Metabolomics Research Group

Current Members:

Amrita K Cheema: Georgetown University

John M Asara: BIDMC/Harvard Medical School

Thomas Neubert : NYU (EB Liaison)

Chris Turck: Max Planck Institute (Chair)

Former Members

William Wikoff: UC Davis

Vladimir Tolstikov: Eli Lilly

Pavel Aronov: Stanford University

Future Members:

Andrew Patterson : Penn State University

Stephen Brown : University of Michigan

MRG 2013 Inter-Laboratory Study

Design a study that resembles a typical metabolomics experiment

Participants asked:

- **to identify quantitative differences between two groups of samples**
- **without (non-targeted) or with (targeted) spiked-in compound information**

International representation of MRG study respondents

Initial solicitation of interest from metabolomics labs, ABRF members, etc. by email.

Participating Countries

US
Canada
England
Scotland
Ireland
Germany
Spain
Italy
Netherlands
Australia
Japan
South Korea
China
Singapore

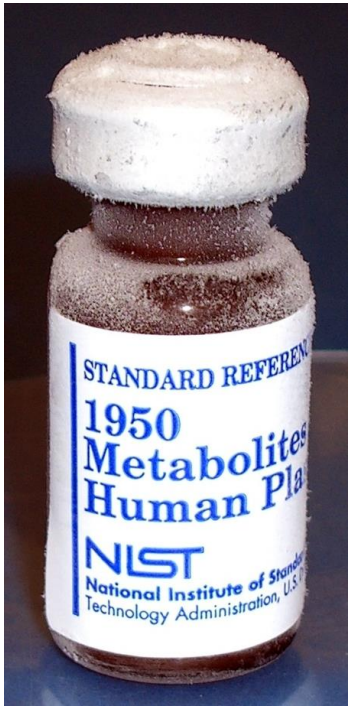
~25% USA & Canada
~35% Europe
~25% Asia

The Association of Biomolecular Resource Facilities
Research • Technology
Communication • Education

Four principles of compound selection

1. Most of the spiked-in compounds should be endogenous with known concentrations in NIST plasma.
2. Compounds should be selected such that they are well distributed in terms of ability to analyze by a particular technique. For example, some compounds should be detectable with ESI+, whereas others should be detectable with ESI-, EI or APCI.
3. Compounds should be selected with a range of difficulty of identification, regardless of technique used.
4. High purity compounds should be chosen.

New NIST plasma standard is an ideal matrix for inter-laboratory studies

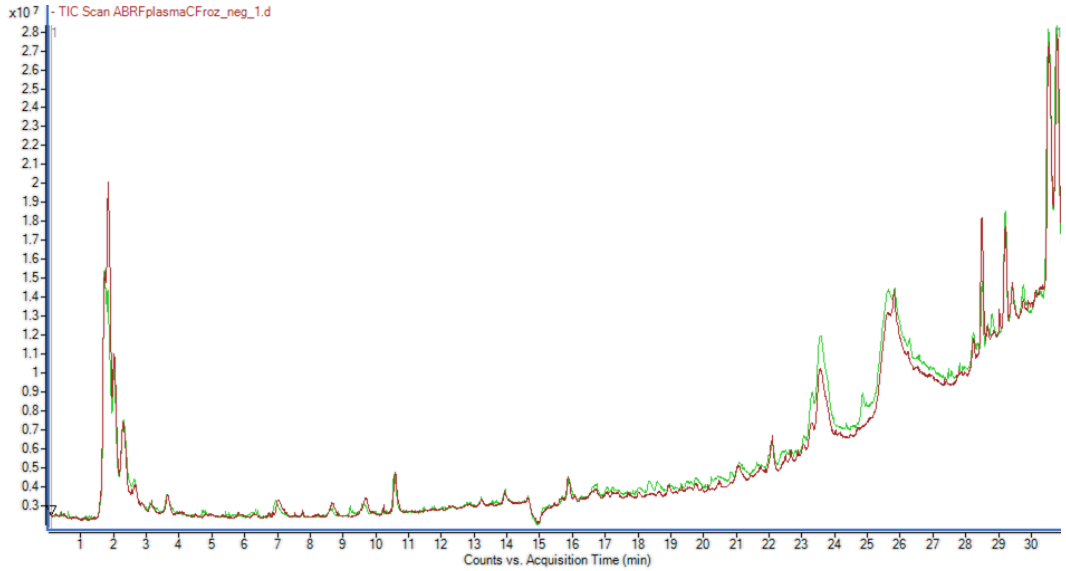


- **Analyzed and validated by several groups on multiple analytical platforms.**
- **Can be used for comparisons over long periods of time.**

NIST has generously donated the plasma that was used for the MRG study.

Lyophilization for sample preparation: Comparison to frozen sample

TIC (-) ESI



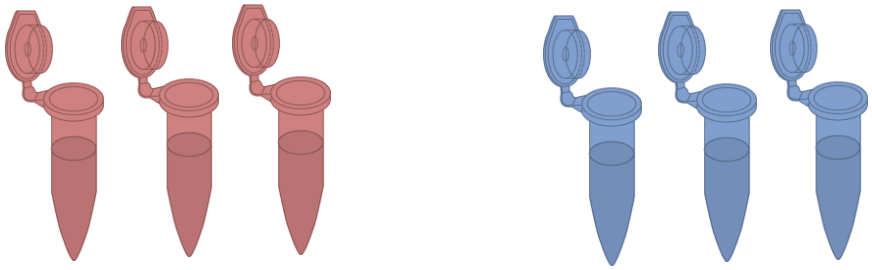
Total ion chromatogram of lyophilized sample superimposes with non-lyophilized sample.

Study Design

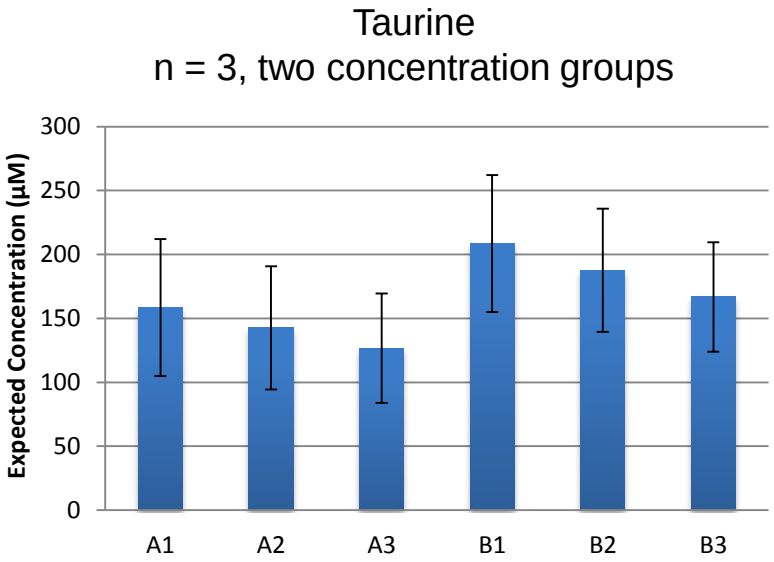
NIST plasma matrix
Pure compounds spiked into each tube

Group A

Group B



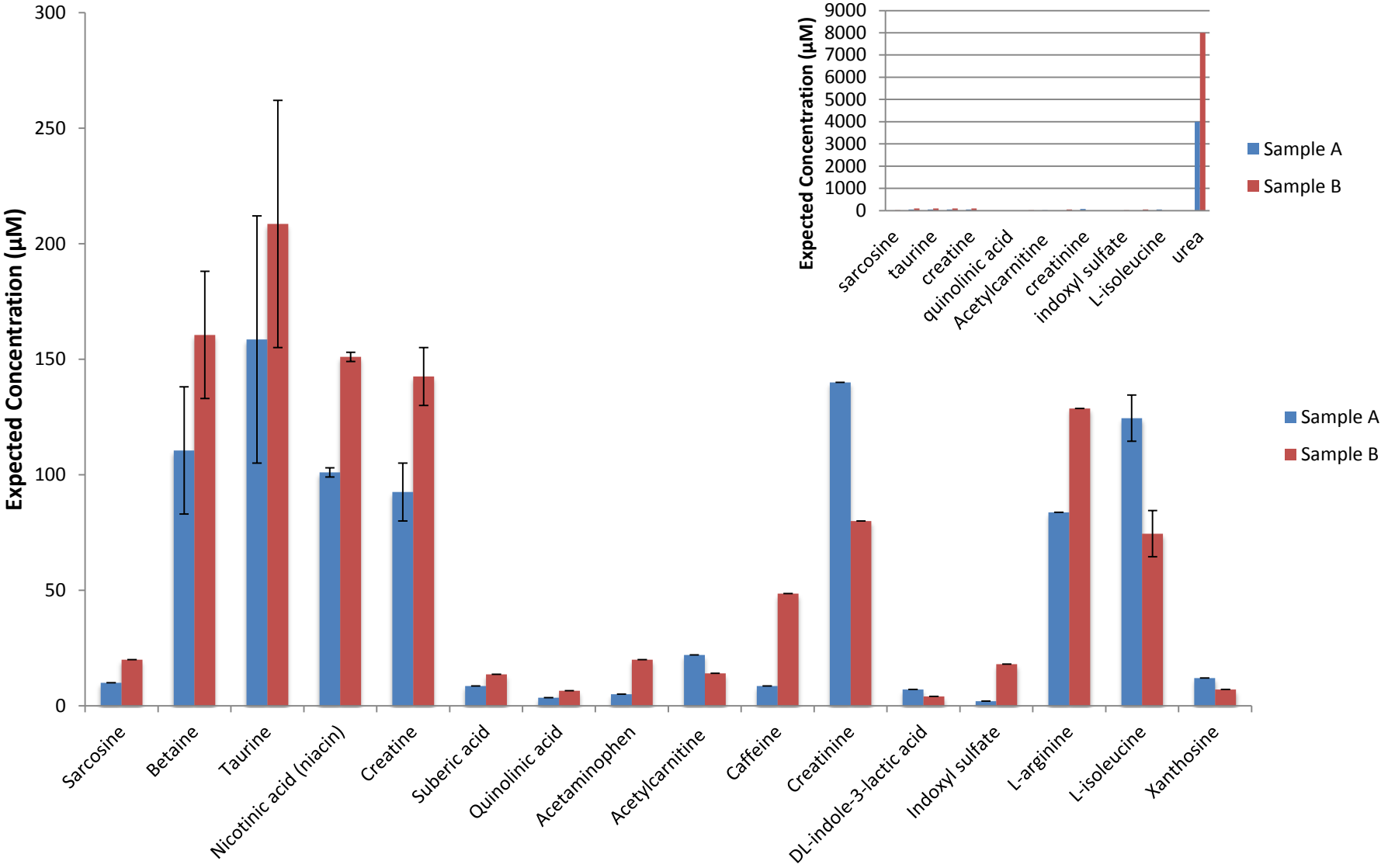
~100 µl per tube



Ratio of A and B = [0.68,0.81], with p < 0.01 after adjusting for endogenous plasma concentration

Enough material to send to approximately 100 participants.
Limitation is the amount of NIST plasma available.

Expected Concentrations of 17 Spiked Metabolites
(Adjusted Based on Endogenous Plasma Concentration)



Expected Concentrations of 17 Spiked Metabolites in Plasma Study Samples

		Spiked Concentration (µM)		Endogenous Concentration (µM)	POS Mode		NEG Mode	
Substance Name	**MW**	**Sample A**	**Sample B**		Ratio A/B		Ratio A/B	
Sarcosine	89.10	10	20	Probably Negligible	0.50	↘		
Betaine	117.15	50	100	33-88	[0.62,0.73]	↘		
Urea	60.06	4000	8000		0.50	↘		
Taurine	125.15	50	100	55-162			[0.68,0.81]	↘
Nicotinic acid (niacin)	123.11	50	100	49-53	[0.66,0.67]	↘		
Creatine	131.14	50	100	30-55	[0.62,0.68]	↘		
Suberic acid	174.20	5	10	3.6			0.63	↘
Quinolinic acid	167.12	3	6	0.47			0.54	↘
Acetaminophen	151.06	5	20	Dose Dependent	0.25	↘		
Acetylcarnitine	203.12	16	8	6	1.57	↗		
Caffeine	194.08	8.50	48.50	Dose Dependent 2-10mg/L	0.18	↘		
Creatinine	113.06	69.98	9.98	70	1.75	↗		
DL-indole-3-lactic acid	205.07	4.2	1.2	2.8			1.75	↗
Indoxyl sulfate	213.01	2	18		0.11	↘		
L-arginine	174.11	3.7	48.7	80	0.65	↘		
L-isoleucine	131.09	54.5	4.5	60-80	[1.59,1.78]	↗		
Xanthosine	284.08	7.00	2	5			1.71	↗

Urea and Indoxyl sulfate were not detected by any of the participating laboratories.

MRG Member Results

		MRG M1	MRG M2	MRG M3		
Substance	Expected Ratio A/B	Observed Ratio A/B	Observed Ratio A/B	Observed Ratio A/B	Observed Ratio A/B	Observed Ratio A/B
Sarcosine	0.5	1.08	0.97	1.38	1.40	1.00
Betaine	[0.62,0.73]	3.53	0.81	2.92	1.84	0.59
Urea	0.5					
Taurine	[0.68,0.81]	0.84	0.28		0.35	3.99
Nicotinic acid (niacin)	[0.66,0.67]	5.11	0.28	5.52	9.38	4.08
Creatine	[0.62,0.68]	0.79	0.50	1.54	2.07	0.87
Suberic acid	0.63	0.17	1.19		0.19	0.53
Quinolinic acid	0.54	0.37	0.90			0.38
Acetaminophen	0.25	8.78	8.06	8.68	8.09	
Acetylcarnitine	1.57	0.72	0.43	0.62	0.48	
Caffeine	0.18	0.78	0.15	0.29	0.20	1.69
Creatinine	1.75	1.61	1.78	1.55	1.65	0.90
DL-indole-3-lactic acid	1.75	0.42	0.51		0.20	1.12
Indoxyl sulfate	0.11					
L-arginine	0.65	0.17	2.10	1.71	1.99	1.26
L-isoleucine	[1.59,1.78]	0.86	0.59	0.75	0.49	0.47
Xanthosine	1.71	0.16	0.60		0.12	0.61

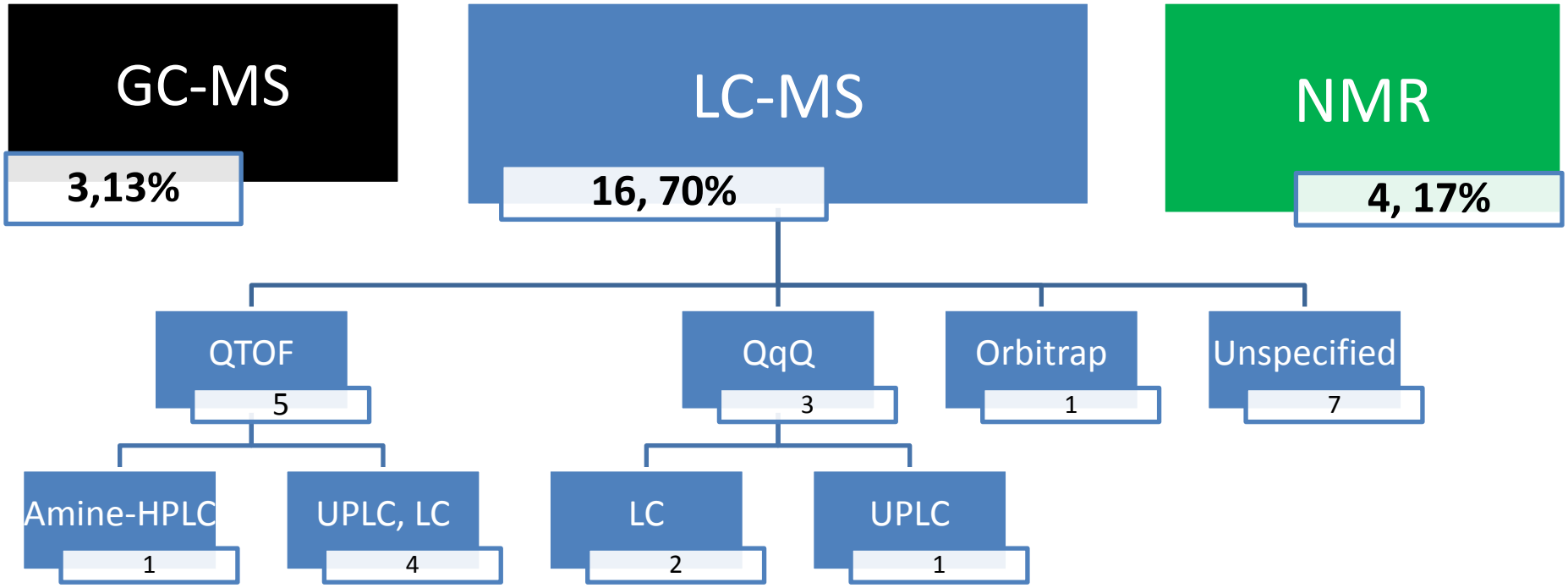
Urea and Indoxyl sulfate were not detected by any of the participating laboratories.

Results Reporting Format

For each compound:

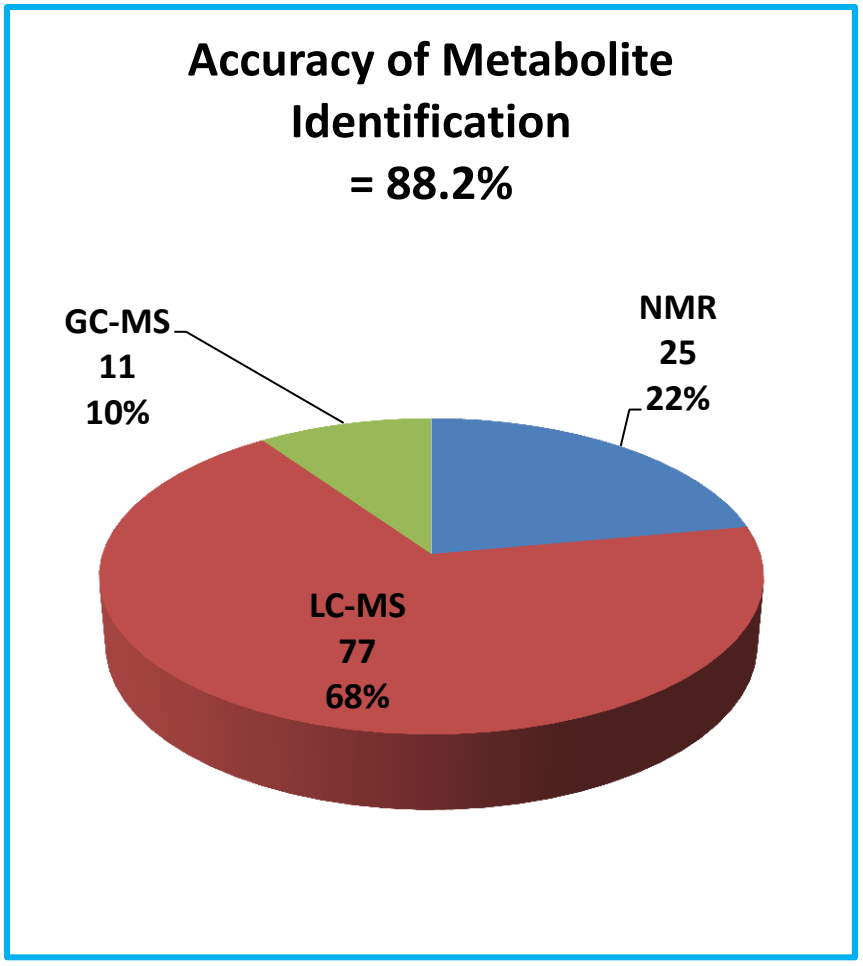
- m/z, ion mode (mass spectrometry)
- Molecular formula (or multiple formulas if ambiguous)
- Fold-change between groups
- Statistical metric for observed difference
- Compound identity

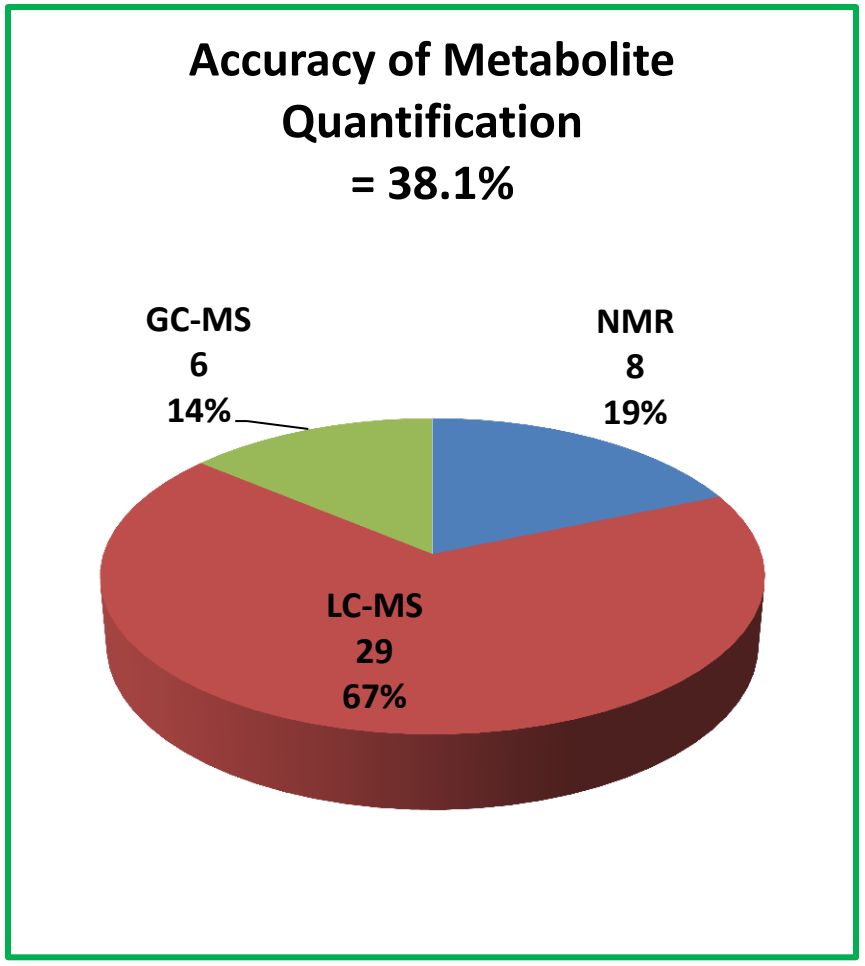
Techniques Used



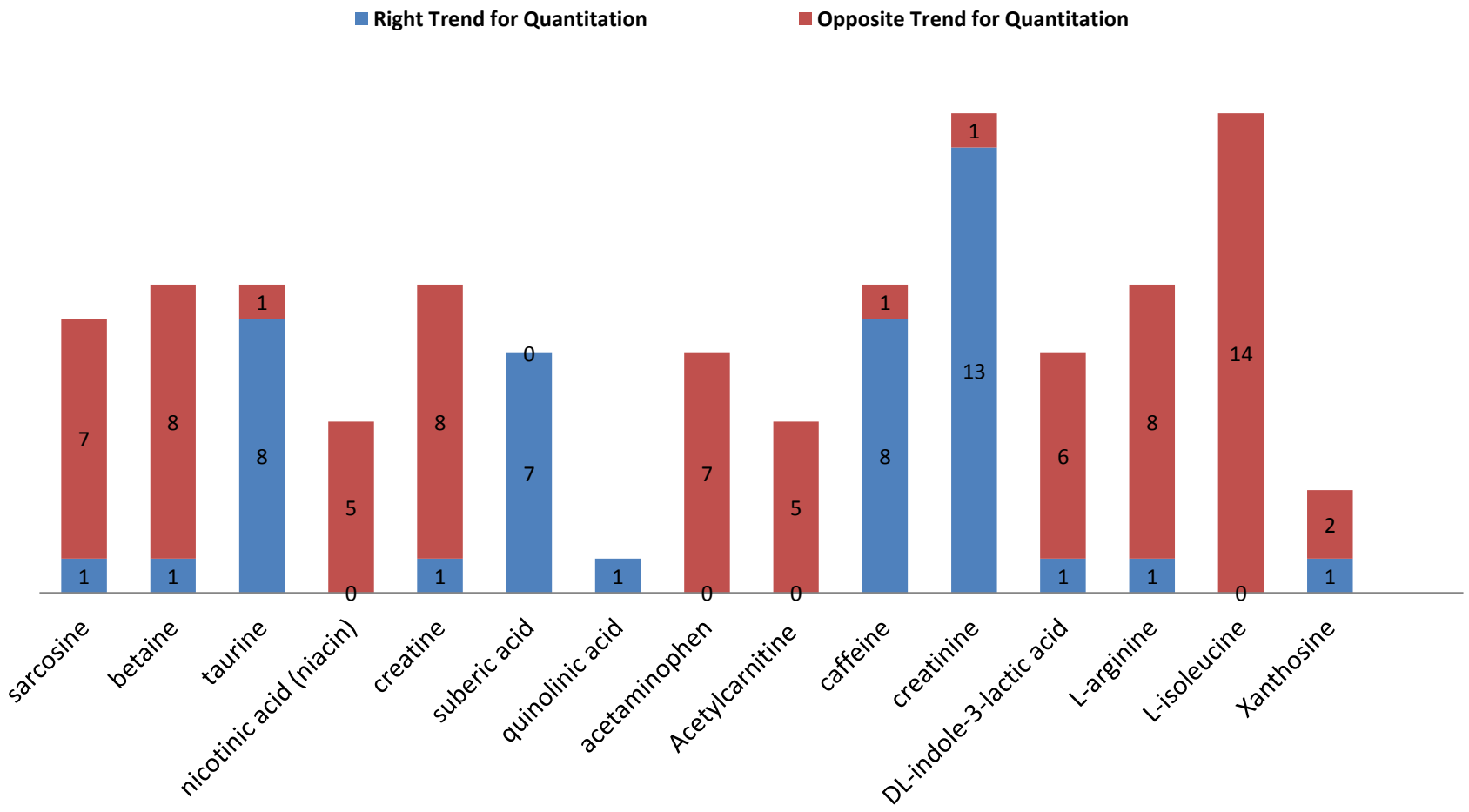
- **Total Participants (including MRG members) = 17**
- **Total Platforms Used = 23**
- **Quantitative Data Returned = 11 (73.3%)**

Performance Measures





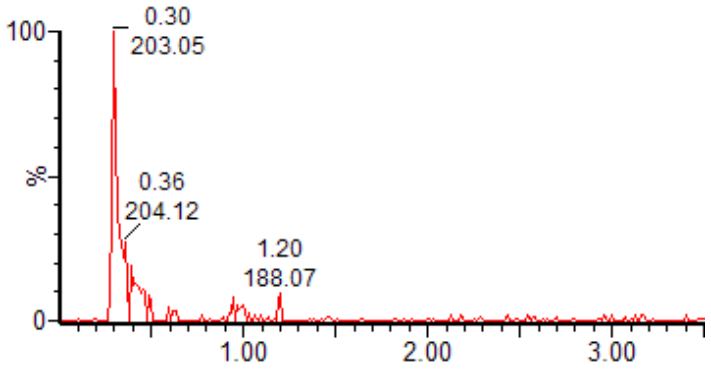
Detection of Spiked Metabolites

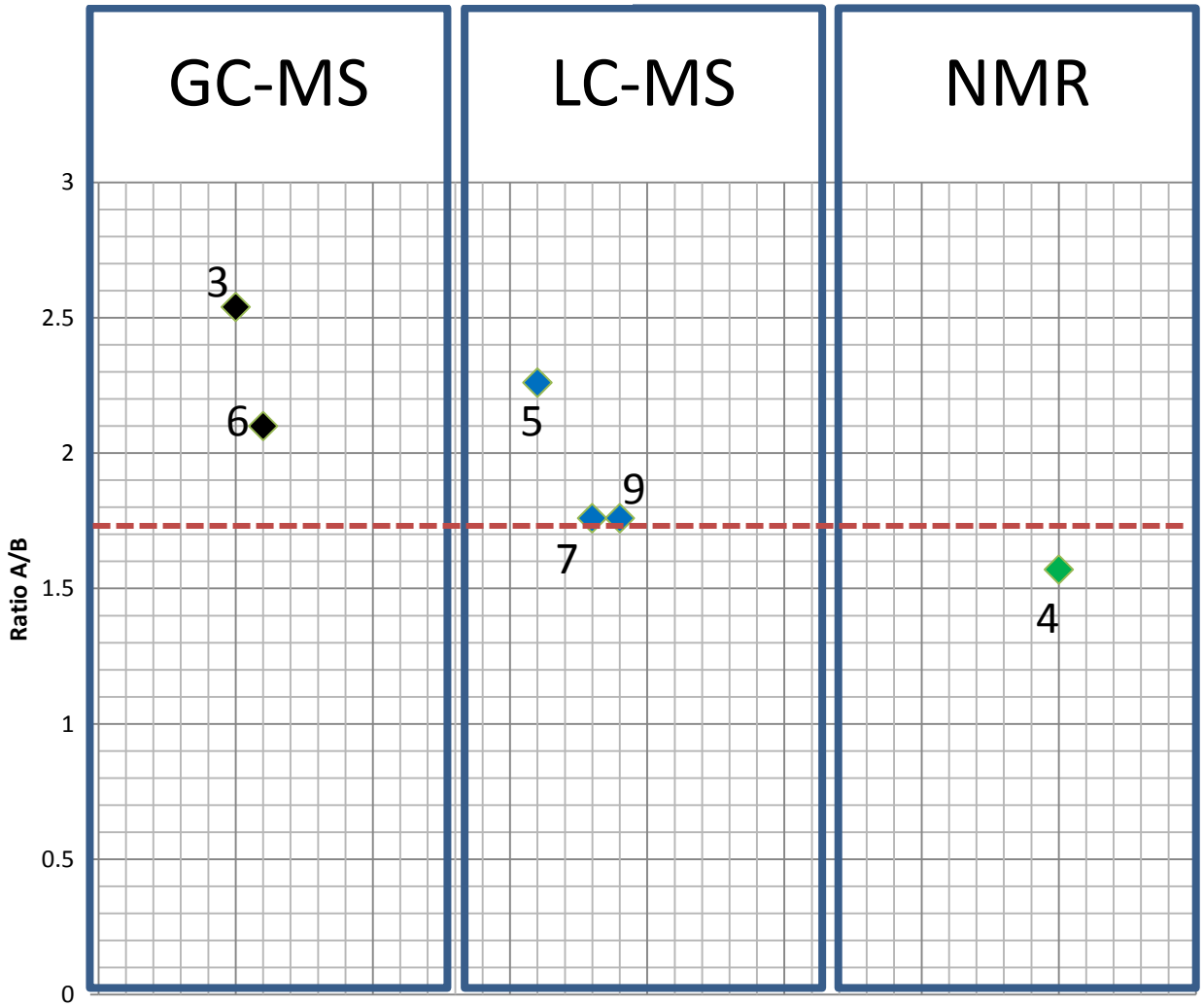


Quantitative Accuracy

creatinine

m/z 114.0667 (ES+)





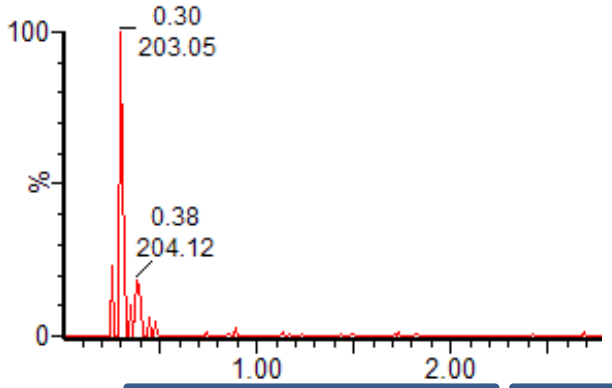
A= 69.98µM
B= 9.98µM
C= 70µM
Expected Ratio A/B
(Dashed Line): 1.75

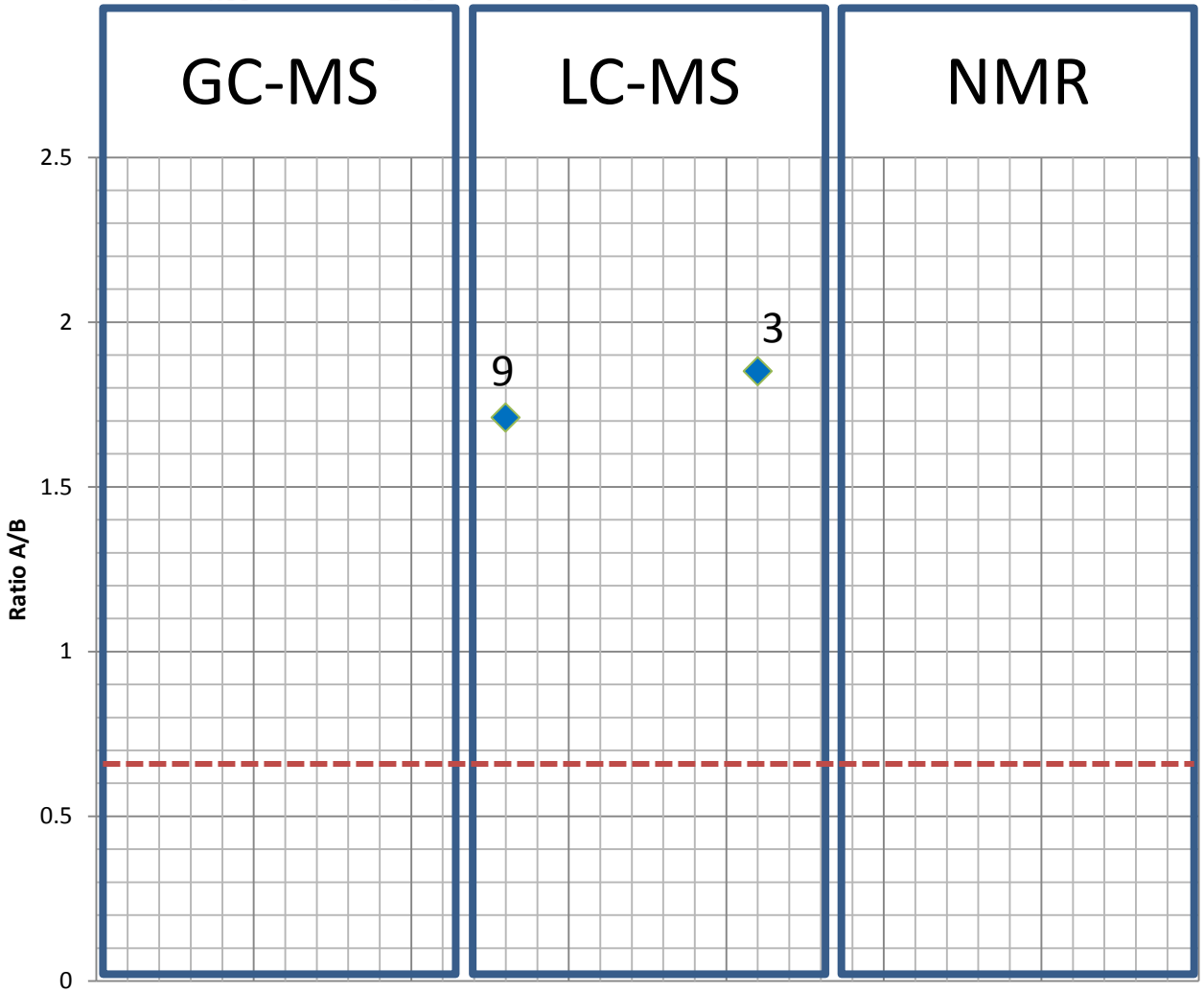
Method
- GC-MS
- LC-MS
- NMR

Quantitative Accuracy

L-arginine

m/z 175.1195 (ES+)





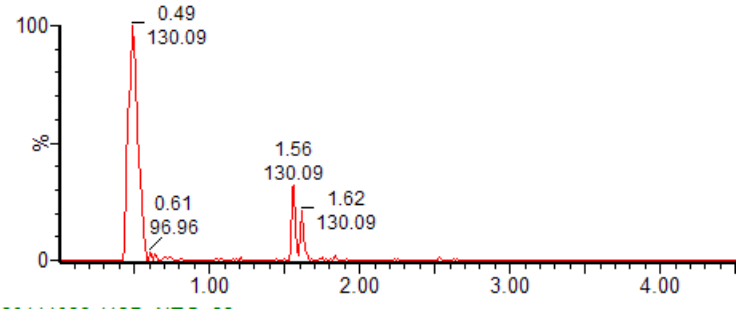
A= 3.7µM
B= 48.7µM
C= 80µM
Expected Ratio A/B
(Dashed Line): 0.65

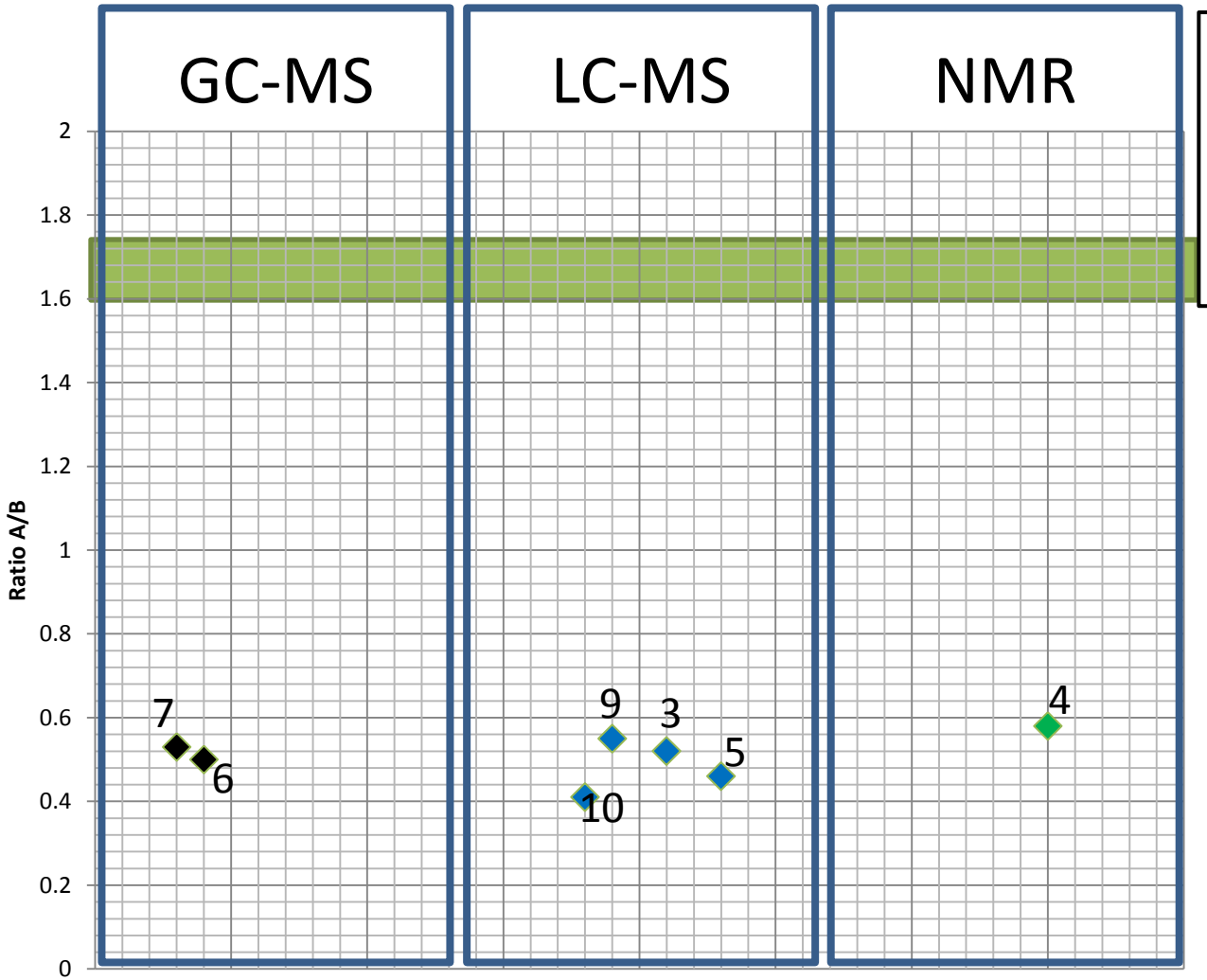
Method
- GC-MS
- LC-MS
- NMR

Quantitative Accuracy

L-isoleucine

m/z 130.0868 (ES-)





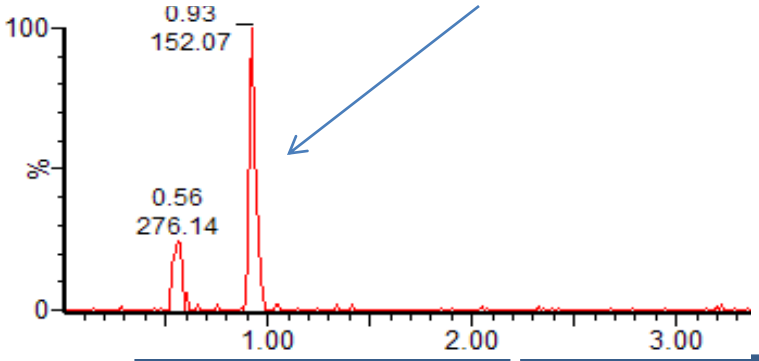
A= 54.5µM
B= 4.5µM
C= 60-80µM
Expected Ratio A/B
(Range): [1.59,1.78]

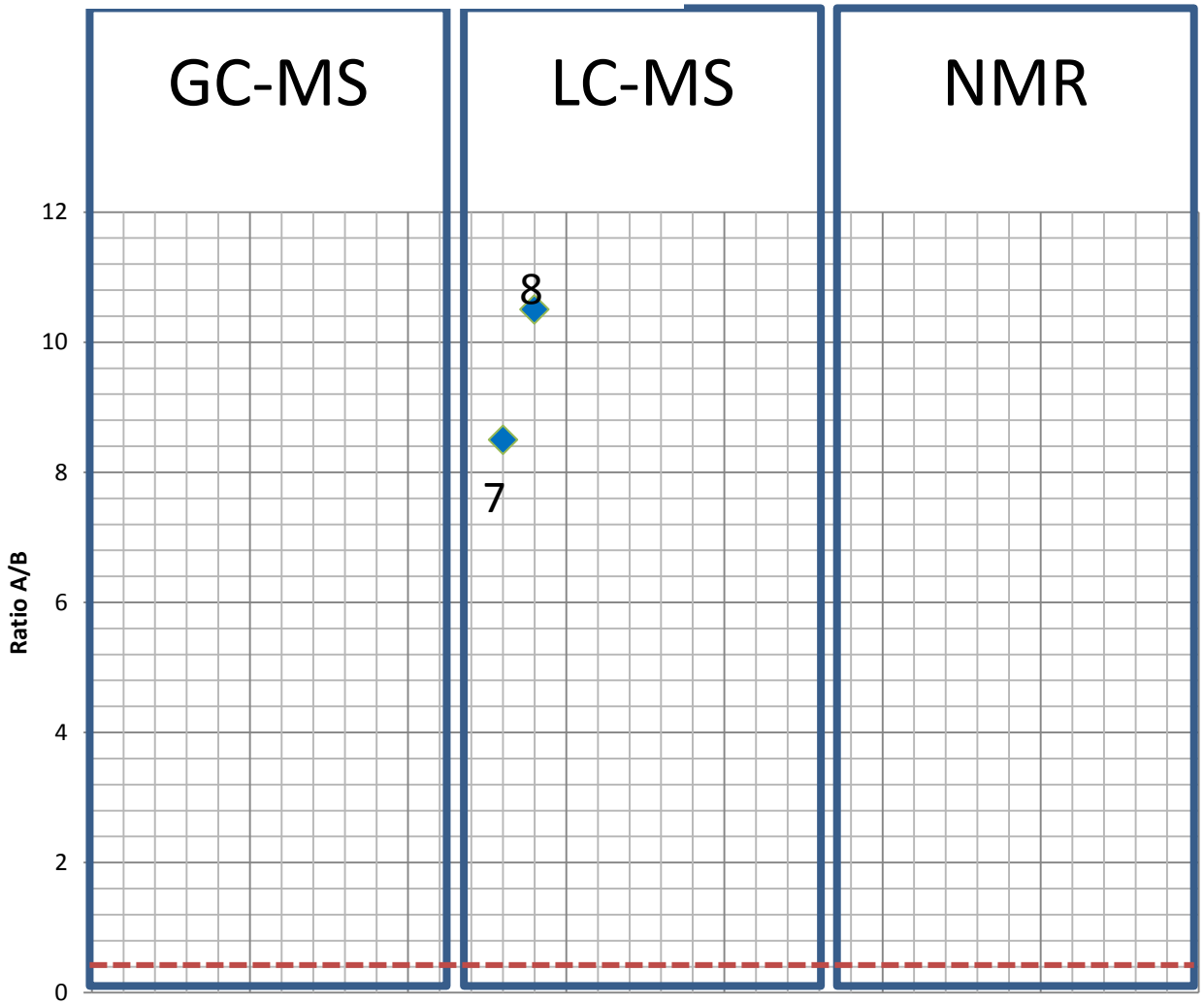
Method
- GC-MS
- LC-MS
- NMR

Quantitative Accuracy

acetaminophen

m/z 152.0712 (ES+)





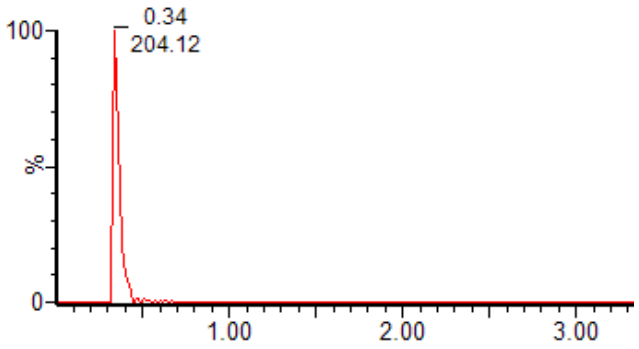
A= 5µM
B= 20µM
C= Dose Dependent
Expected Ratio A/B
(Dashed Line): 0.25

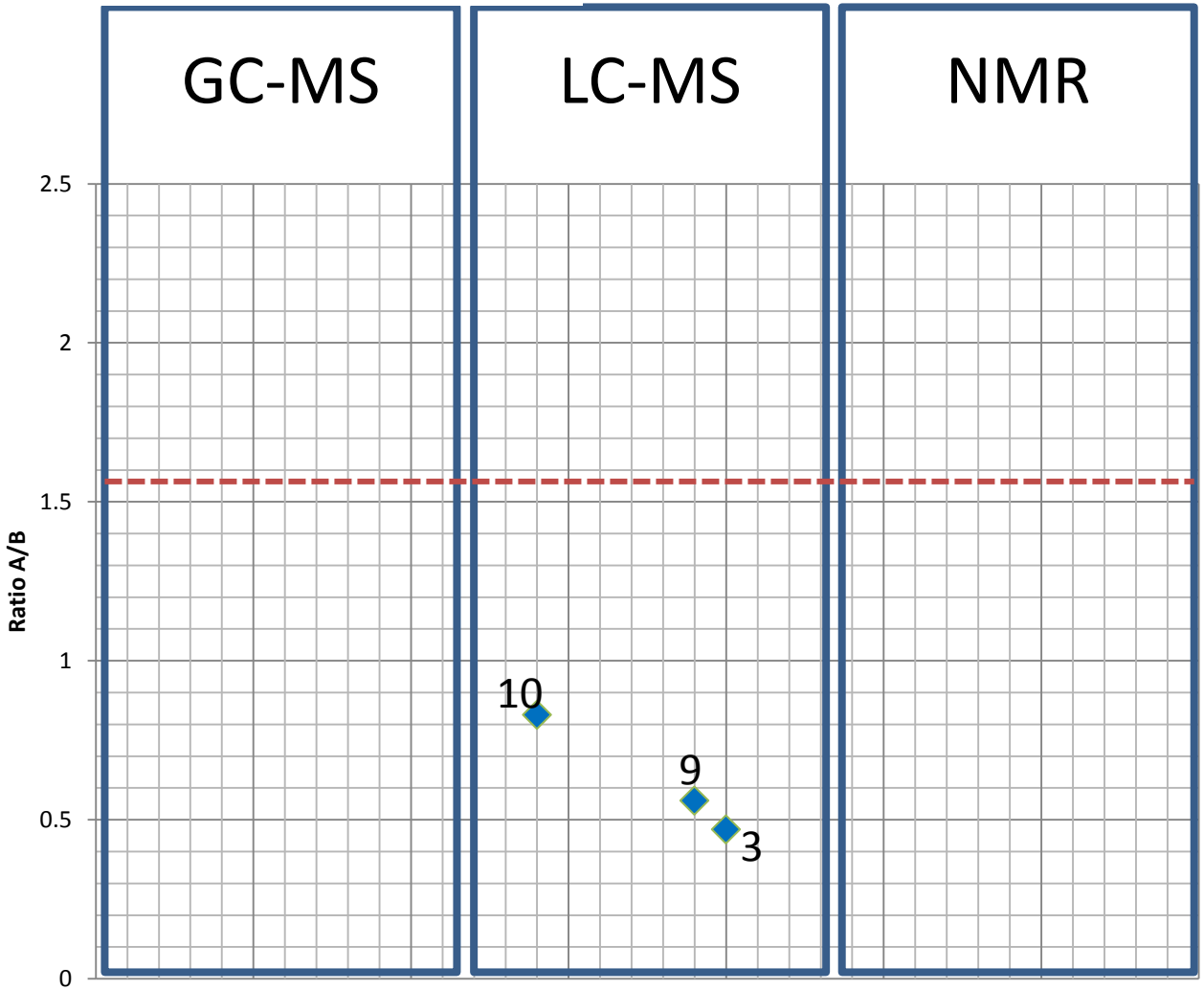
Method
- GC-MS
- LC-MS
- NMR

Quantitative Accuracy

acetylcarnitine

m/z 204.1236 (ES+)





A= 16µM
B= 8µM
C= 6µM
Expected Ratio A/B
(Dashed Line): 1.57

Method
- GC-MS
- LC-MS
- NMR

Conclusions

- LC-MS was the most commonly used platform to analyze study samples.
- For the LC-MS platforms, the metabolite detection accuracy was dependent on the protocol used for sample processing as well as the analytical conditions (column chemistry, mobile phase, etc.).
- The quantification trends were quite consistent for the laboratories that used LC-MS platforms.
- Quantitative data for Taurine, Suberic acid, Caffeine, and Creatinine were most consistent across laboratories and analytical platforms.
- Quantification of metabolites with high endogenous plasma concentrations turned out to be the most challenging.
- A combination of platforms increased the accuracy and overall rate of detection.
  - Average Detection Rate is 31.76%.
    - Average Detection Rate is 22.59% for untargeted and 39.71% for targeted methods, a 75% increase in detection rate.
  - Using 2 different platforms, the detection rates were 52.9% and 64.7%, respectively.
  - Using different separation systems in conjunction with MS-based platforms resulted in the highest detection rate (88.2%).