LC-MS Bioanalysis From minutes to seconds

Scott Summerfield

“Imagination is the only weapon in the war against reality.”

Lewis Carroll, Alice in Wonderland

– Chromatogram from RapidSep separation of stereoisomers

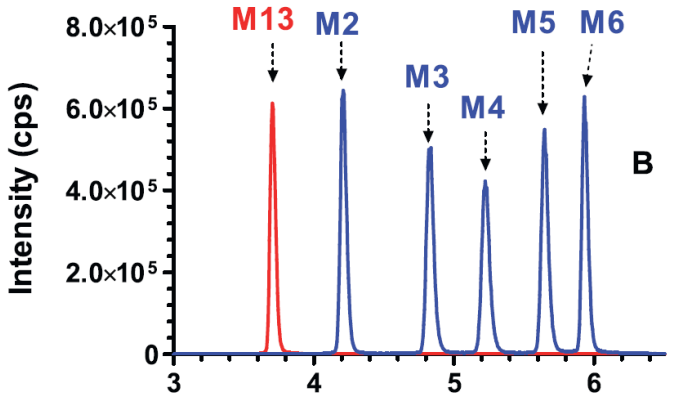
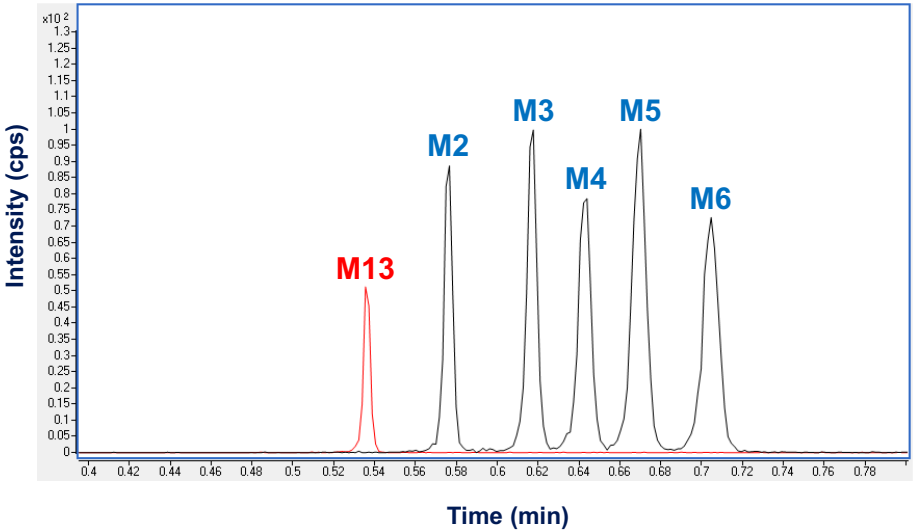


Fig. 2. Reverse phase chromatograms of GSK1278863 (Method A; m/z = 392 → 291; (A)) and its metabolites M2 though M6, M13 (Method B; m/z = 424 → 323 for M2 though M6 and 440 → 339 for M13; (B)) fortified into human plasma at 1000 ng/mL.

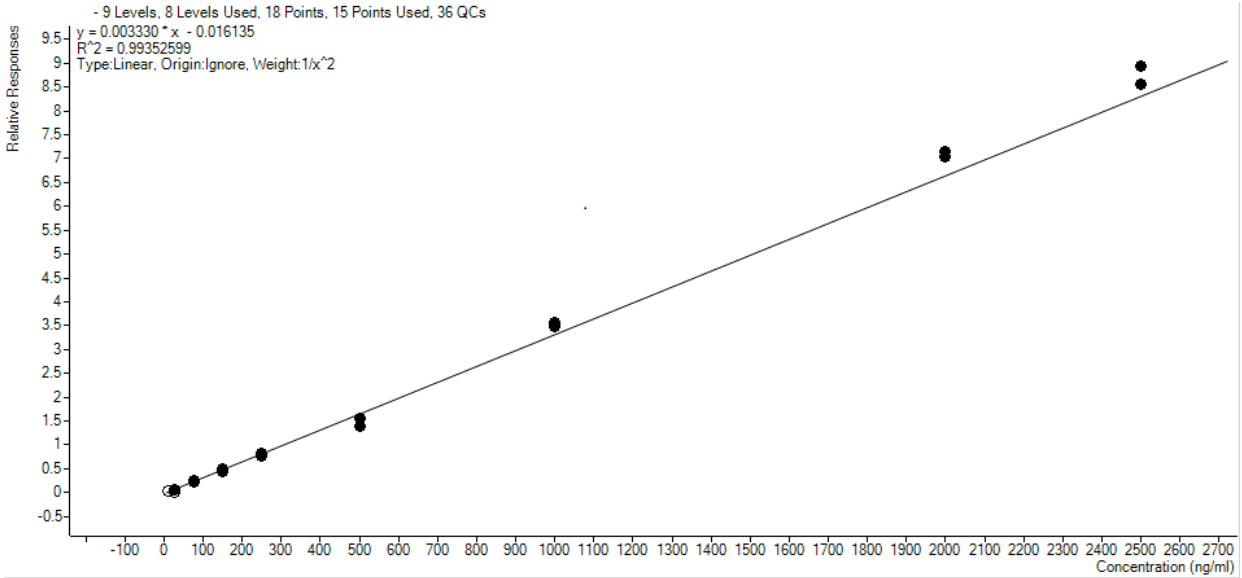


Same column and (100 x 2.1mm HSS) LC conditions as original method but (TOF-MS instead of QqQ)

Licea-Perez, J Chromatogr B Analyt Technol Biomed Life Sci. (2016)

Results Faster LC-MS Method

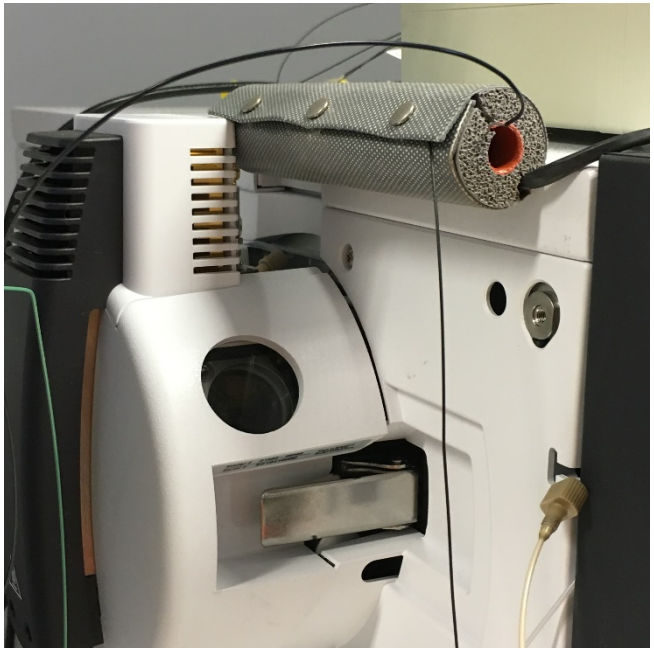
Comparable linearity and stats for all analytes



QC (ng/mL)	25	75	500	2000	2500
Bias (%)	15.01	1.60	-6.50	6.36	4.70
CV (%)	8.05	9.02	4.97	5.92	4.35

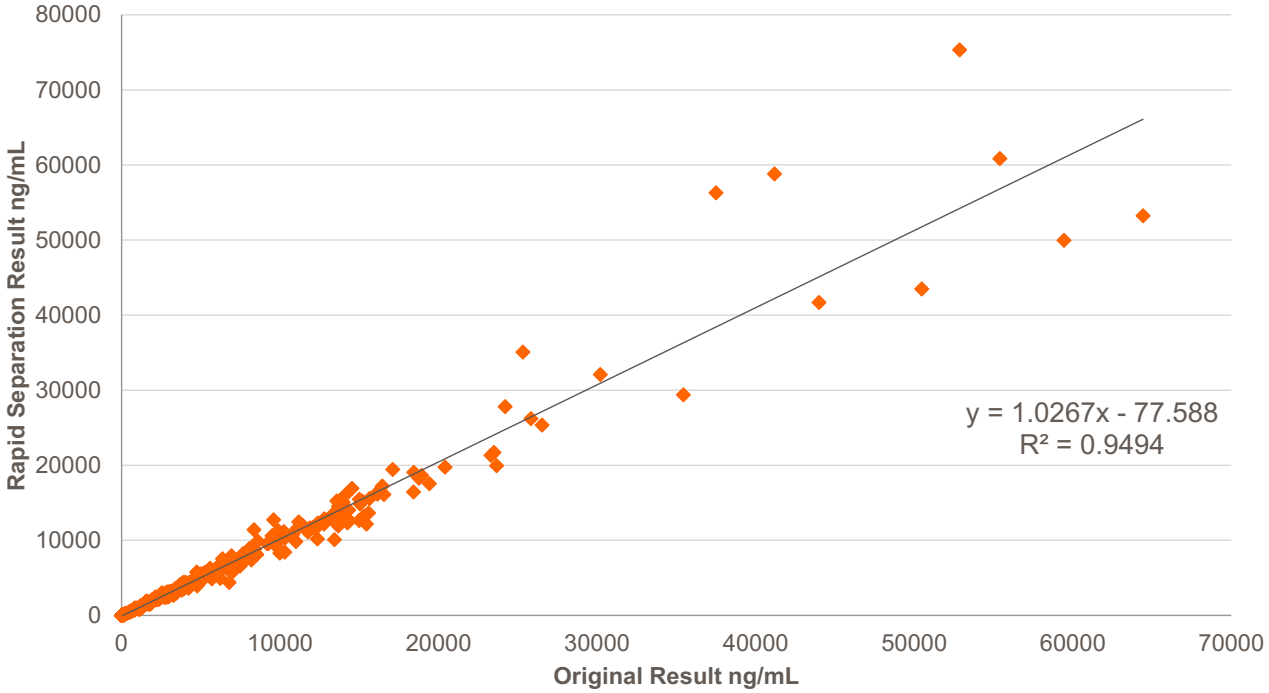
Rapid LC-MS setup

- Agilent 1290 with dual injector coupled to Agilent 6545 QTOF
- Minimal system dead volume
- Column connected directly into ion source in order to minimise post-column dispersion
- Initial evaluations performed on 10mm – 100mm columns



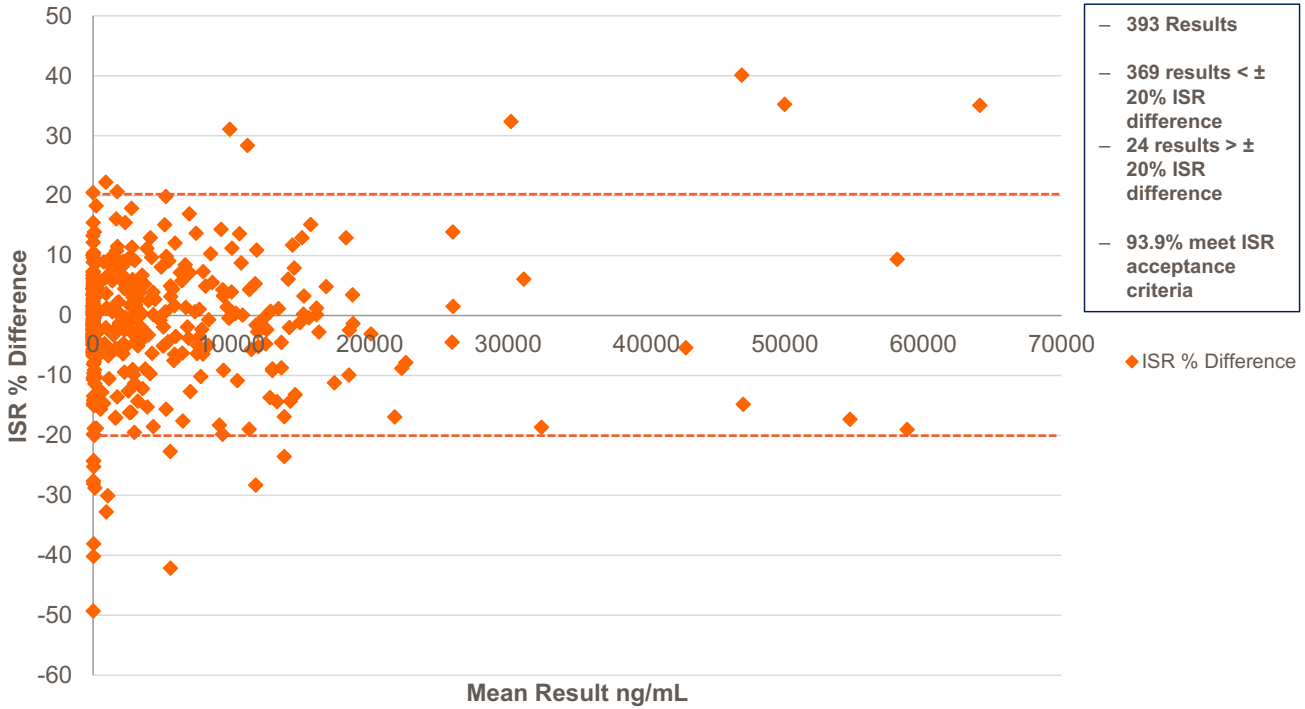
Comparison of Results

Non-GLP Safety Studies Supporting Candidate Selection



Incurred Sample Reproducibility

Non-GLP Safety Studies Supporting Candidate Selection



Matrix and Recovery

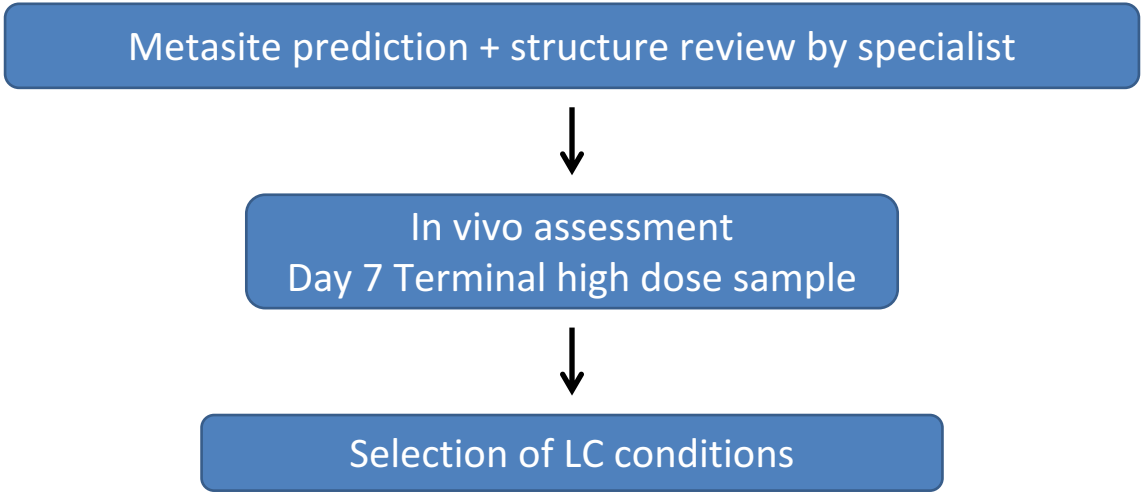
No Significant Differences observed between conventional and rapid LC-MS

Compound A	RapidSep Gradient A			RapidSep Gradient B			Conventional Chromatography		
	QC2	QC3	QC4	QC2	QC3	QC4	QC2	QC3	QC4
% Mean Recovery	**58.6**	**62.3**	**69.4**	**63.4**	**63.4**	**69.8**	**58.4**	**63.5**	**66.7**
%CV	5.5	2.2	1.6	4.9	3.5	2.4	3.6	2.9	1.8
Mean Matrix Factor	**1.02**	**0.99**	**0.99**	**0.99**	**0.98**	**0.99**	**1.00**	**1.00**	**1.03**
%CV	7.1	3.0	4.1	5.1	3.8	3.1	4.1	4.1	0.8

Compound B	RapidSep Gradient A			RapidSep Gradient B			Conventional Chromatography		
	QC2	QC3	QC4	QC2	QC3	QC4	QC2	QC3	QC4
% Mean Recovery	**59.7**	**65.6**	**67.0**	**64.3**	**63.1**	**69.2**	**60.1**	**62.2**	**67.9**
%CV	6.4	3.2	4.5	5.4	1.3	4.6	5.5	4.7	3.8
Mean Matrix Factor	**0.90**	**0.87**	**0.90**	**0.98**	**0.96**	**0.94**	**1.01**	**0.98**	**0.98**
%CV	6.6	6.9	8.4	3.1	2.2	1.9	5.7	2.7	6.2

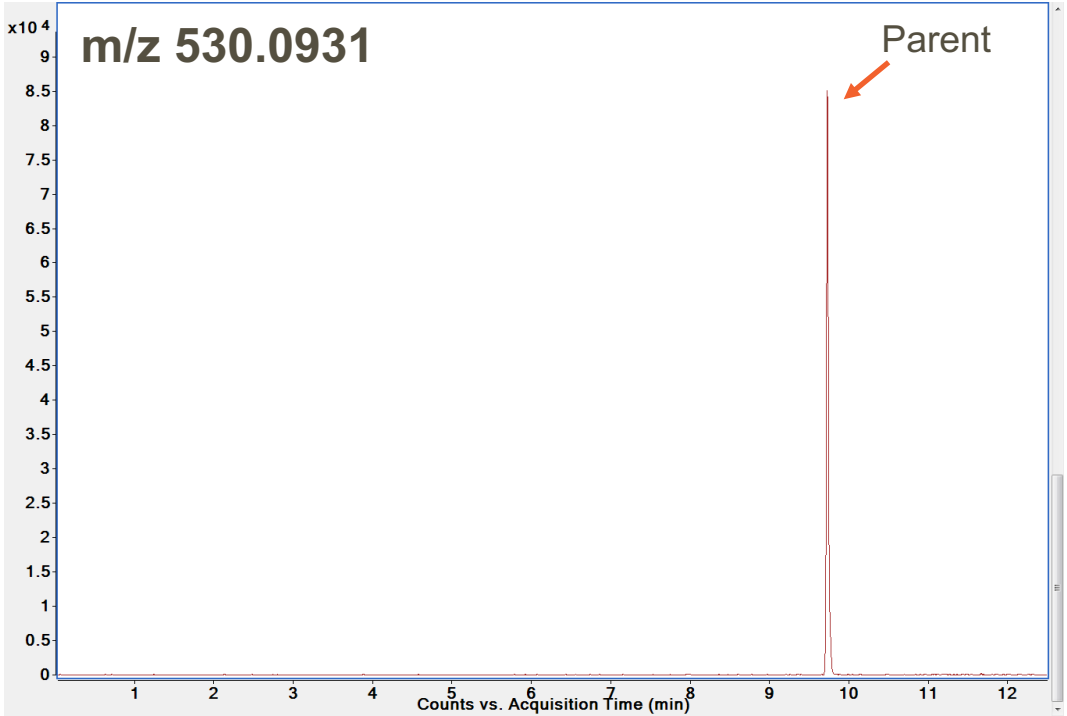
Screening Strategy

Assessment circulating isobaric interferences



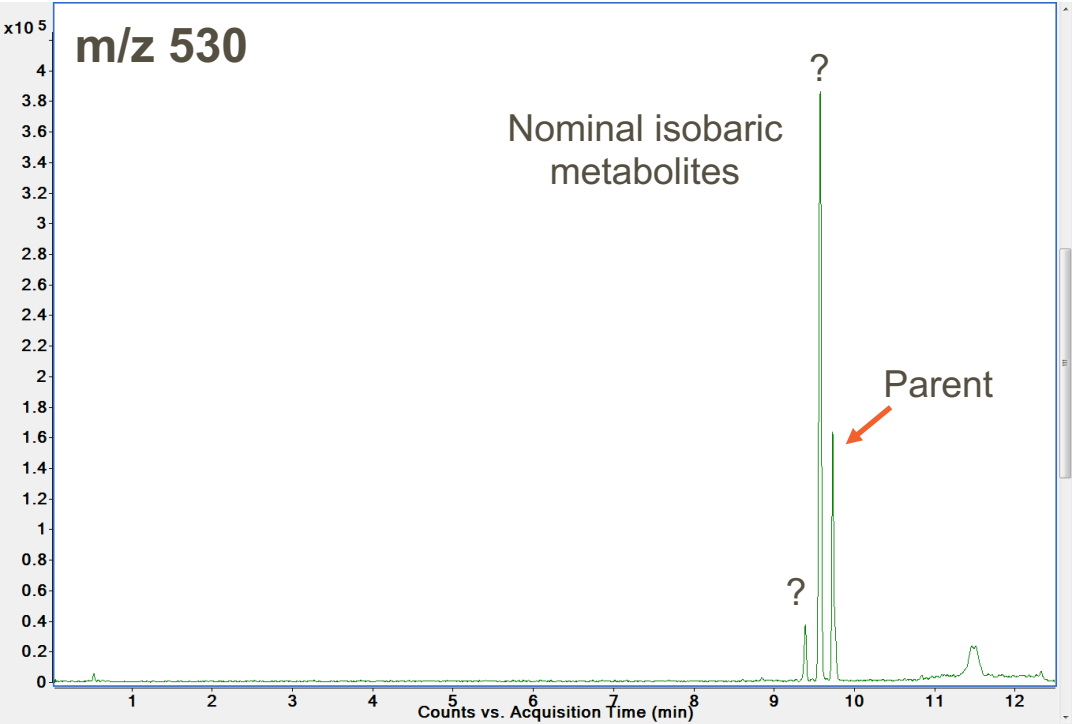
In Vivo Screening

LC-TOF MS



In Vivo Screening

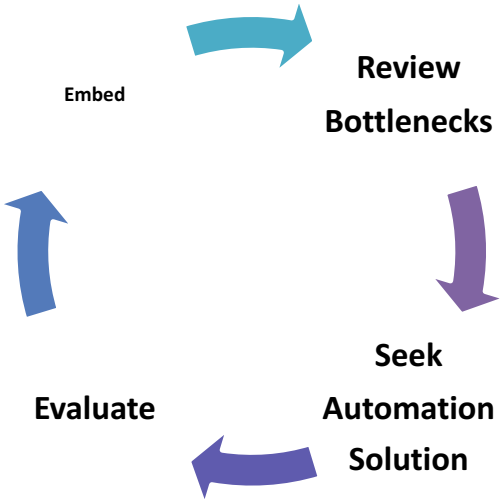
LC-TOF MS



“Why, sometimes I've believed as many as six impossible things before breakfast.”

Lewis Carroll, Alice in Wonderland

– Companion automation to complement faster LC-MS/MS



Why Bother With Faster Separations?

Fidelity of separation is a key target

- Sustainability
  - Reduced solvent consumption (per assay)
- Laboratory Footprint
  - Fewer (but higher end) MS systems supporting bioanalysis
  - Faster scans speeds to complement sensitivity
- MS utilisation and Automation
  - Greater internal capacity
  - Reduction of monotonous tasks and focus on data quality and data integrity
- Wider Adoption?
  - 2D separations

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