	2	108.96	289.2202	20586	326.0782	15660	342.0731	1639	
14	13	1	MET STAB - Midazolam - TOF	5	2	117.54	289.2202	20170	326.0782	16033	342.0731	1660	
15	14	1	MET STAB - Midazolam - TOF	6	2	126.66	289.2202	21896	326.0782	16270	342.0731	1961	
16	15	1	MET STAB - Midazolam - TOF	7	2	135.38	289.2202	0	326.0782	0	342.0731	0	
17	16	1	MET STAB - Midazolam - TOF	8	2	143.97	289.2202	38	326.0782	35	342.0731	304	
18	17	1	MET STAB - Midazolam - TOF	1	3	152.58	289.2202	31169	326.0782	23636	342.0731	924	
19	18	1	MET STAB - Midazolam - TOF	2	3	161.54	289.2202	20535	326.0782	18175	342.0731	1432	
20	19	1	MET STAB - Midazolam - TOF	3	3	170.32	289.2202	21169	326.0782	19609	342.0731	1975	
21	20	1	MET STAB - Midazolam - TOF	4	3	178.88	289.2202	19473.5	326.0782	14961	342.0731	1745	
22	21	1	MET STAB - Midazolam - TOF	5	3	187.47	289.2202	19944	326.0782	16225	342.0731	2044	
23	22	1	MET STAB - Midazolam - TOF	6	3	196.59	289.2202	21645	326.0782	16923	342.0731	1815	
24	23	1	MET STAB - Midazolam - TOF	7	3	205.71	289.2202	0	326.0782	0	342.0731	0	
25	24	1	MET STAB - Midazolam - TOF	8	3	214.43	289.2202	0	326.0782	21	342.0731	0	
26	25	1	MET STAB - Midazolam - TOF	1	4	222.99	289.2202	26194	326.0782	21271	342.0731	2408	
27	26	1	MET STAB - Midazolam - TOF	2	4	232.11	289.2202	22814	326.0782	16462	342.0731	2598	
28	27	1	MET STAB - Midazolam - TOF	3	4	240.95	289.2202	21227	326.0782	13507.5	342.0731	3846	
29	28	1	MET STAB - Midazolam - TOF	4	4	249.65	289.2202	18232	326.0782	4928	342.0731	5090	
30	29	1	MET STAB - Midazolam - TOF	5	4	258.15	289.2202	20417	326.0782	2246	342.0731	6953	
31	30	1	MET STAB - Midazolam - TOF	6	4	267.28	289.2202	21351.5	326.0782	1617	342.0731	6578	

High-throughput TOF Data Analysis



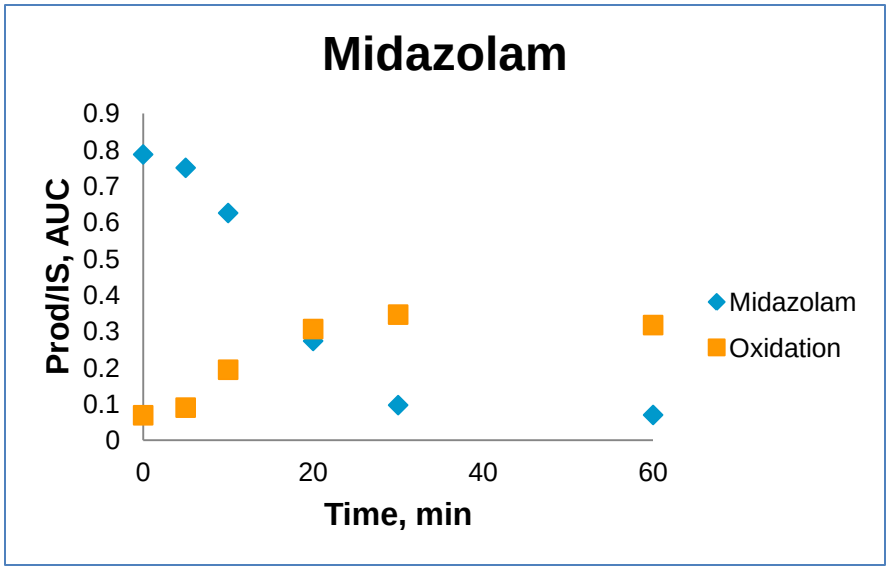
Sip	Sequence	PlateName	Row	Column	Injection	Mass IS	Abundance IS	Mass 1	Abundance 1	Mass 1 [+O, +15.995]	Abundance 1 [+O, +15.995]
1	1	MET STAB - Midazolam - TOF	1	1	9.92	289.2202	33395	326.0782	24326	342.0731	674
2	1	MET STAB - Midazolam - TOF	2	1	20.2	289.2202	24512	326.0782	19041	342.0731	2215
3	1	MET STAB - Midazolam - TOF	3	1	29.29	289.2202	22010	326.0782	18801	342.0731	2930
4	1	MET STAB - Midazolam - TOF	4	1	37.9	289.2202	22279	326.0782			
5	1	MET STAB - Midazolam - TOF	5	1	46.7	289.2202	21361	326.0782			
6	1	MET STAB - Midazolam - TOF	6	1	55.68	289.2202	22154	326.0782			
7	1	MET STAB - Midazolam - TOF	7	1	64.79	289.2202	0	326.0782			
8	1	MET STAB - Midazolam - TOF	8	1	73.49	289.2202	0	326.0782			
9	1	MET STAB - Midazolam - TOF	1	2	82.43	289.2202	31275	326.0782			
10	1	MET STAB - Midazolam - TOF	2	2	91.27	289.2202	22037	326.0782			
11	1	MET STAB - Midazolam - TOF	3	2	100.38	289.2202	22886	326.0782			
12	1	MET STAB - Midazolam - TOF	4	2	108.96	289.2202	20586	326.0782			
13	1	MET STAB - Midazolam - TOF	5	2	117.54	289.2202	20170	326.0782			
14	1	MET STAB - Midazolam - TOF	6	2	126.66	289.2202	21896	326.0782			
15	1	MET STAB - Midazolam - TOF	7	2	135.38	289.2202	0	326.0782			
16	1	MET STAB - Midazolam - TOF	8	2	143.97	289.2202	38	326.0782			
17	1	MET STAB - Midazolam - TOF	1	3	152.58	289.2202	31169	326.0782			
18	1	MET STAB - Midazolam - TOF	2	3	161.54	289.2202	20535	326.0782			
19	1	MET STAB - Midazolam - TOF	3	3	170.32	289.2202	21169	326.0782			
20	1	MET STAB - Midazolam - TOF	4	3	178.88	289.2202	19473.5	326.0782			
21	1	MET STAB - Midazolam - TOF	5	3	187.47	289.2202	19944	326.0782			
22	1	MET STAB - Midazolam - TOF	6	3	196.59	289.2202	21645	326.0782			
23	1	MET STAB - Midazolam - TOF	7	3	205.71	289.2202	0	326.0782			
24	1	MET STAB - Midazolam - TOF	8	3	214.43	289.2202	0	326.0782			
25	1	MET STAB - Midazolam - TOF	1	4	222.99	289.2202	26194	326.0782			
26	1	MET STAB - Midazolam - TOF	2	4	232.11	289.2202	22814	326.0782			
27	1	MET STAB - Midazolam - TOF	3	4	240.95	289.2202	21227	326.0782			
28	1	MET STAB - Midazolam - TOF	4	4	249.65	289.2202	18232	326.0782			
29	1	MET STAB - Midazolam - TOF	5	4	258.15	289.2202	20417	326.0782			
30	1	MET STAB - Midazolam - TOF	6	4	267.28	289.2202	21351.5	326.0782			

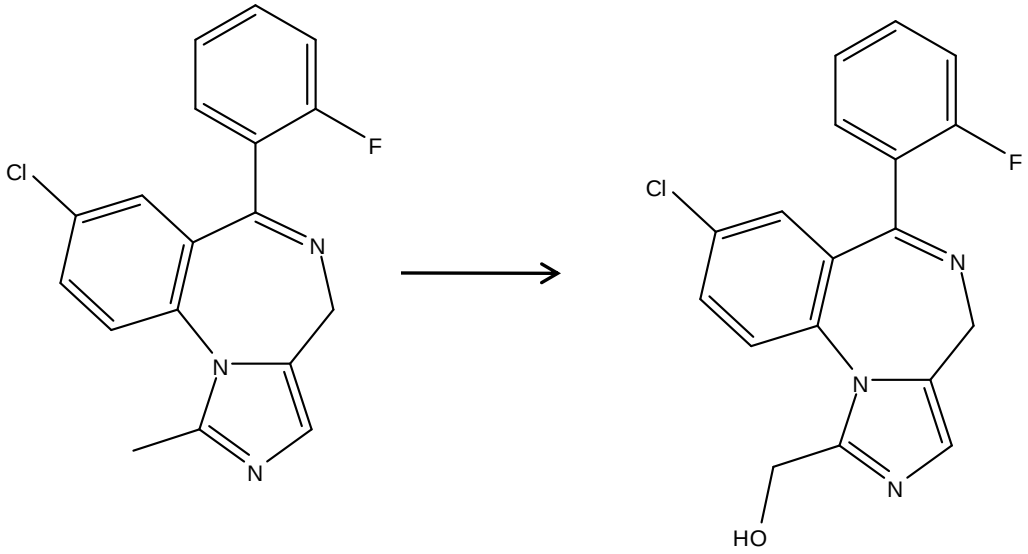




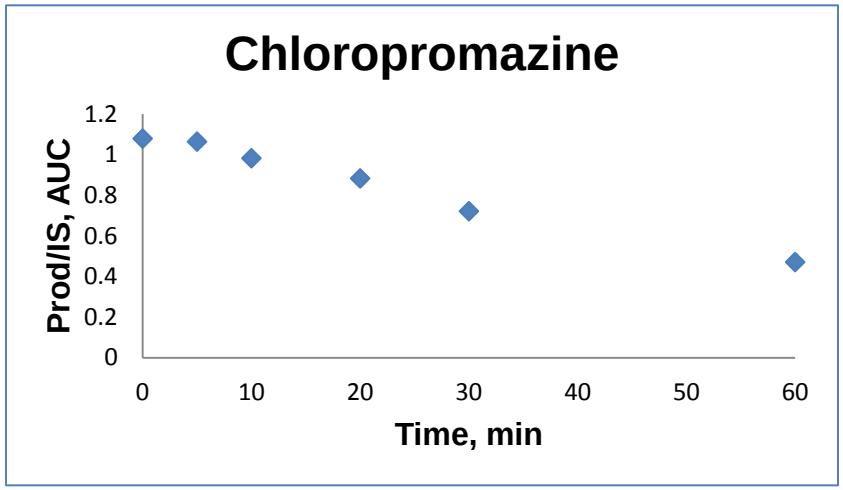
- Import mass table or formulas
- Select metabolites of interest
- Obtain fully-integrated peak areas

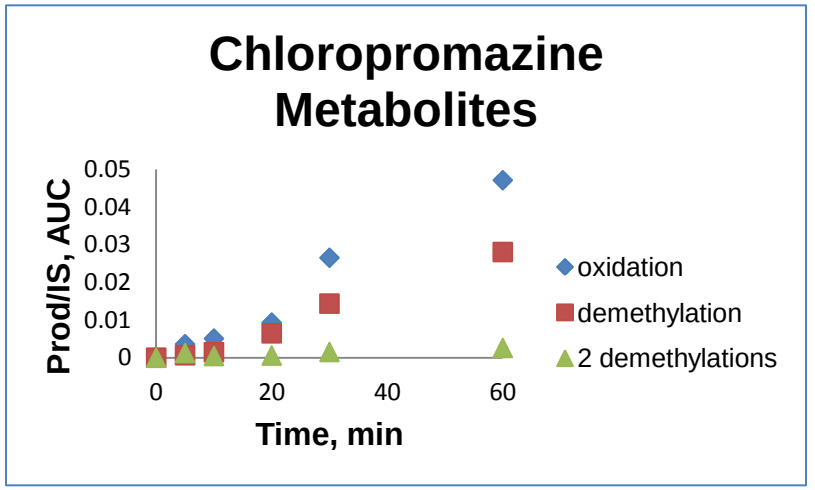
Metabolite Analysis Results - Midazolam

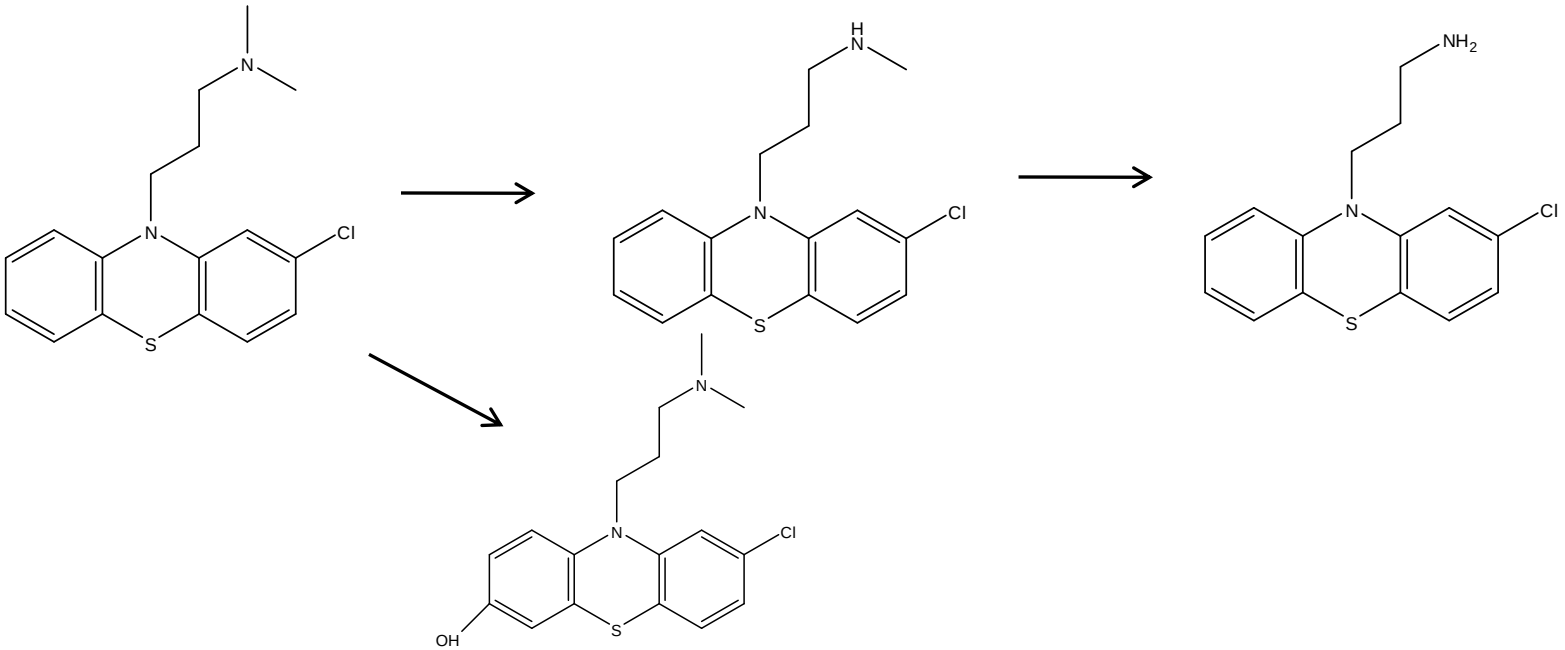




Metabolite Analysis Results - Chloropromazine

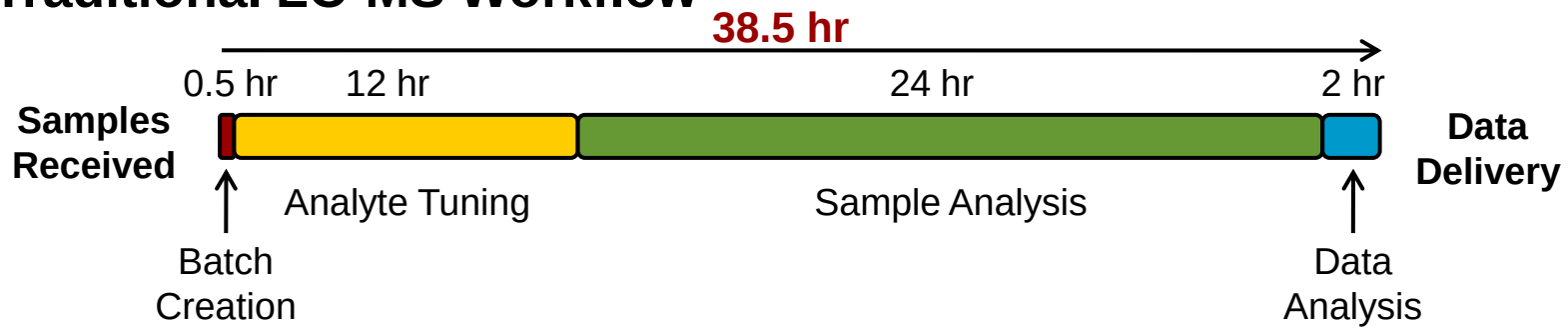




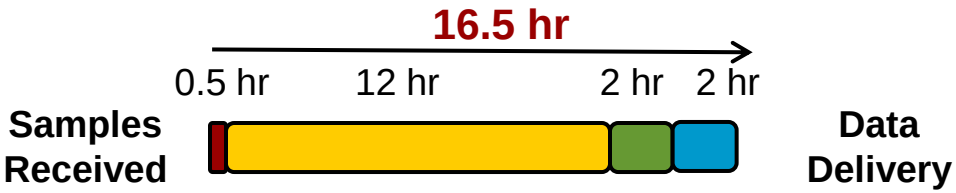


Reducing Bottlenecks in ADME Sample Analysis using Solid Phase Extraction with a Quadrupole Time-of-Flight Mass Spectrometer

Traditional LC-MS Workflow

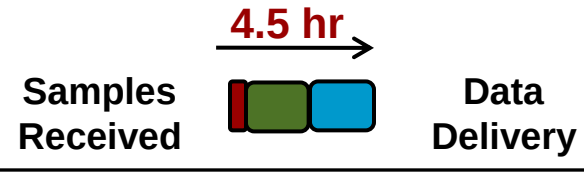


RapidFire 300 MS QQQ Work Flow



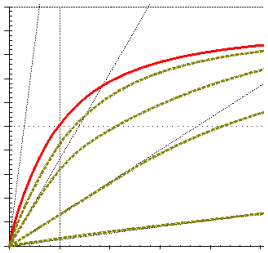
Adapted from oral presentation by Panos Hatsis, Novartis, 58th ASMS Conference on Mass Spectrometry and Allied Topics, May 23 - 27, 2010

RapidFire 360 TOF Work Flow



NOVARTIS

RapidFire Resolves Bottlenecks…

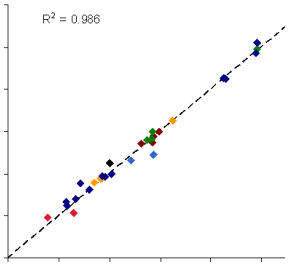


Improved Data Quality:

- Throughput allows for
  - Multiple concentrations: run full IC_{50}s
  - Multiple time points
- Avoids surrogate substrates, indirect and coupled assays

Maximizes Productivity:

- Fastest data turnaround
  - 10x data generated per FTE compared to LC-MS
- Rapid assay development in HTS ⇒ faster time to answer



Strong Correlation with Traditional Technologies:

- LC-MS/MS: CYP450 inhibition, metabolic stability, etc
- Optical-probe or radioactivity-based detectors

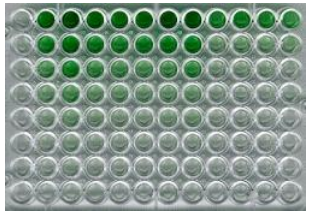
Advantages of RapidFire

Lower Operational Costs:

- Solvents: 6 s @3.0 mL/min = 0.25 mL (~2¢/sample)
- Cartridge: $200/cartridge or
  ~ 3000 samples/cartridge (~7¢/sample)

Minimal Reagent & Disposal Costs in HTS:

- Only native substrate & enzyme are required
- No antibodies, luminescent or radioactive reagents, kits



Fits Existing Workflows

- Designed to operate similar to a plate reader