



# High-Throughput Analysis of Five Therapeutic Drug Monitoring Assays using Ultra Fast SPE-MS/MS

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# Disclosures

- Relevant Financial Relationship(s):  
None
  
- Off Label Usage:  
None

# Outline

- Introduction
- Extraction method using the RapidFire
- Validation Data
- Conclusions

## Why the RapidFire vs. Turboflow?

- Get patients their results as fast as possible
- Free up instrumentation
- Cost savings

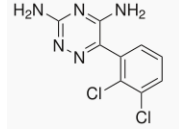
## Why Therapeutic Drug Monitoring?

- Non-endogenous compounds
- High volume test
- High drug concentrations

# High Volume TDM Assays in the Toxicology Lab

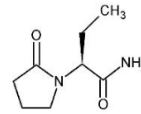
- Lamotrigine - Anticonvulsant

- ~ 40,000 samples annually (110 daily)



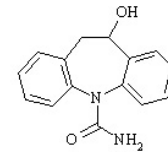
- Levetiracetam - Anticonvulsant

- ~ 60,000 samples annually (160 daily)



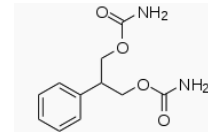
- Oxcarbazepine Metabolite (OMHC) - Anticonvulsant

- ~ 18,000 samples annually (50 daily)



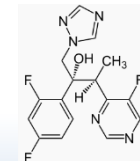
- Felbamate - Anticonvulsant

- ~ 2,000 samples annually (6 daily)



- Voriconazole - Antifungal

- ~ 10,000 samples annually (25 daily)



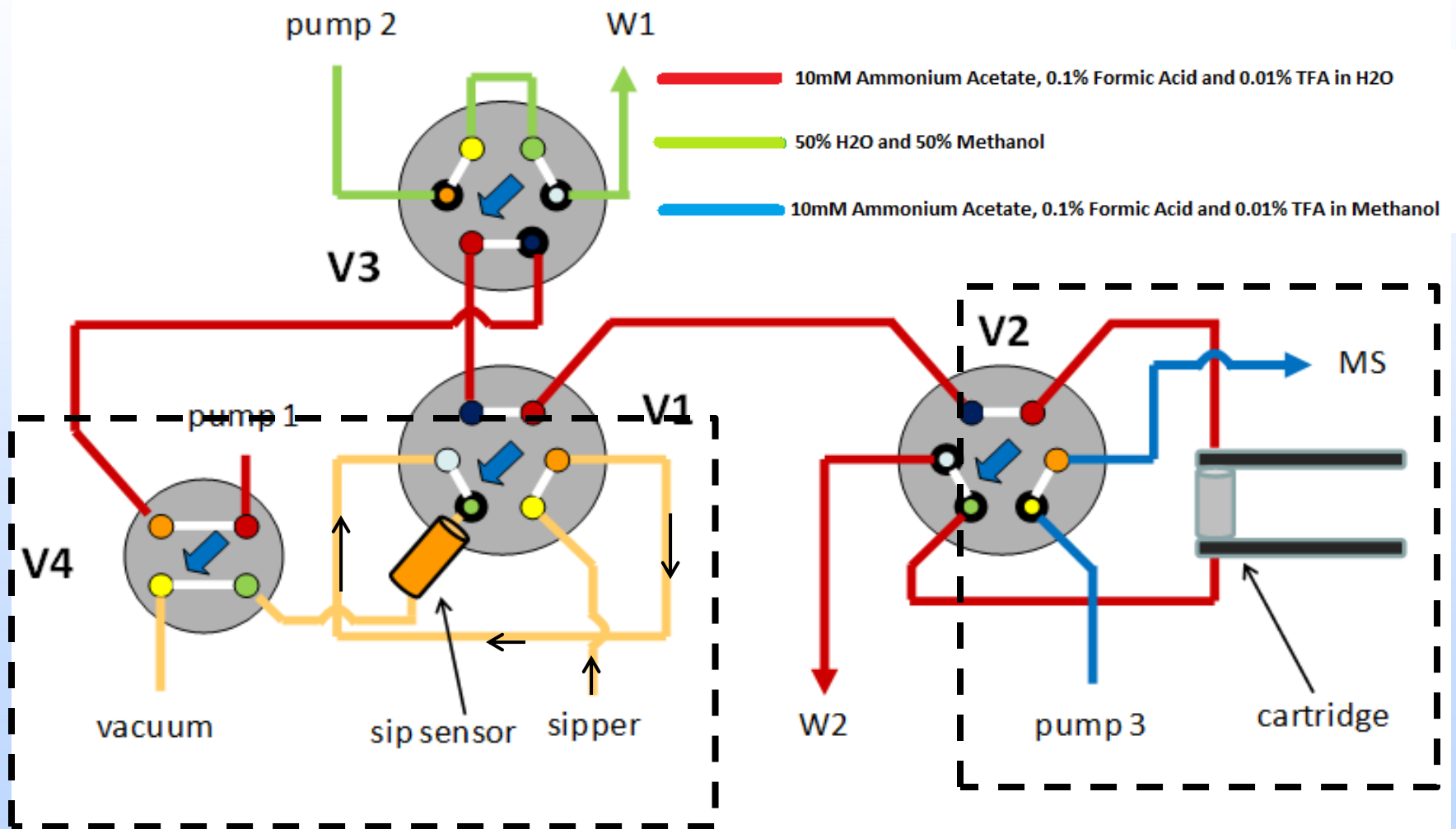
# Extraction Method

- 50  $\mu\text{L}$  serum
- Crash with 200  $\mu\text{L}$  acetonitrile containing deuterium labeled IS
- Vortex/Centrifuge
- Remove 30  $\mu\text{L}$  of supernatant and dilute with 600  $\mu\text{L}$  of mobile phase A.
- Done on a Hamilton Starlet – 96 well plate format

# Agilent RapidFire 300 and 6490 QQQ

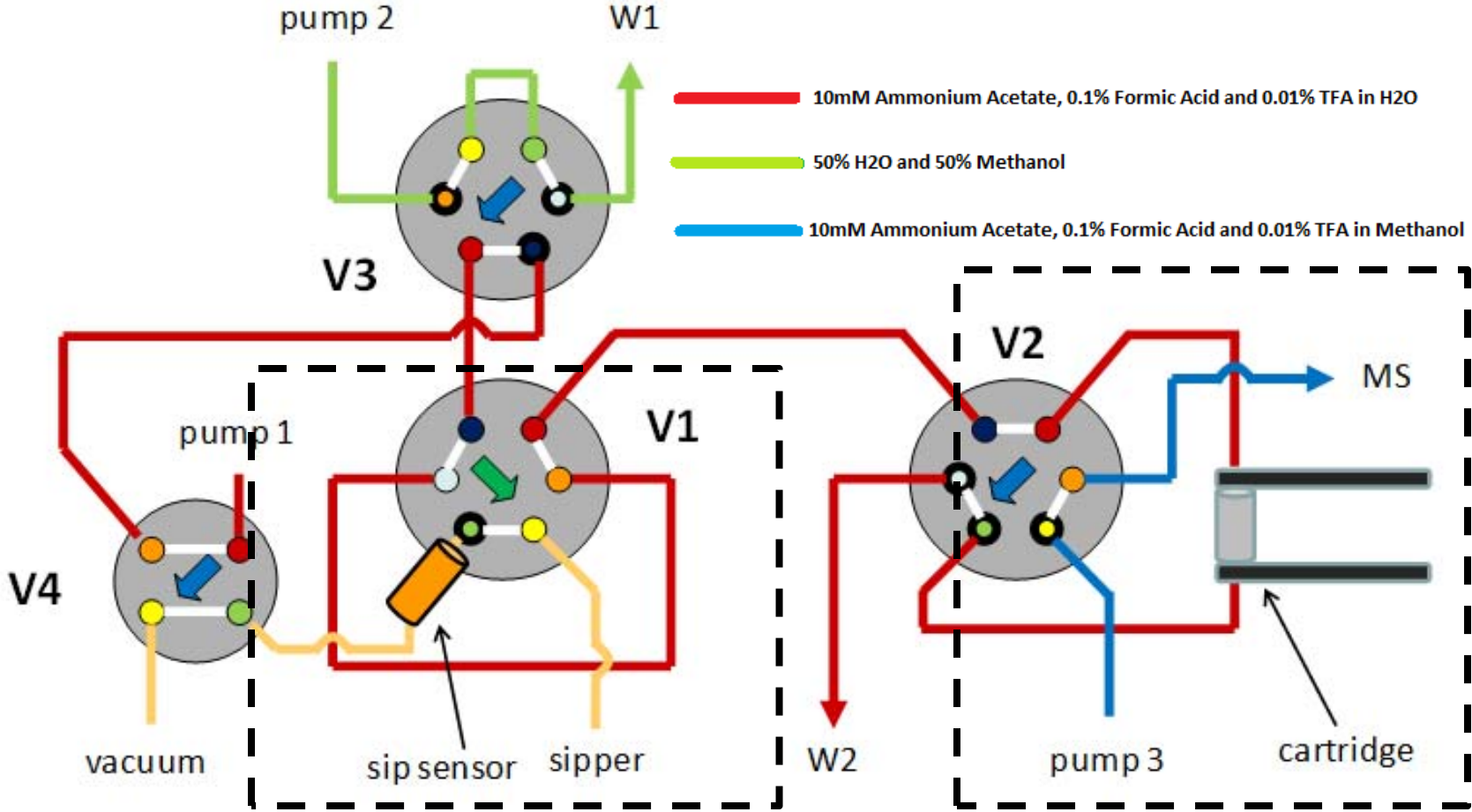


# States 1&4: Aspirate & Re-equilibrate

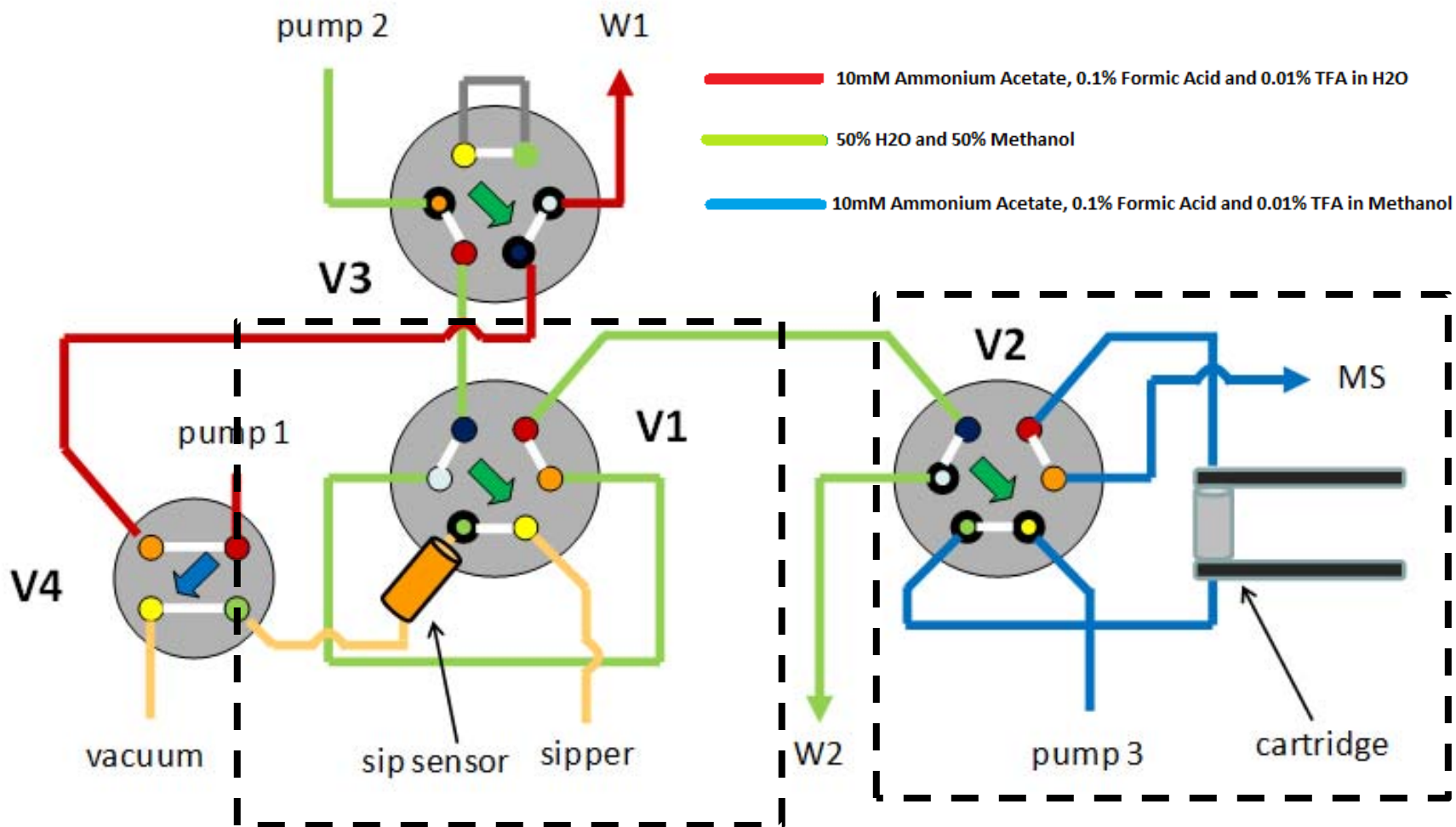




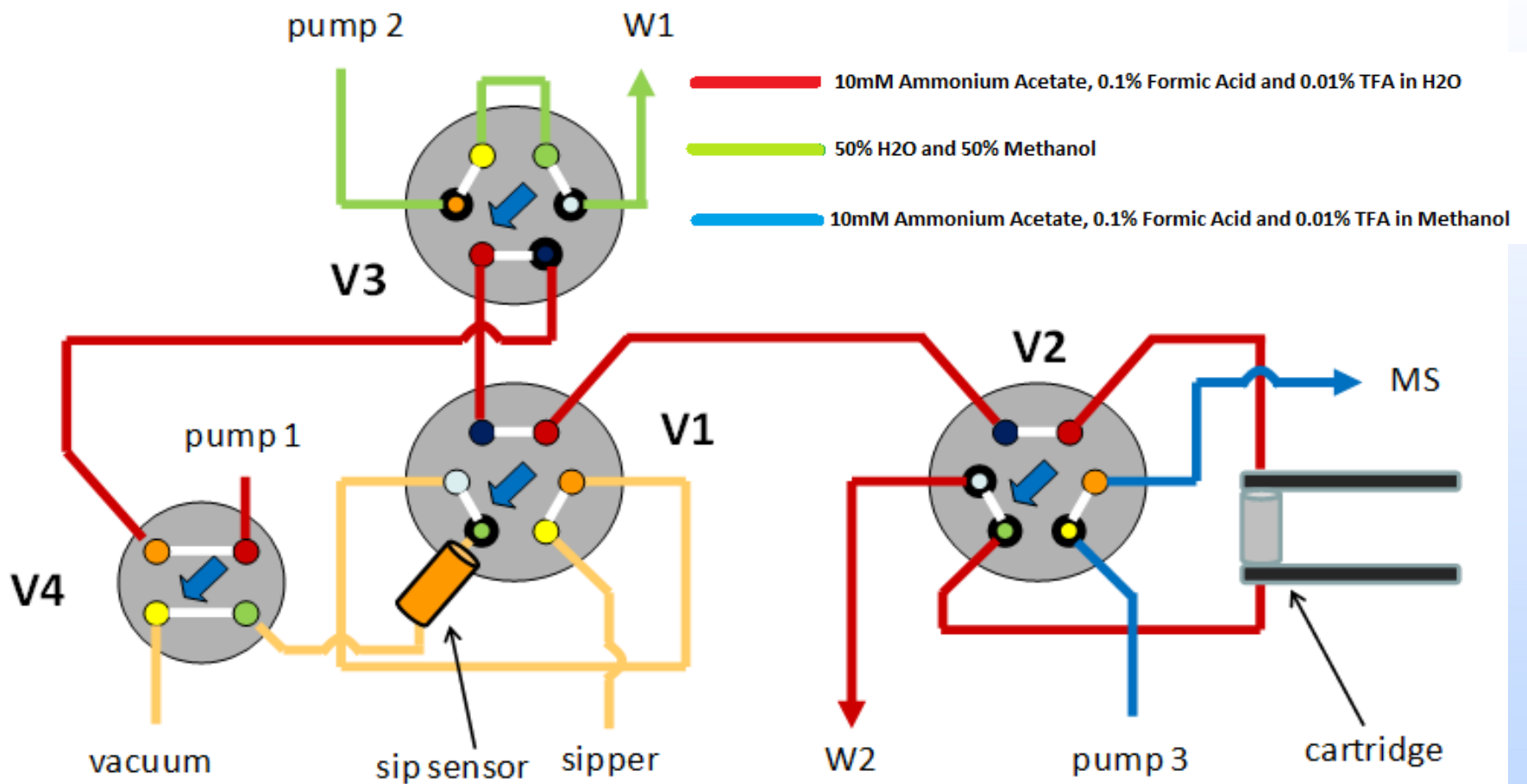
# State 2: Load/Wash



# State 3: Elute



# States 1&4: Aspirate & Re-equilibrate



# RapidFire Methods

Step	Lamotrigine	Levetiracetam	OMHC	Felbamate	Voriconazole
Inject	600 ms	600 ms	600 ms	600 ms	600 ms
Load	3500 ms	3500 ms	3500 ms	3500 ms	3500 ms
Wash	0 ms	0 ms	0 ms	0 ms	0 ms
Elute	3500 ms	4000 ms	4000 ms	4000 ms	3500 ms
Equil	500 ms	1000 ms	1000 ms	500 ms	1000 ms
<b>Total (sec):</b>	<b>8.0</b>	<b>9.0</b>	<b>9.0</b>	<b>8.5</b>	<b>8.5 (x2) = 17</b>

# Standard Curve Responses

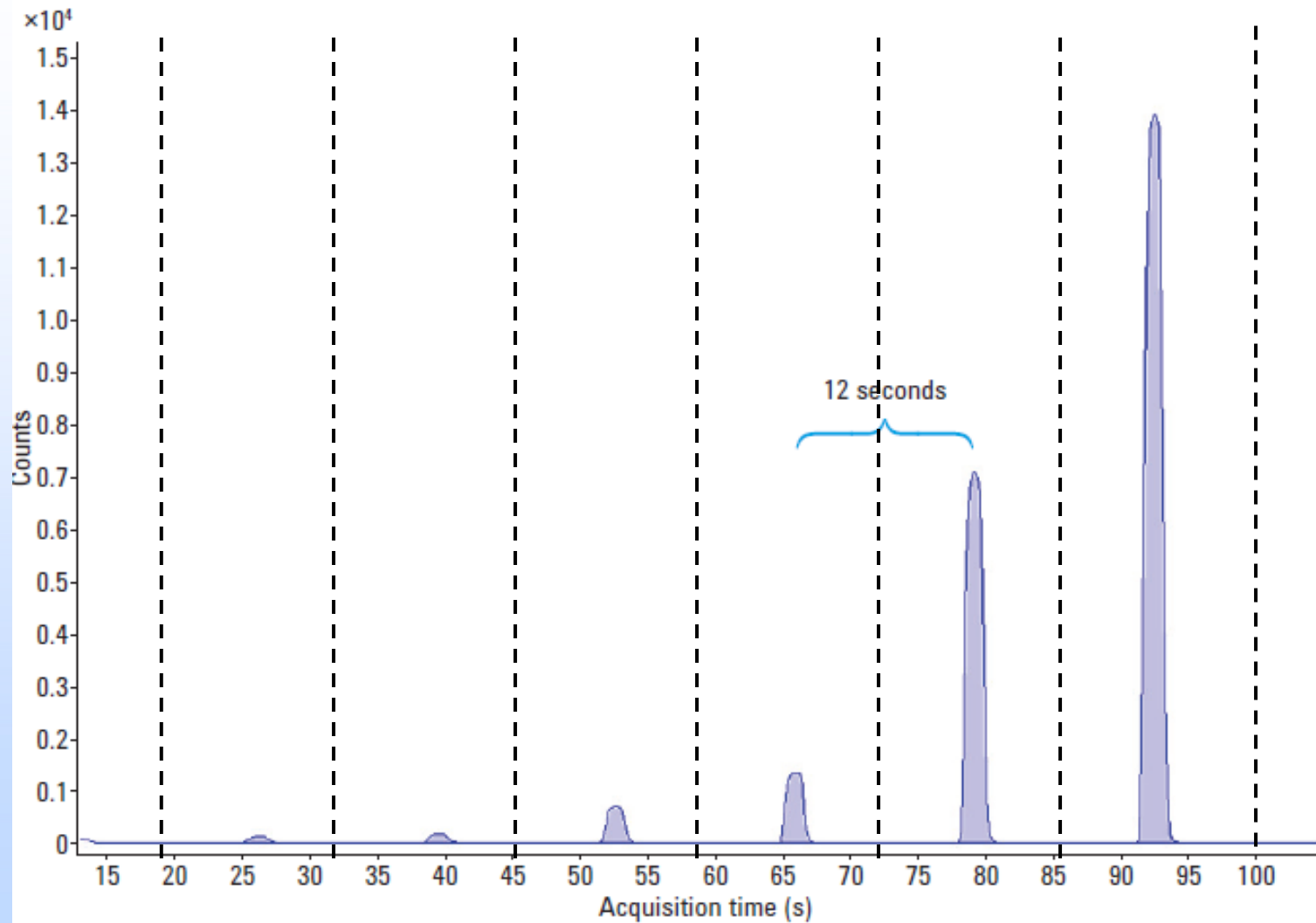
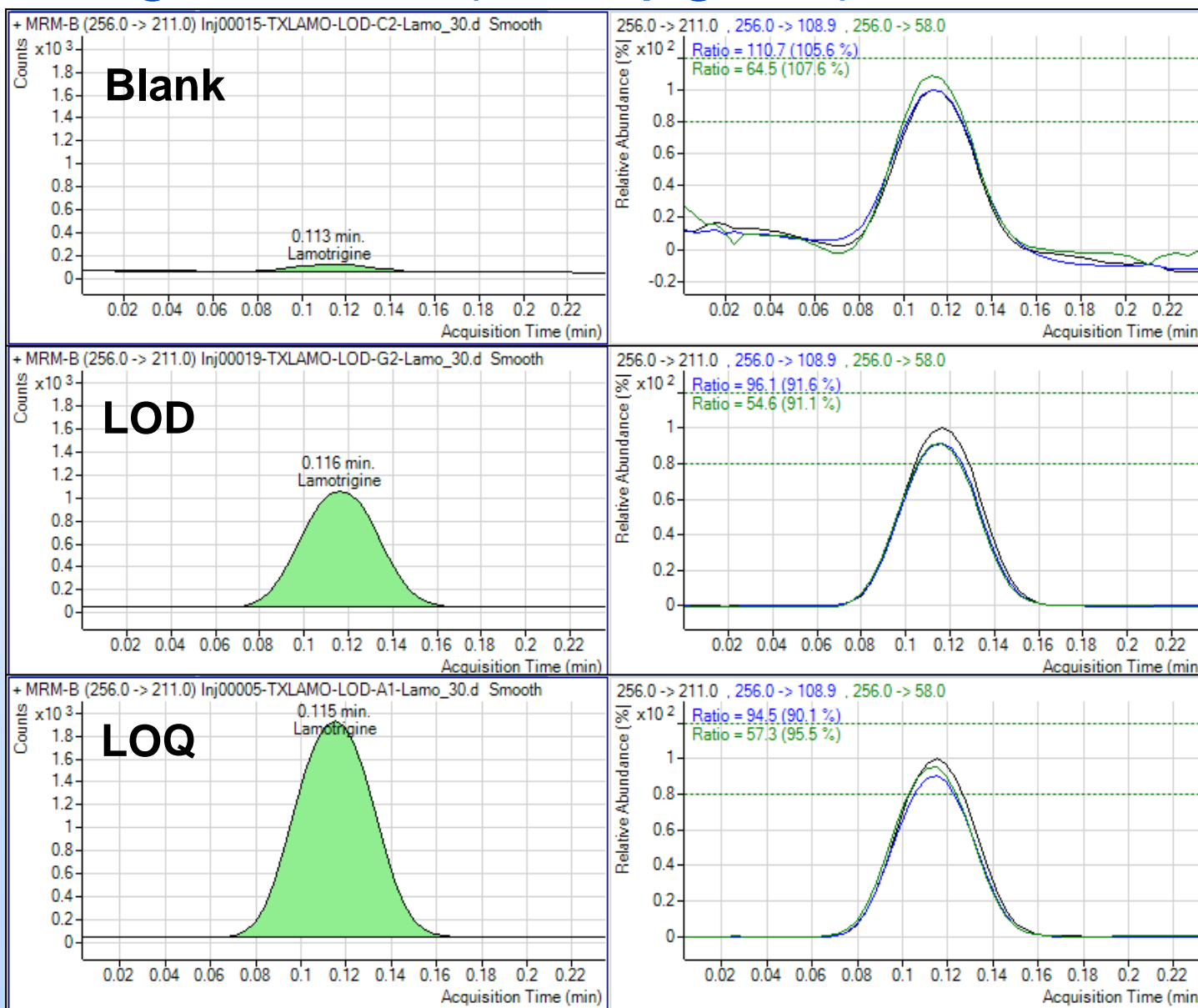


Figure 2. Standard curve showing injection cycle times of 12 seconds.

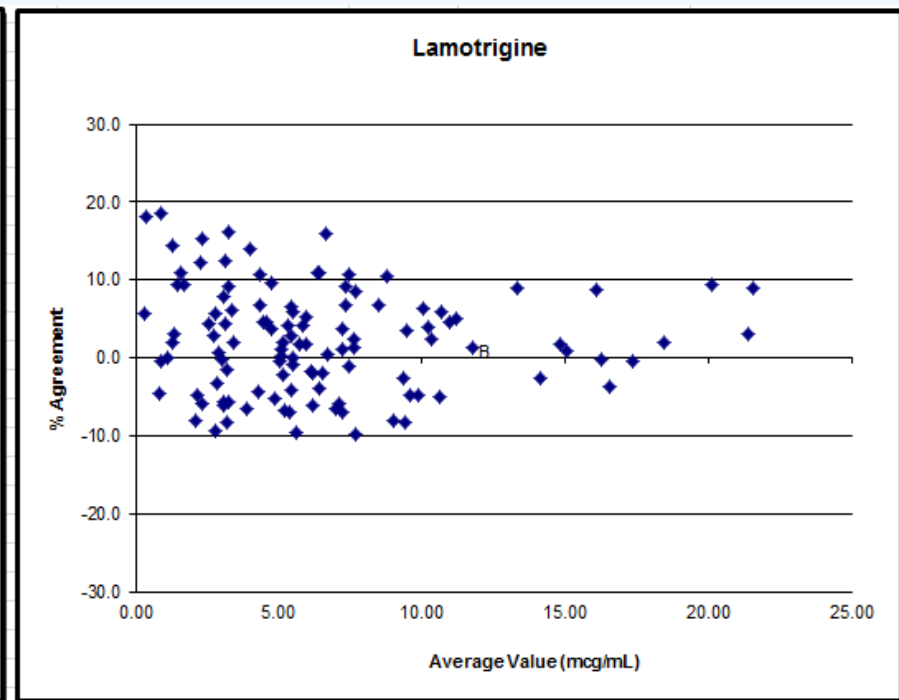
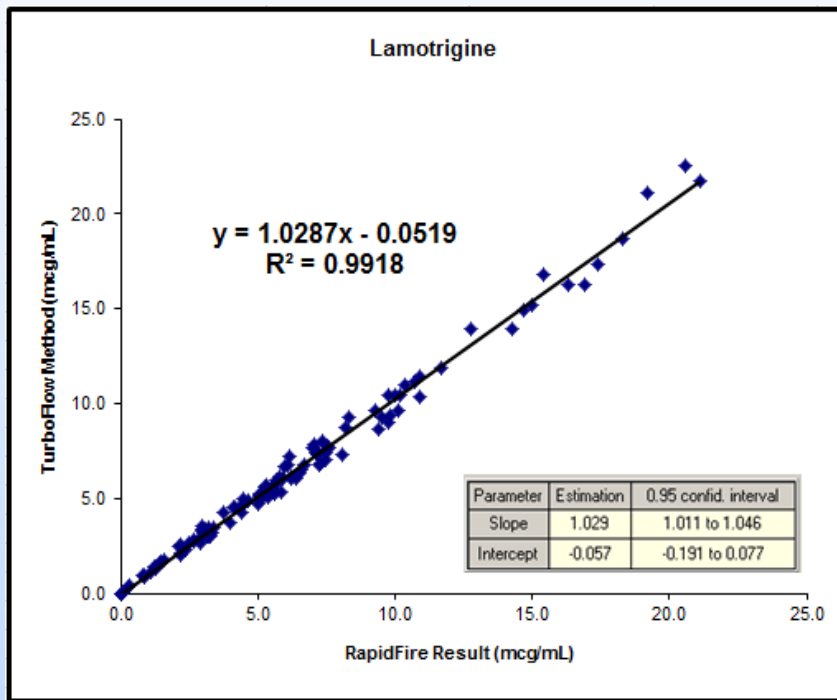
# Validation Data

# Lamotrigine LOQ (0.20 $\mu\text{g}/\text{mL}$ )



# Lamotrigine (n=100)

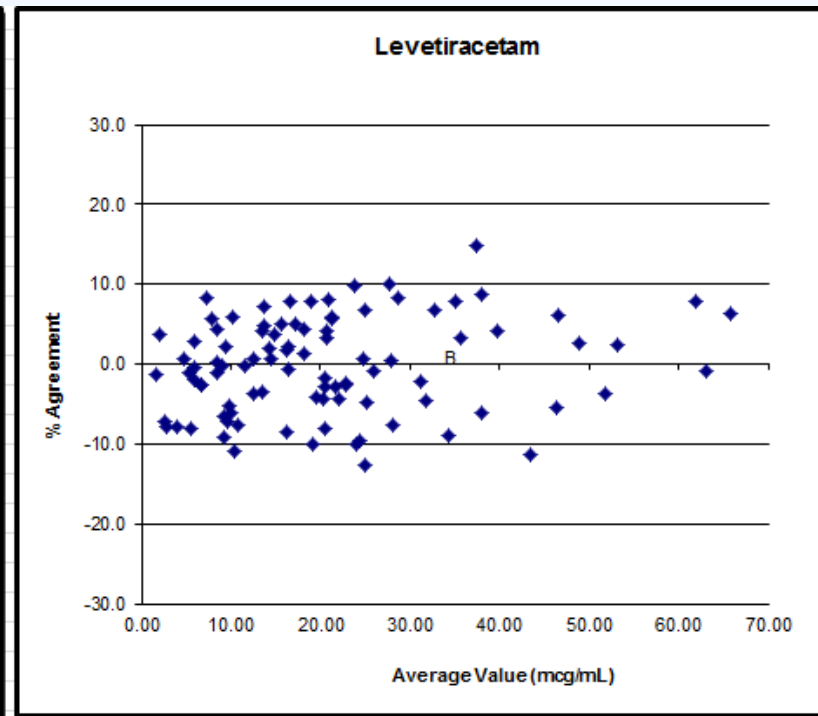
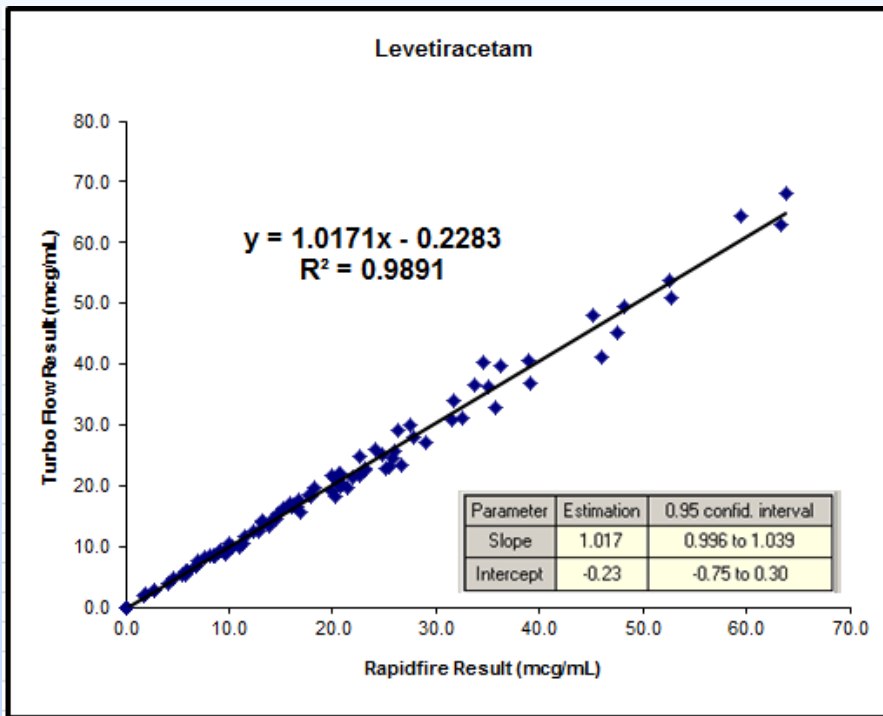
- Linear Range: 0.20 µg/mL – 25.0 µg/mL





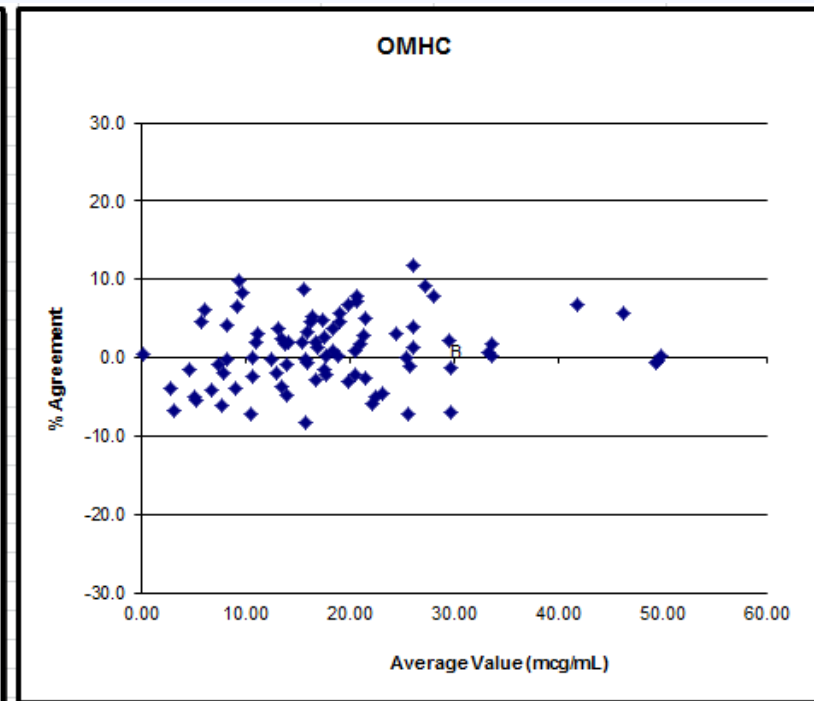
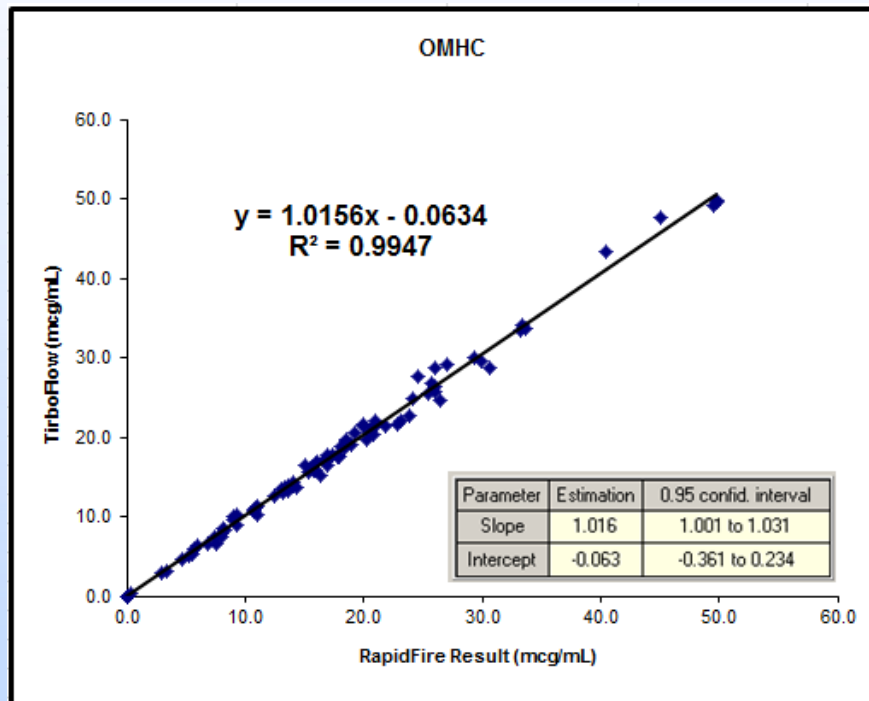
# Levetiracetam (n=100)

- Linear Range: 1.0 µg/mL – 100.0 µg/mL



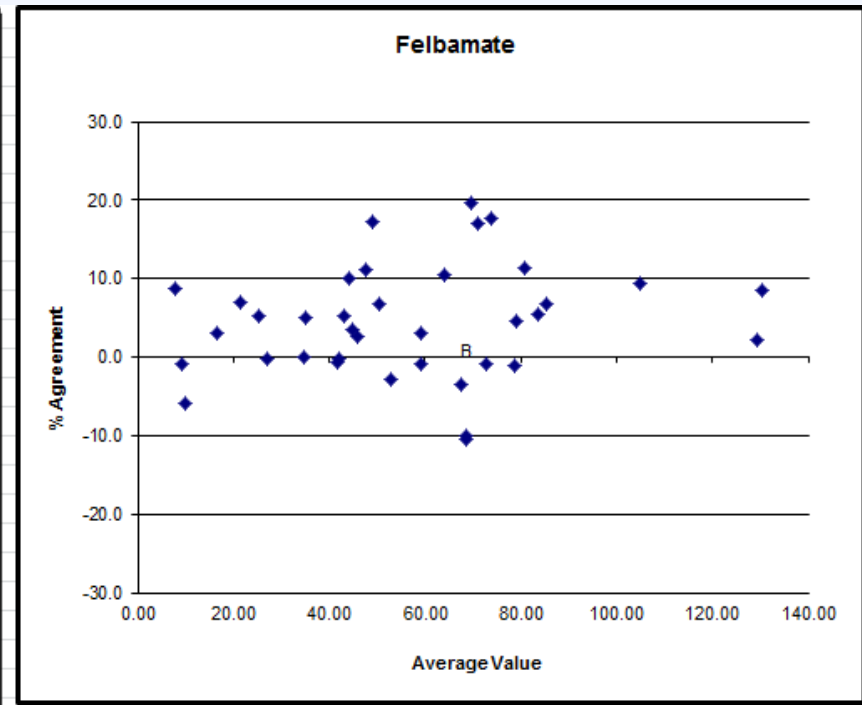
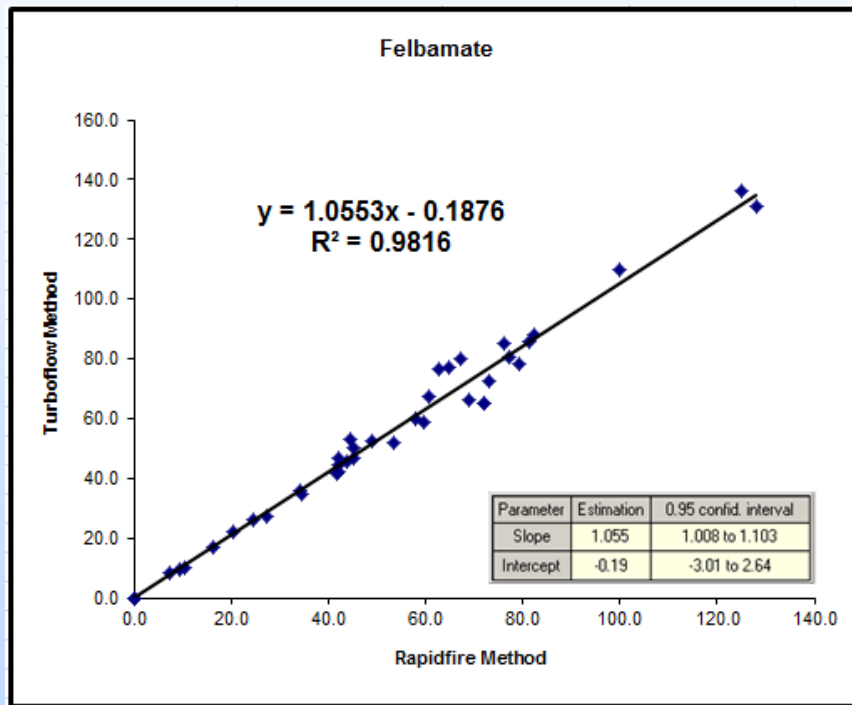
# OMHC (n=100)

- Linear Range: 1.0 µg/mL – 50.0 µg/mL



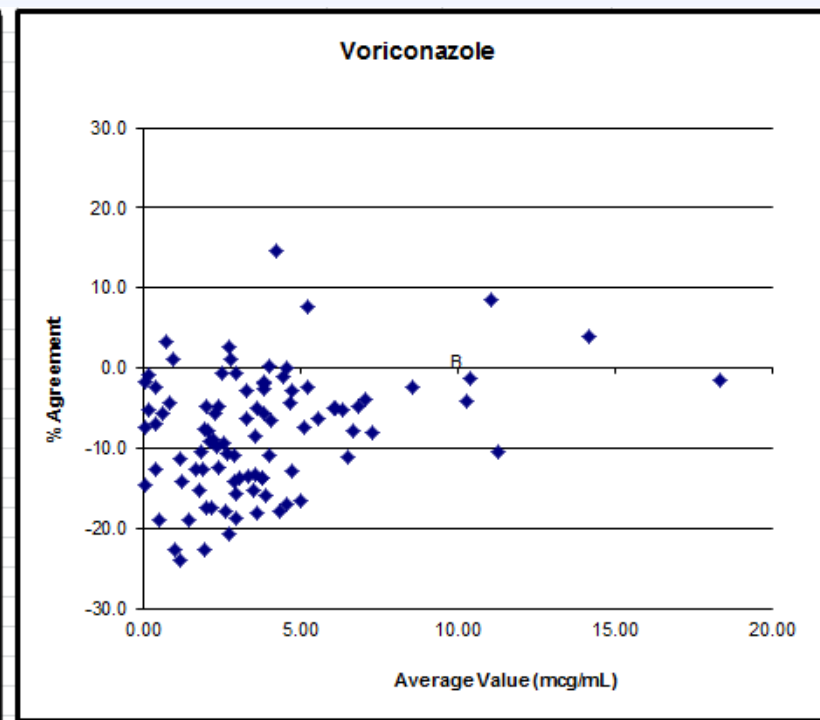
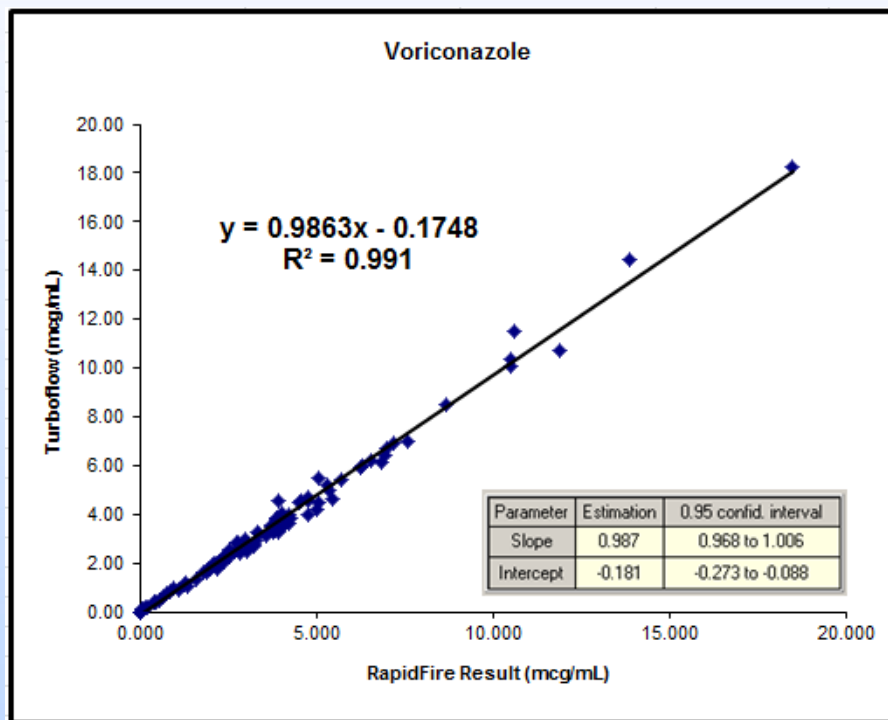
# Felbamate (n=40)

- Linear Range: 5.0 µg/mL – 150.0 µg/mL

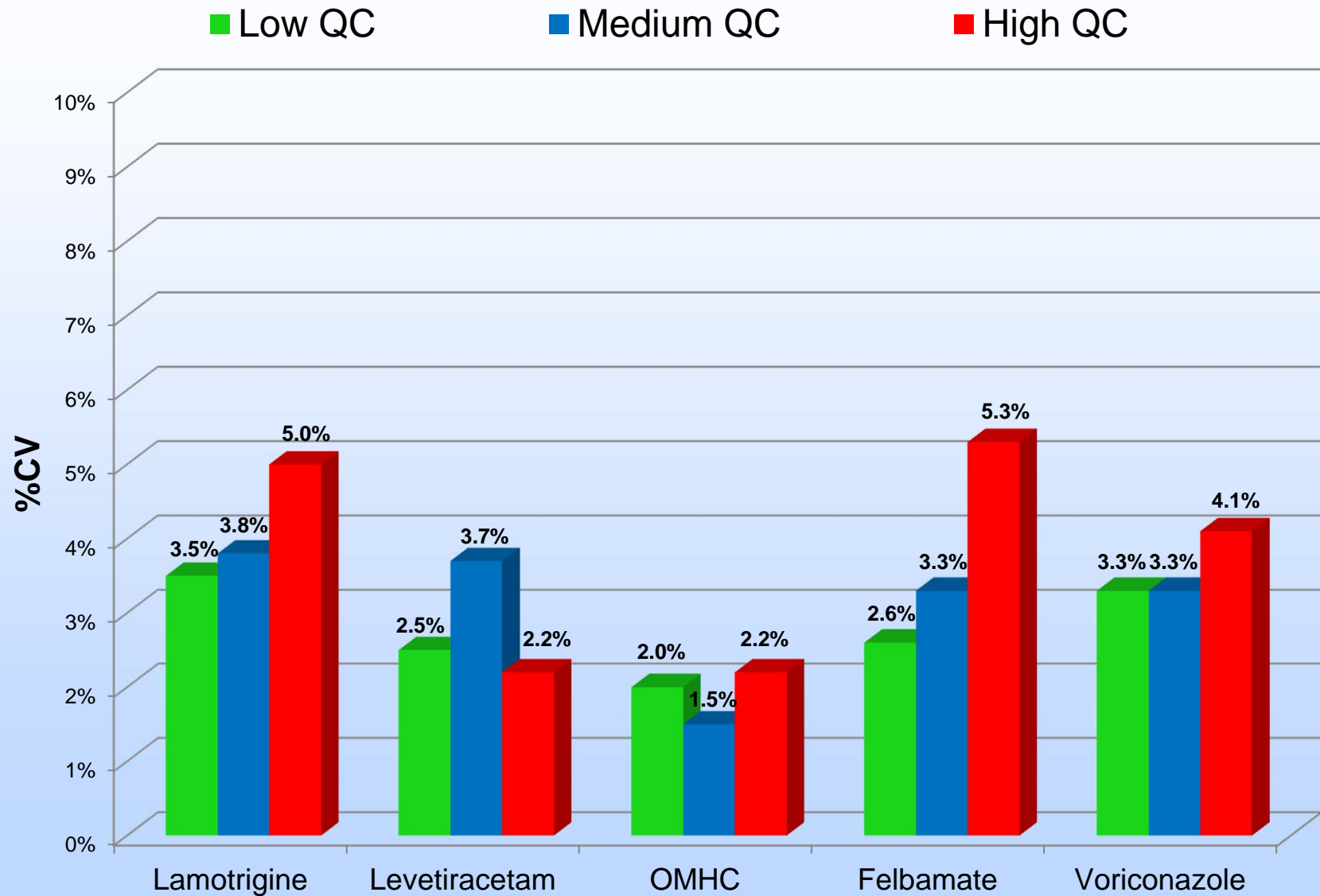


# Voriconazole (n=100)

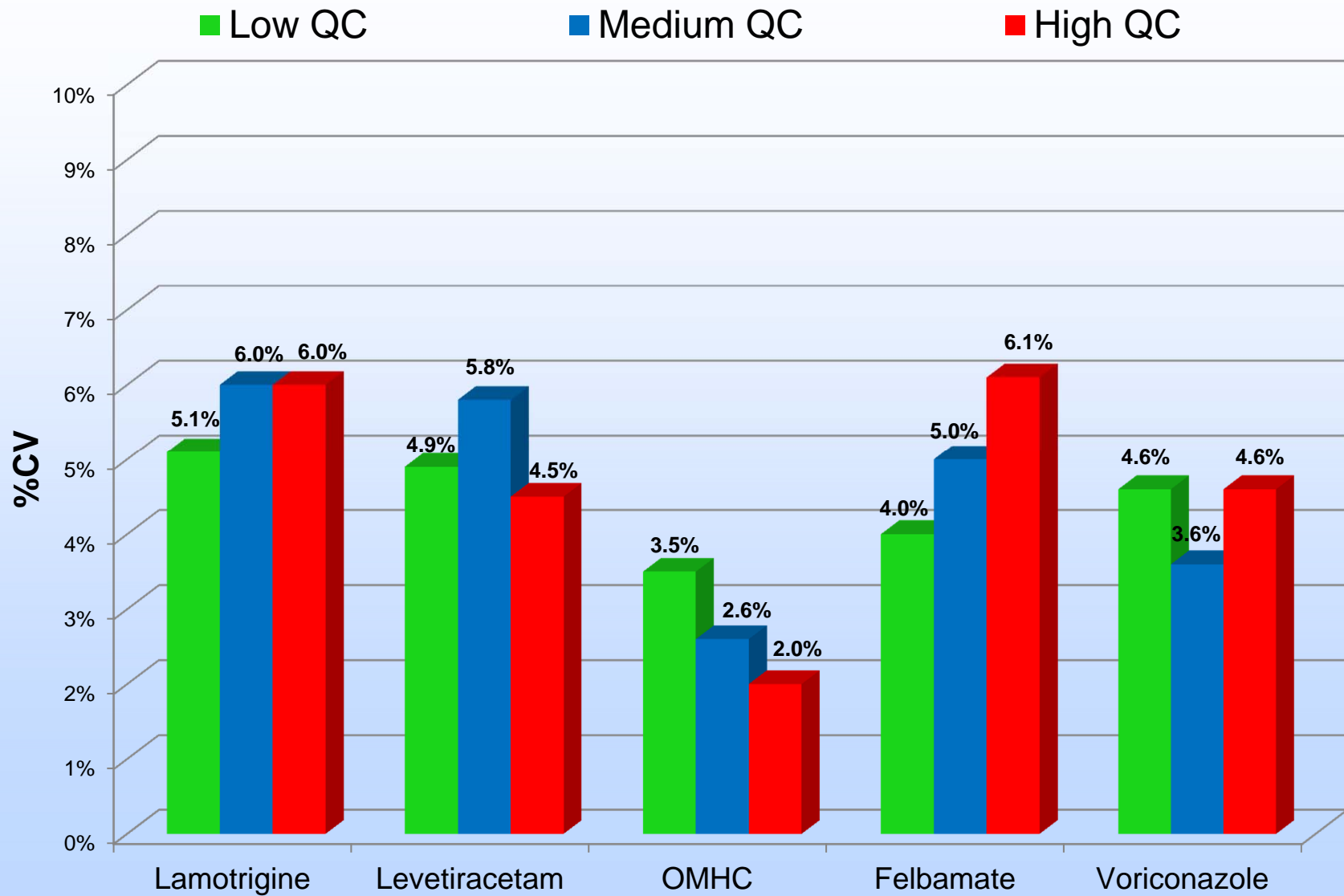
- Linear Range: 0.10 µg/mL – 20.0 µg/mL



# Within Run Precision (n=20)



# Between Run Precision (n=20)



# Compounds shown not to interfere

## Drugs Tested at 100 µg/mL for Interference

1 ACETOMINOPHEN	34 AZITHROMYCIN
2 ATORVASTIN	35 ROSUVASTATIN
3 AMOXICILLIN	36 CLOPIDOGREL
4 LISINOPRIL	37 SIMVASTATIN
5 HYDROCHLOROTHIAZIDE	38 METOPROLOL
6 FUROSIMIDE	39 ARIPIPRAZOLE
7 ALPRAZOLAM	40 LEVOTHYROXINE
8 AMLODIPINE	41 COCAINE*
9 METFORMIN	42 LSD OXO LSD
10 SERTRALINE	43 CATHINONE ANALOGS*
11 IBUPROFEN	44 FELBAMATE
12 CEPHALEXIN	45 CARBAMAZEPINE
13 ZOLPIDEM	46 CLONAZAPAM
14 PREDNISONE	47 GABAPENTIN
15 HYDROCODONE	48 OMHC
16 ESOMEPRAZOLE	49 PHENYTOIN
17 LORAZEPAM	50 PRIMIDONE
18 CITALOPRAM	51 TIAGABINE
19 PROXOXYPHENE	52 TOPIRAMATE
20 WARFARIN	53 VALPROIC ACID
21 BUPIVACAINE	54 ZONISAMIDE
22 OPIATES*	55 POSACONAZOLE
23 BENZODIAZAPINES*	56 FLUCONAZOLE
24 BARBS*	57 ITRACONAZOLE
25 AMPHS*	58 OH-ITRACONAZOLE
26 THC	59 LAMOTRIGINE
27 FENTANYL	60 CLONAZEPAM
28 NORFEENTANYL	61 MIDAZOLAM
29 METHADONE	62 CHLORDIAZEPOXIDE
30 EDDP	63 DIAZEPAM
31 PCP	64 NORDIAZEPAM
32 KETAMINE	65 C1011
33 NORKETAMINE	66 LEVETERICETAM

### AMPHS INCLUDE:

MDMA  
MDA  
Amphetamine  
Methamphetamine  
Pseudoephedrine  
Phentermine

### BARBS INCLUDE:

Pentobarbital  
Phenobarbital  
Secobarbital  
Butalbital  
Amobarbital  
Mephobarbital

### COCAINE INCLUDES:

Cocaine  
Benzoyllecgonine  
Norcocaine  
m-Hydroxybenzoyllecgonine  
Levamisole  
Cocaethylene

### CATHINONE ANALOGS:

Methylone  
Mephedrone  
MDPV  
Ethylone

### BENZOS INCLUDE:

Oxazepam  
Nordiazepam  
Lorazepam  
Hydroxyethyl-Flurazepam  
a-Hydroxy-Alprazolam  
a-Hydroxy-Triazolam  
Temazepam  
7-Aminoclonazepam  
7-Aminoflunitrazepam  
Clonazepam  
Diazepam  
Nordiazepam  
Midazolam  
Chlordiazepoxide  
Flurazepam  
Flunitrazepam

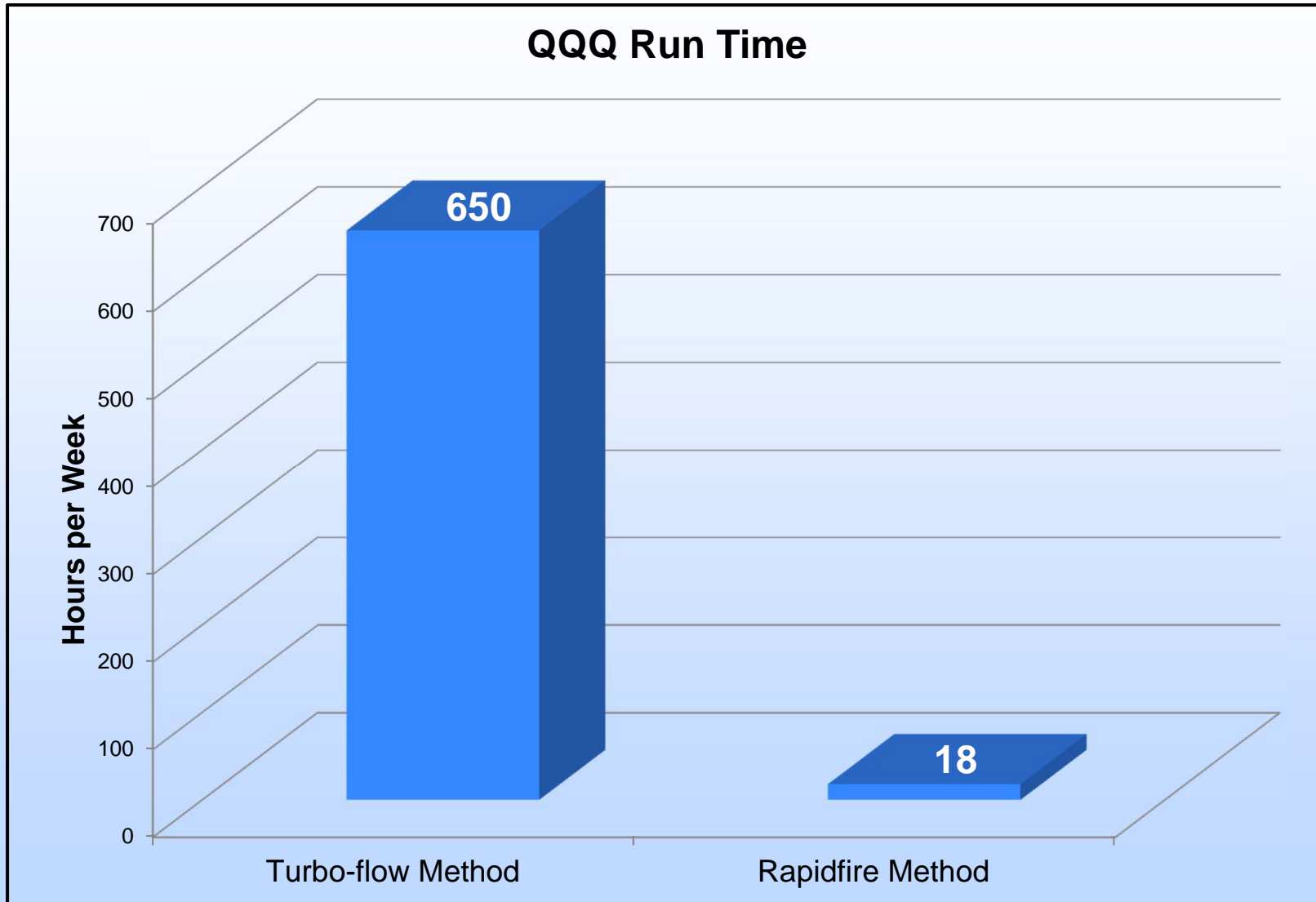
### OPIATES INCLUDE:

Hydromorphone  
Oxycodone  
Codeine  
Morphine  
6-MonoacetylMorphine

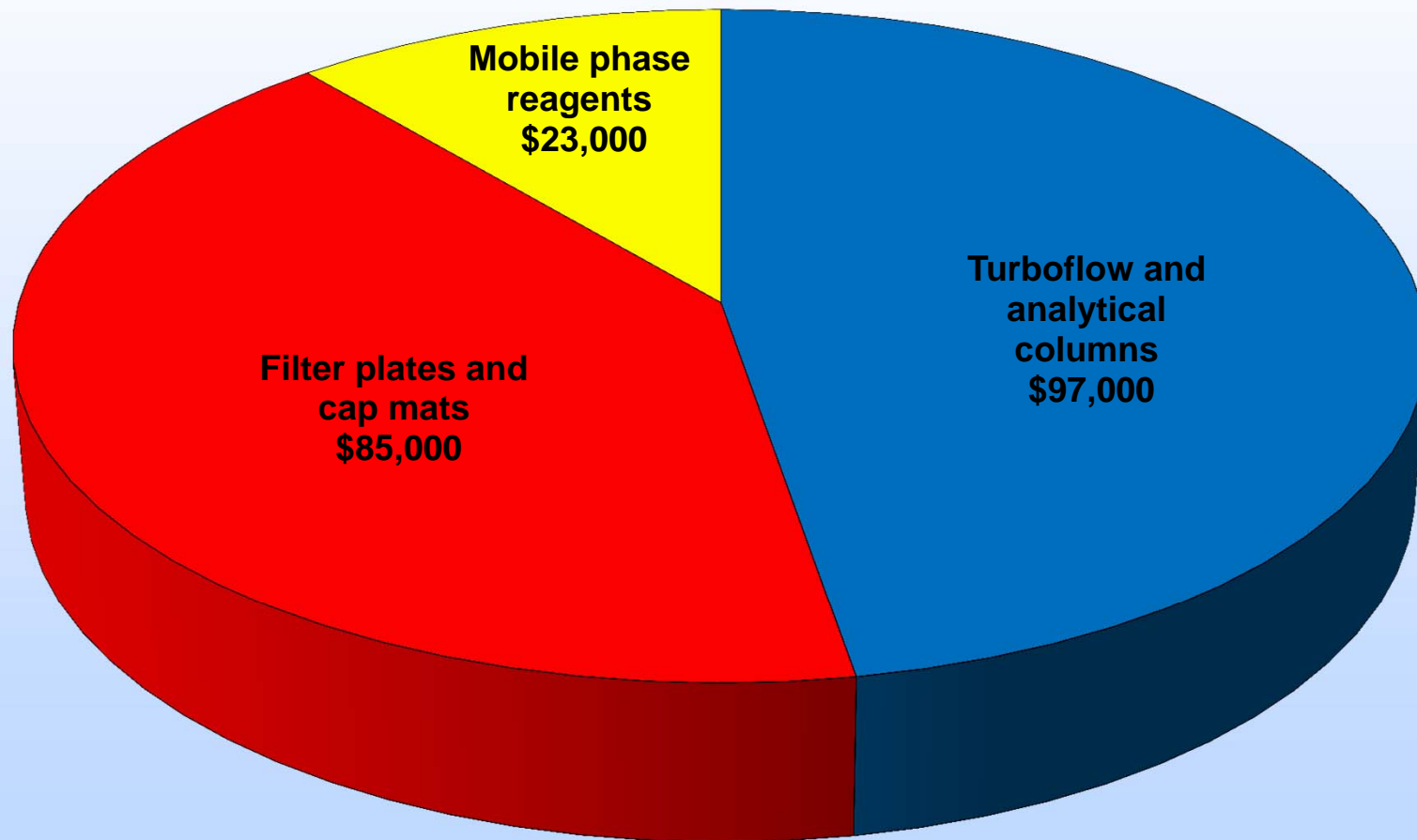
What benefits did we realize?



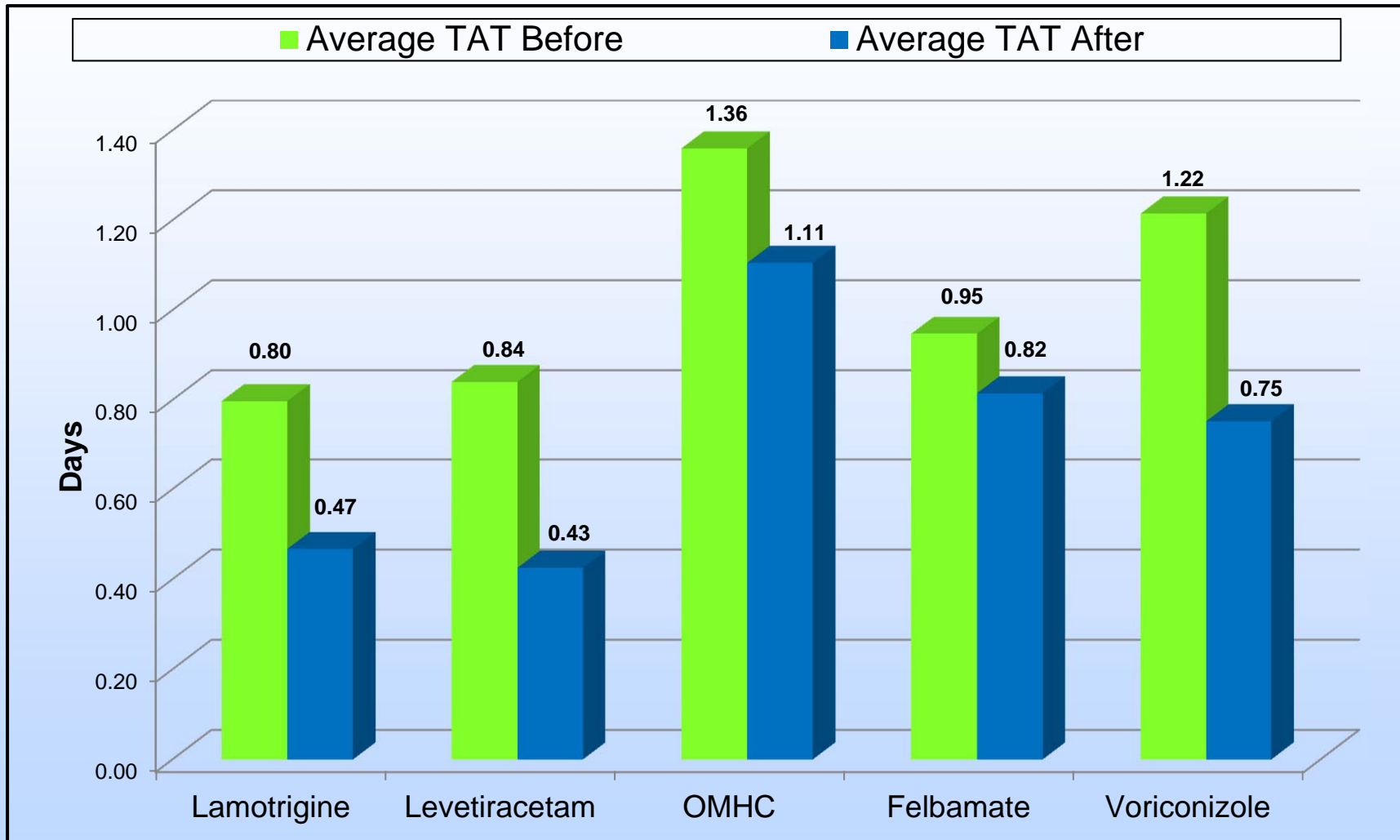
# Mass Spec Time Savings: 97%



# Estimated consumables annual cost savings: \$205,000



# Turn Around Time Comparison:



# Projects in Progress...

- Immunosuppressants:
  - Tacrolimus
  - Cyclosporin
  - Sirolimus
  - Everolimus
- Busulfan
- Methotrexate
- Lacosamide
- Pregabalin
- Rufinamide

# Special Thanks

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## Questions & Discussion