

High-Throughput SPE- MS/MS - A Viable Approach For Validated Bioanalytical Analysis?

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RapidFire & Integrated workflows

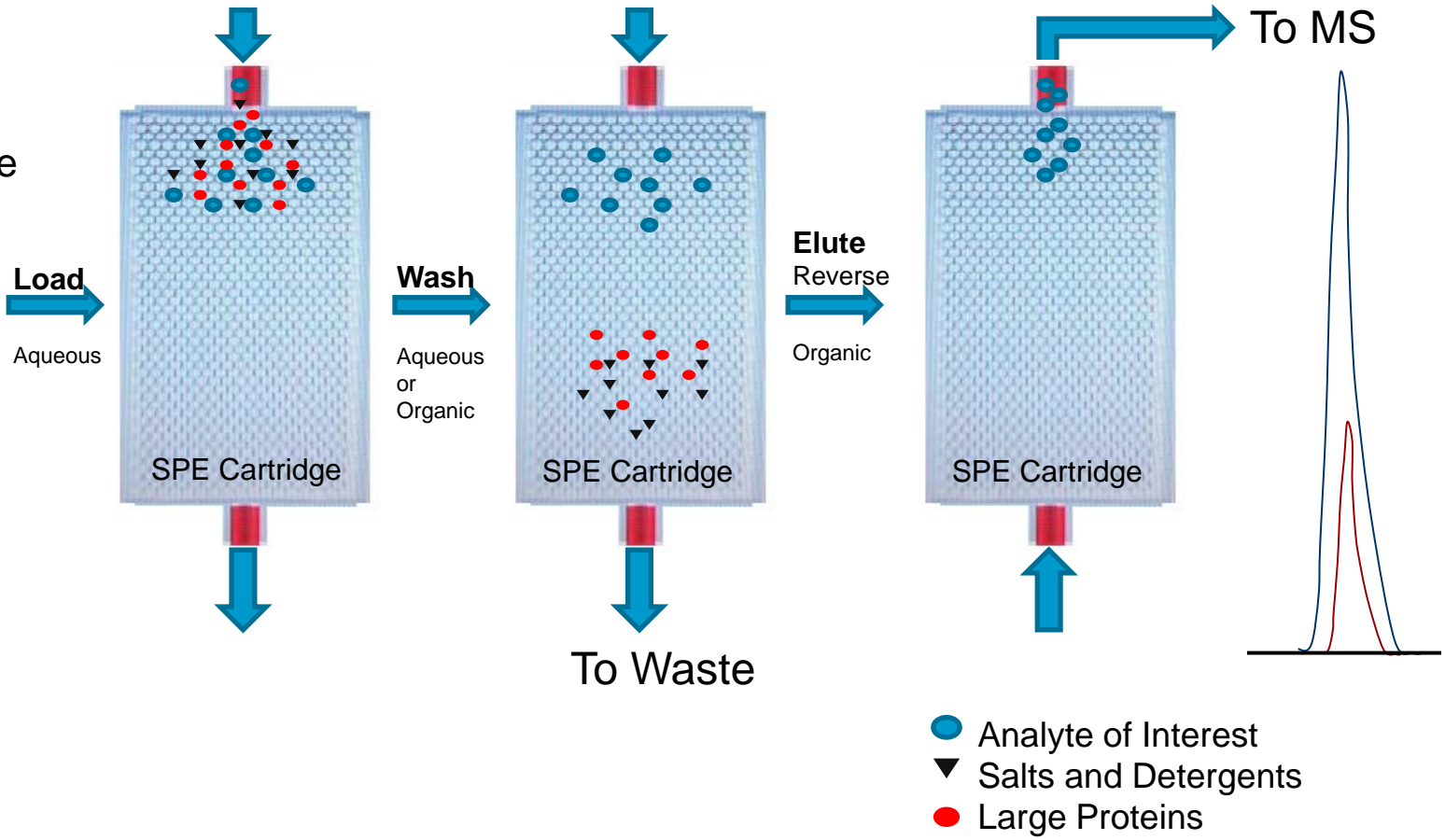
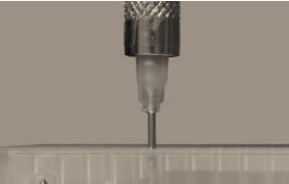
Topics

- SPE/MS/MS Overview
 - RapidFire/MS/MS
- Demonstration Data
 - Immunosuppressant panel
 - Methadone & EDDP
 - Benzodiazepine panel
 - Antiepileptic panel
- Clinical Lab Testimonial
- Summary
- Acknowledgements

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What is SPE/MS/MS?

Aspirate Sample



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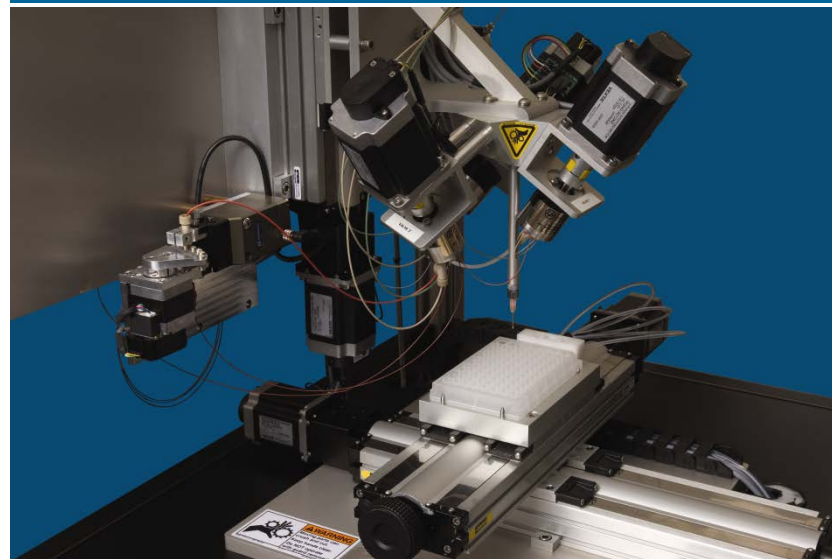
RapidFire Mass Spectrometry

Ultra-fast autosampler & online SPE system

- Orthogonal to HPLC/MS
- Reusable SPE cartridge
- Integrates with ESI and APCI MS instruments: QQQ or TOF
- Cycle time = 7-16 seconds/sample

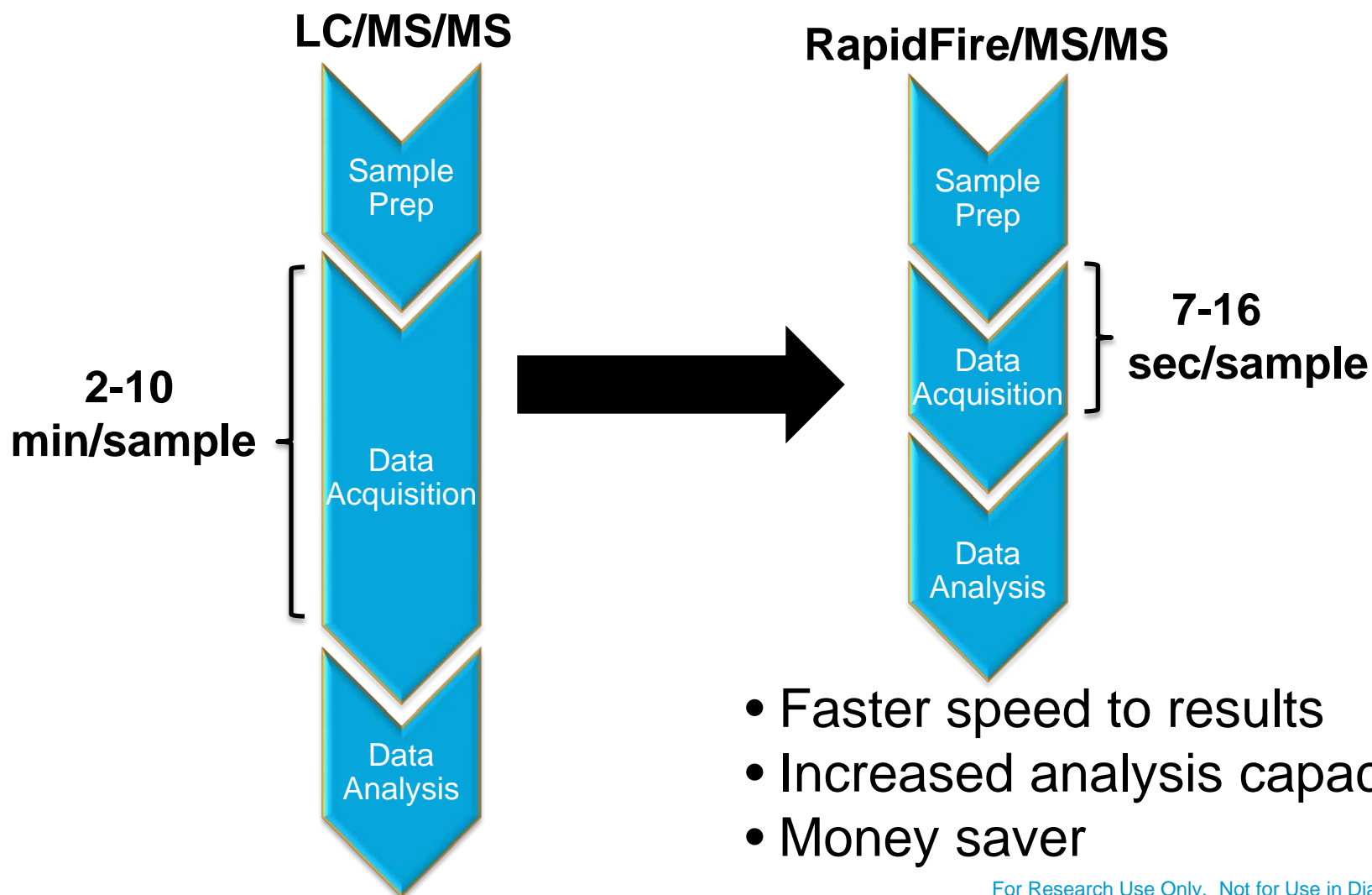
Compatible matrices

- Serum
- Plasma
- Whole blood
- Urine



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RapidFire/MS/MS Decreases Data Acquisition Time



- Faster speed to results
- Increased analysis capacity
- Money saver

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Immunosuppressant Panel (4 Analytes, 12 MRMs)

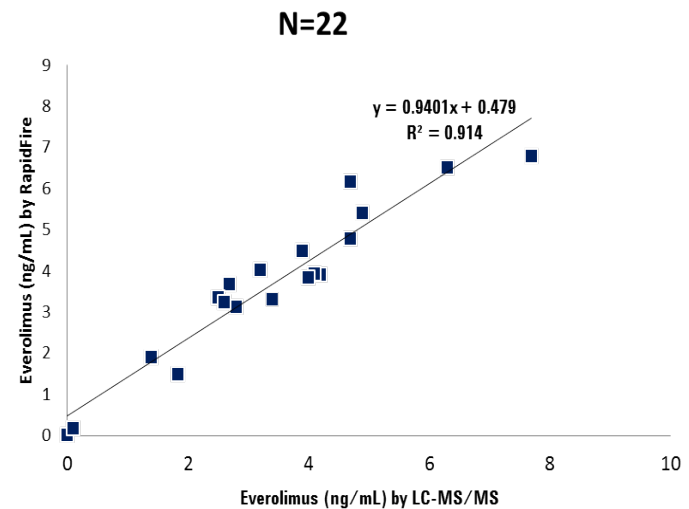
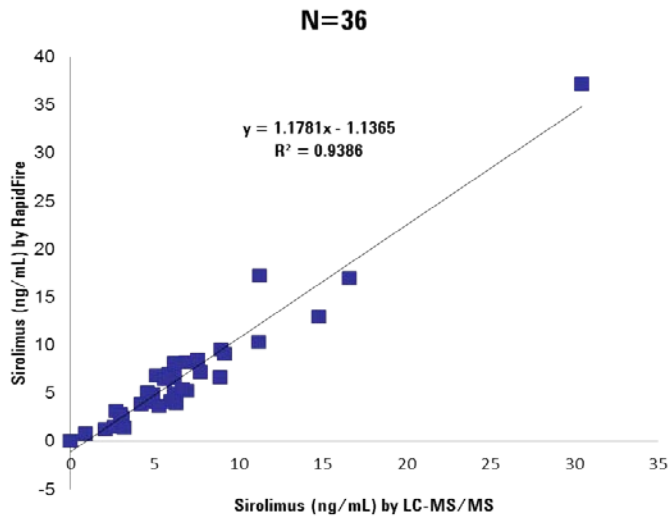
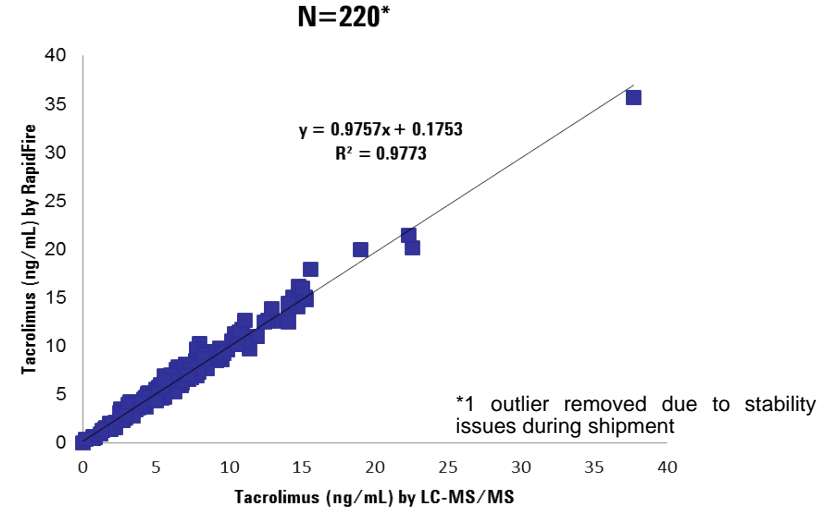
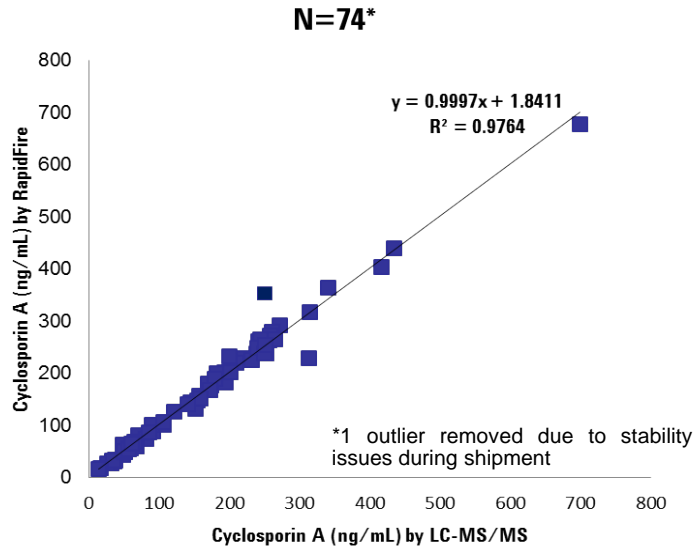
Compound	Q1	Q3	Dwell	Fragmentor	CE
Tacrolimus quant	821.9	768.5	10	145	17
Tacrolimus qual	821.9	786.5	10	145	13
Ascomycin	809.6	756.5	10	125	17
Everolimus quant	975.6	908.6	10	170	15
Everolimus qual	975.6	926.8	10	170	10
Everolimus-d4	979.6	912.7	10	180	10
Sirolimus quant	931.6	864.6	10	175	12
Sirolimus qual	931.6	882.2	10	175	8
Sirolimus-d3	934.6	864.5	10	180	18
Cyclosporin A quant	1219.8	1202.8	10	170	12
Cyclosporin A qual	1202.9	1184.8	10	200	30
Cyclosporin A-d4	1223.9	1206.9	10	170	12

RapidFire Conditions

- <13 seconds per sample
- C18 Cartridge
- Buffers:
 - A: 10mM ammonium acetate + 0.01% TFA + 0.09% Formic Acid
 - Wash Solvent: 50% MeOH
 - B: 10mM ammonium acetate in MeOH + 0.01% TFA + 0.09% Formic Acid

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Immunosuppressants – Blinded Human Samples



Data from collaboration with Pierre Wallemacq, UCL, Brussels

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Quantitative Analysis: Dilute & Shoot

Methadone & EDDP in Urine

- **Sample preparation**

- Dilute & Shoot (1:200 with H₂O:MeOH)

- **RapidFire analysis**

- RapidFire 300 + Agilent 6460 Triple quad

- Solvent A: Water + 0.01% TFA + 0.09% Formic Acid

- Solvent B and C: 50% Methanol : 50% IPA + 0.01% TFA + 0.09% Formic Acid

- Total sample cycle time = 11 sec

- C18 Cartridge

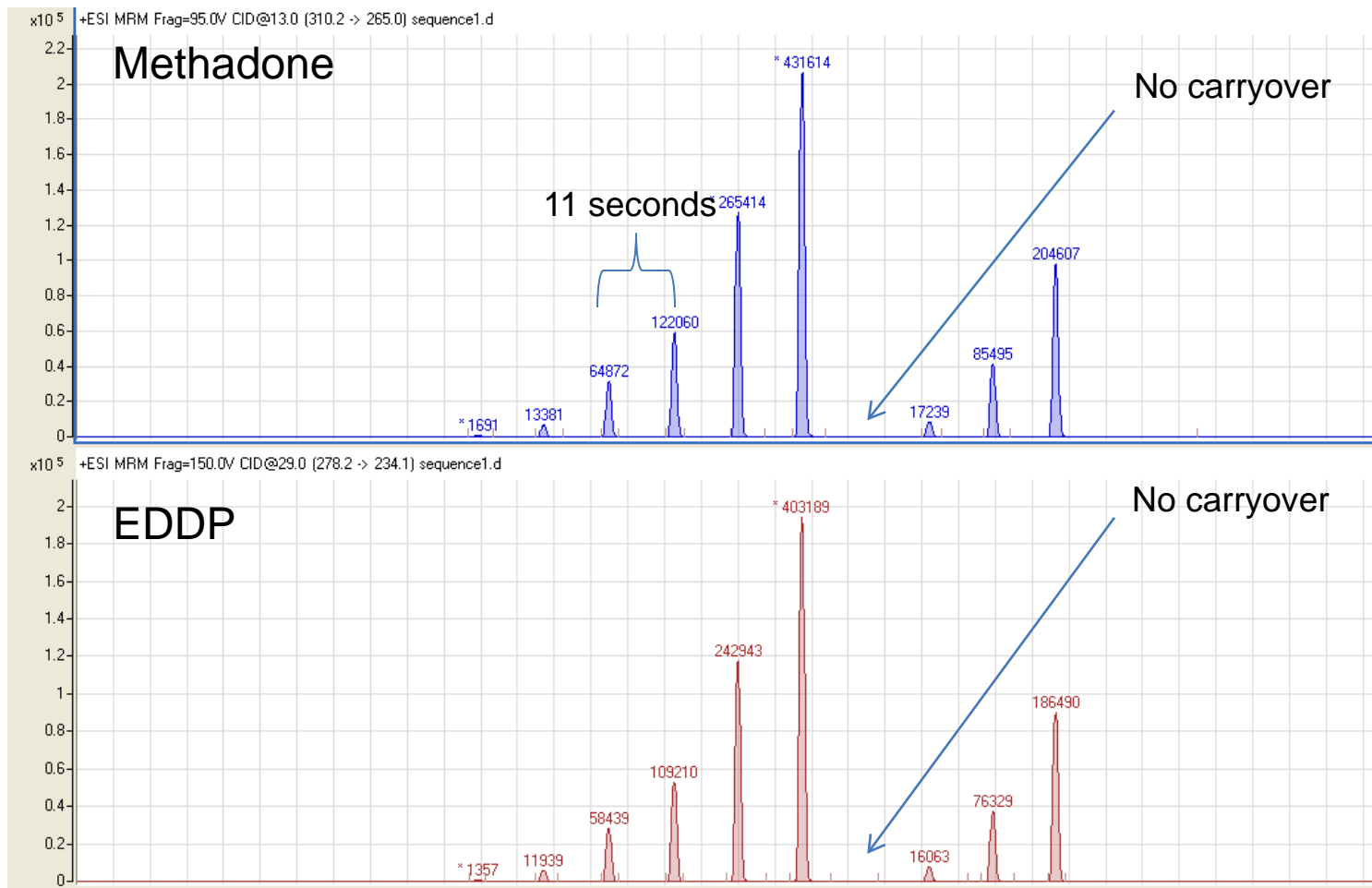
- LOQ = 100 ng/ml

Compound	Q1	Q3
Methadone_d3	313.2	268.3
Methadone Quant	310.2	105.1
Methadone Qual	310.2	265.0
EDDP-d3	281.2	234.1
EDDP Quant	278.2	234.1
EDDP Qual	278.2	219.1

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Calibrators & Carryover Assessment

Methadone & EDDP in Urine



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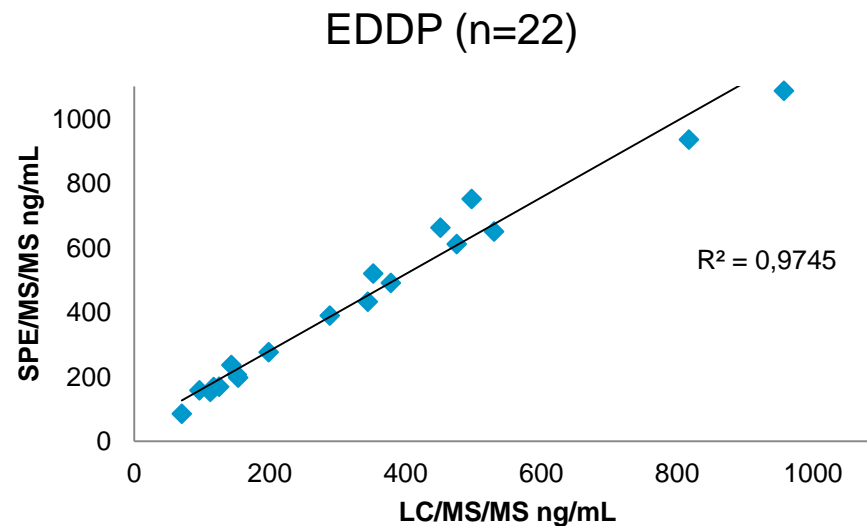
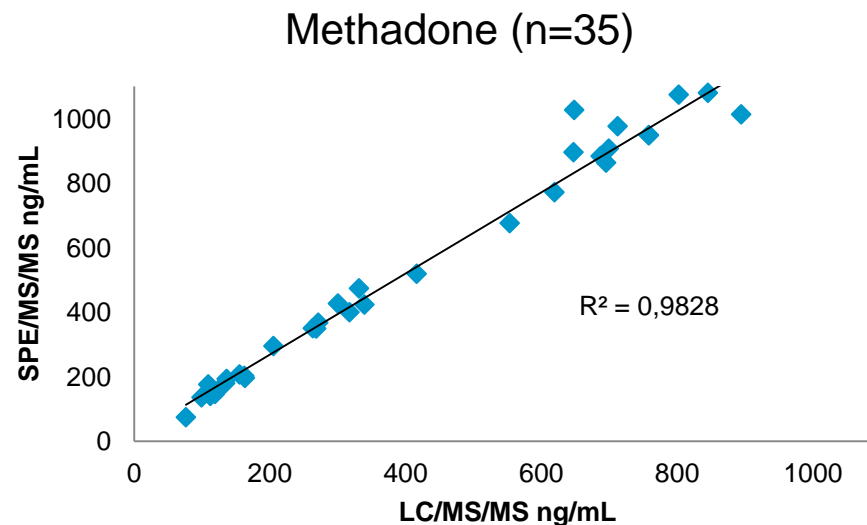
Correlation Analysis

LC/MS/MS vs. SPE/MS/MS

Results Summary

- 282 samples in total
- Methadone
 - 101 positive by LC/MS/MS
 - 101 positive by SPE/MS/MS
 - 0 false positives
- EDDP
 - 103 positive by LC/MS/MS
 - 103 positive by SPE/MS/MS
 - 0 false positives

Dominion Diagnostics: Source of blinded human samples and LC/MS/MS data



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Quantitative Analysis: Enzymatic Hydrolysis

Benzodiazepines in Urine (7 Analytes, 16 MRMs)

• Sample preparation

- Enzyme Hydrolysis → Dilute & shoot.
- 1:50 dilution (water)

• RapidFire analysis

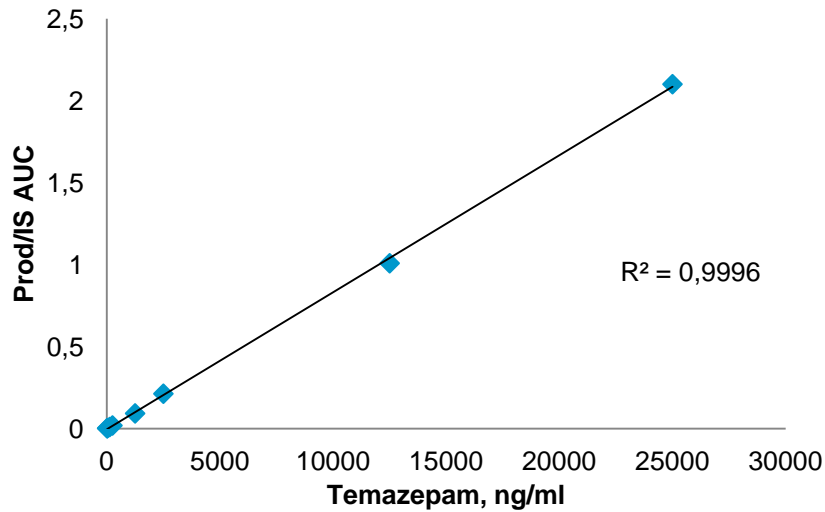
- RapidFire 300 + Agilent 6490 Triple quad
 - Solvent A: H₂O
 - Solvent B: 10% ACN
 - Solvent C: (1:1) MeOH: IPA + 0.1% Formic Acid
- C4
- Total sample cycle time = 13 sec
- LOQ = 50 ng/mL

Analyte	Q1	Q3
α - Hydroxy Alprazolam	325.1	297
	325.1	215.9
Lorazepam	321	275
	321	229
Alprazolam	309.1	281
	309.1	205
Temazepam	301.1	255.1
	301.1	177
Oxazepam	287.1	241
	287.1	104
7-Amino Clonazepam	286.1	222.1
	286.1	121.1
Nordiazepam	271.1	165
	271.1	140
d5 – Nordiazepam	276.1	165.1
	276.1	140.1

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Representative Data

Temazepam in Urine



Calibration Curve

Temazepam ng/ml	Intraday % Accuracy	Intraday % Precision	Interday % Accuracy	Interday % Precision
50	104.09	4.55	105.37	3.82
250	96.18	4.44	93.71	4.36
500	96.66	6.35	100.14	6.00
2500	101.35	4.60	102.81	6.70
6250	102.75	0.79	99.40	4.24
12500	100.49	2.52	101.66	3.75

Accuracy & Precision Results

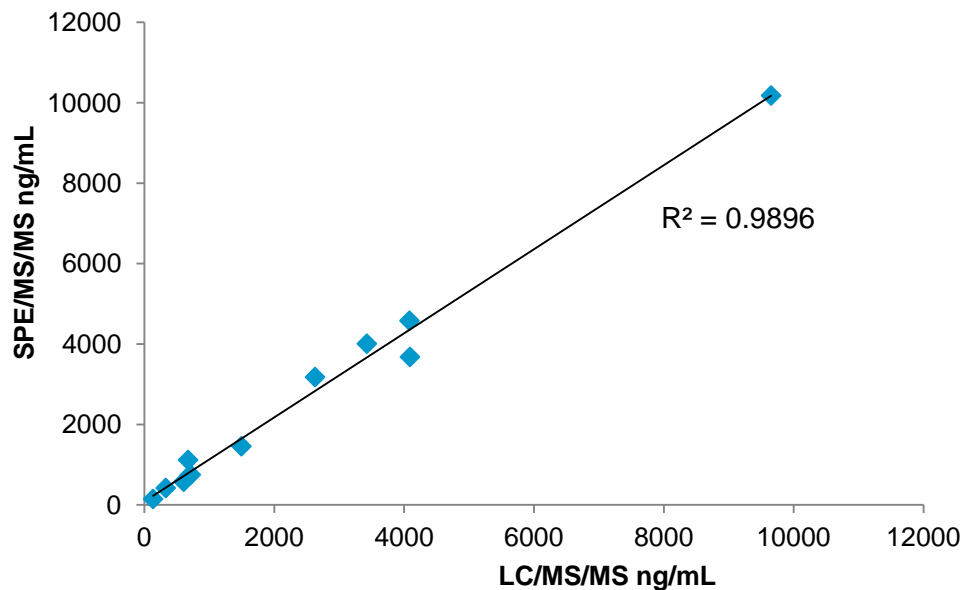
N = 4

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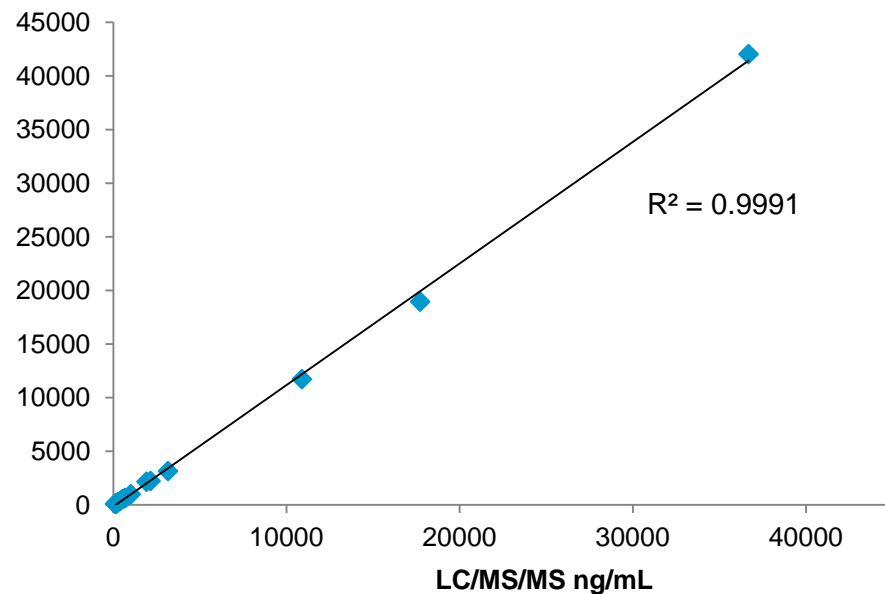
Correlation Analysis

LC/MS/MS vs. SPE/MS/MS

Oxazepam (n=12)



Temazepam (n=16)



*Dominion Diagnostics:
Source of blinded human samples*

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Antiepileptic Panel in Serum (6 Analytes, 15 MRMs)

• Sample preparation

- 1:2 crash with MeCN then centrifuge
- 1:50 dilution in H₂O

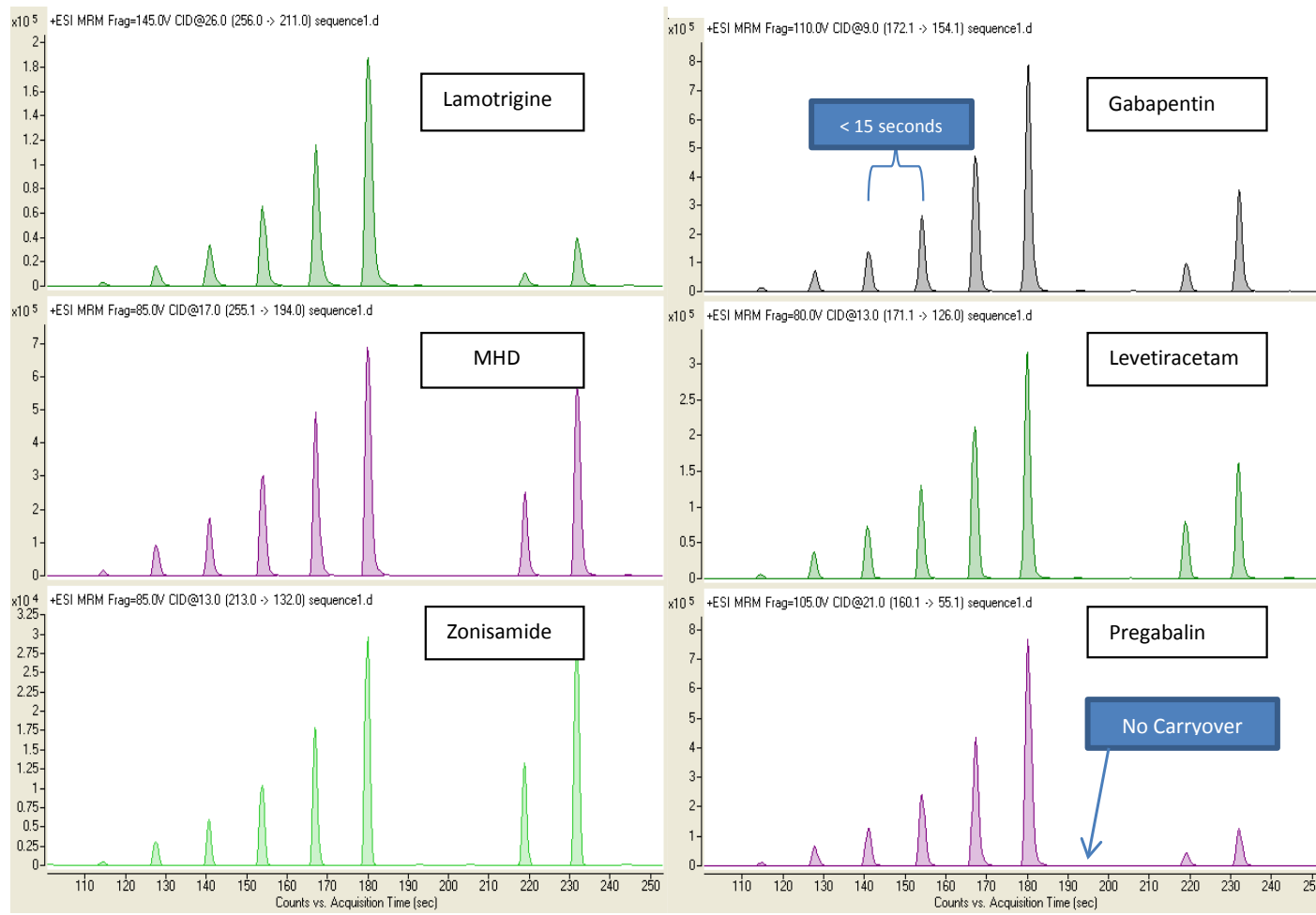
• RapidFire analysis

- RapidFire 300 + Agilent 6460 Triple quad
 - Solvent A: 0.1% Formic acid in H₂O
 - Solvent B/C: (1:1) MeOH: IPA + 0.1% Formic Acid
- C18
- Total sample cycle time = 14 sec
- LOQ = 1 mcg/ml

Compound	Q1	Q3
Lamotrigine Quant	256	211
Lamotrigine Qual	256	108.9
MHD Quant	255.1	194
MHD Qual	255.1	165
Zonisamide Quant	213	132
Zonisamide Qual	213	51.1
Gabapentin-d10	182.2	164.1
Gabapentin Quant	172.1	154.1
Gabapentin Qual	172.1	137
Levetiracetam-d6	177.1	132
Levetiracetam Quant	171.1	126
Levetiracetam Qual	171.1	69
Pregabalin-d6	166.2	148.1
Pregabalin Quant	160.1	55.1
Pregabalin Qual	160.1	83

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Anti-Epileptic Panel Standard Curves and QCs

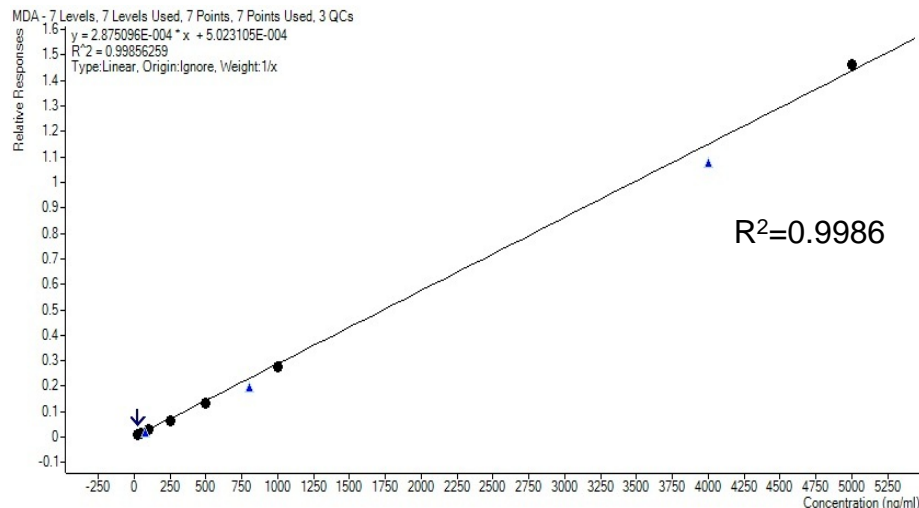


- Simultaneous analysis
- No carry over for any analytes

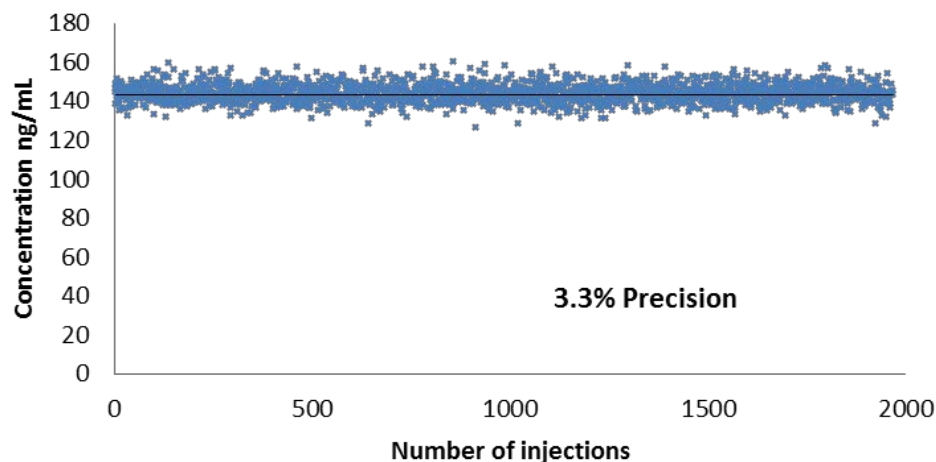
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Representative Calibration Curve & Robustness

MDMA in Urine



MDMA Calibration Curve



MDMA Robustness Experiment

- 2000 sequential injections
- Same SPE cartridge & system

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

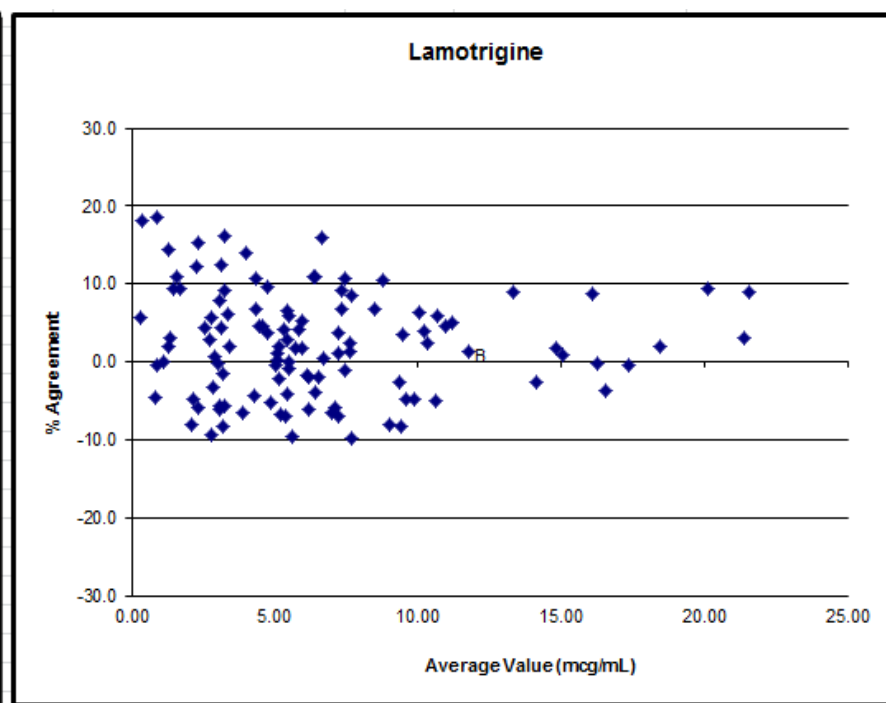
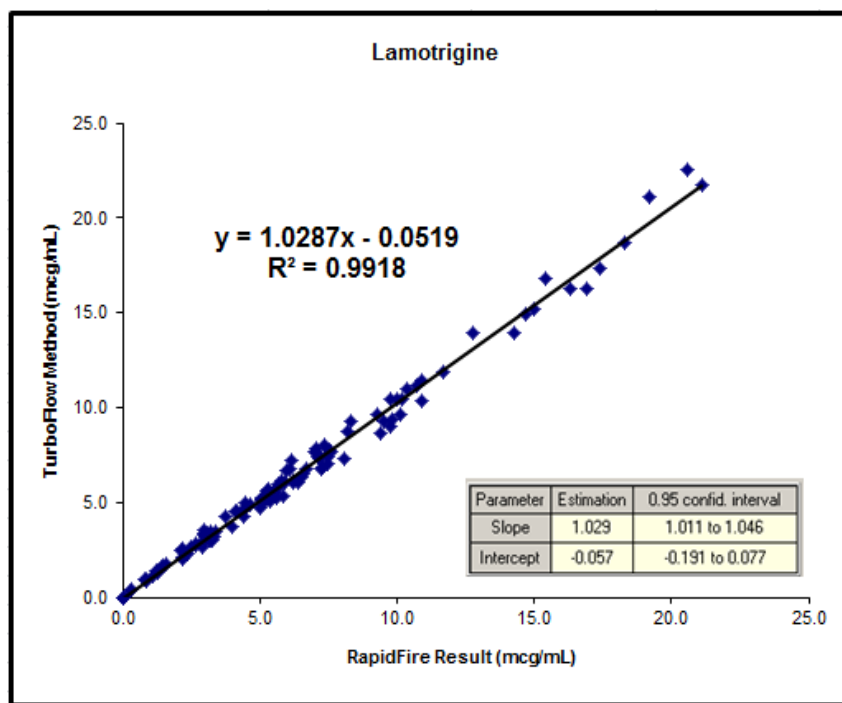
Extraction Method

- 50 μL serum
- Crash with 200 μL acetonitrile containing deuterium labeled IS
- Vortex/Centrifuge
- Remove 30 μL of supernatant and dilute with 600 μL of mobile phase A.
- 96 well plate format

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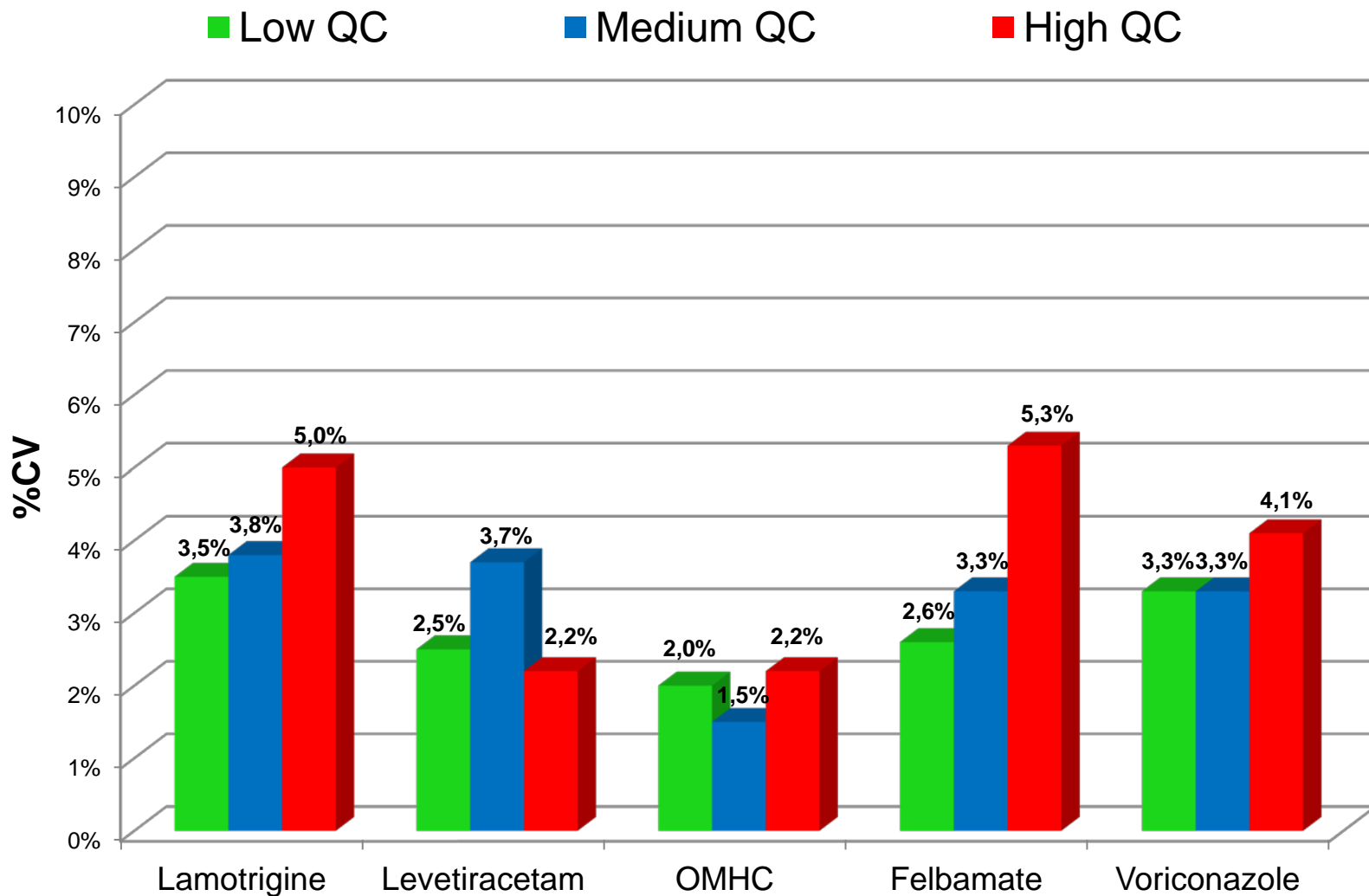
Lamotrigine (n=100)

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



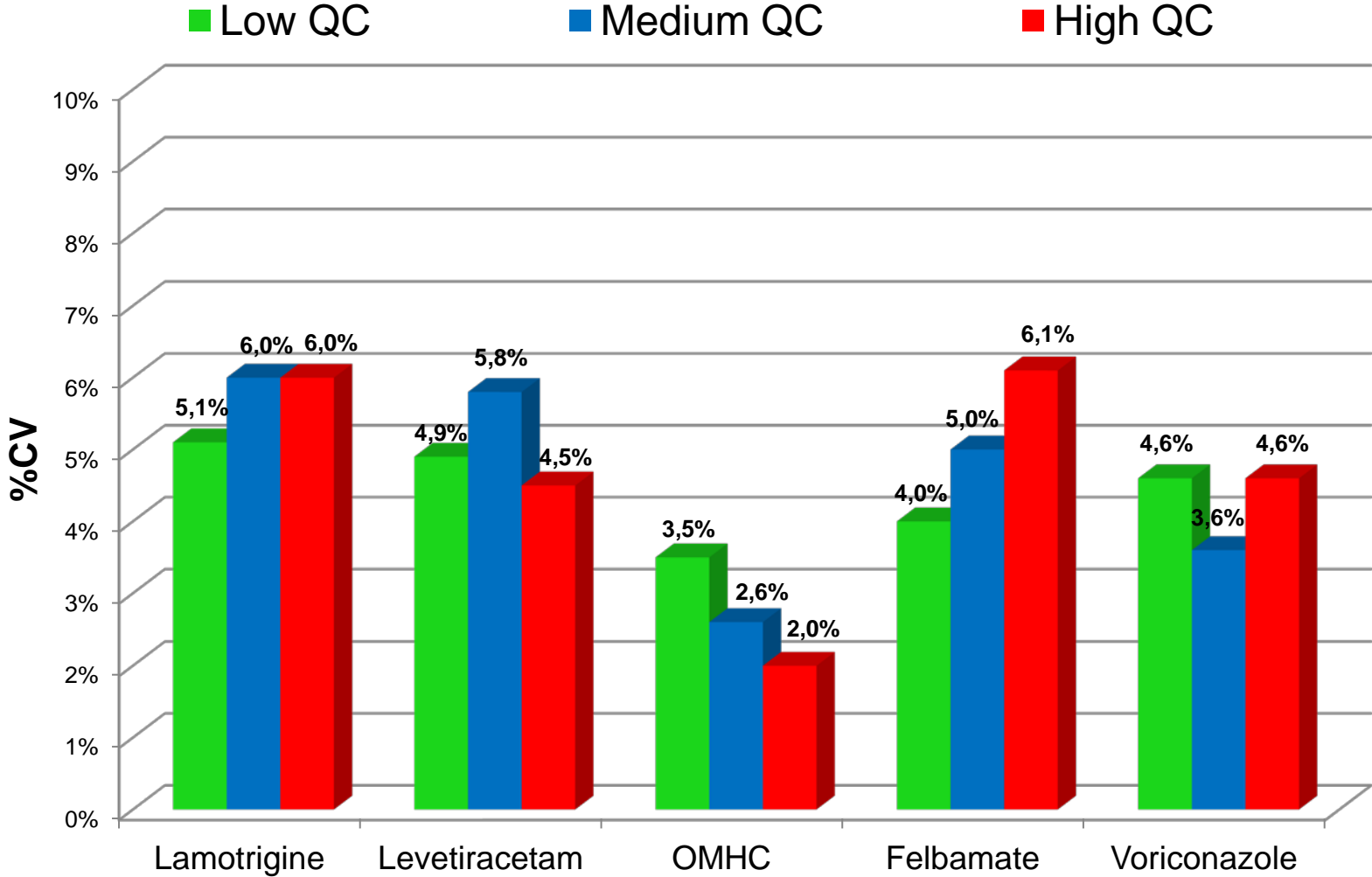
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Within Run Precision (n=20)



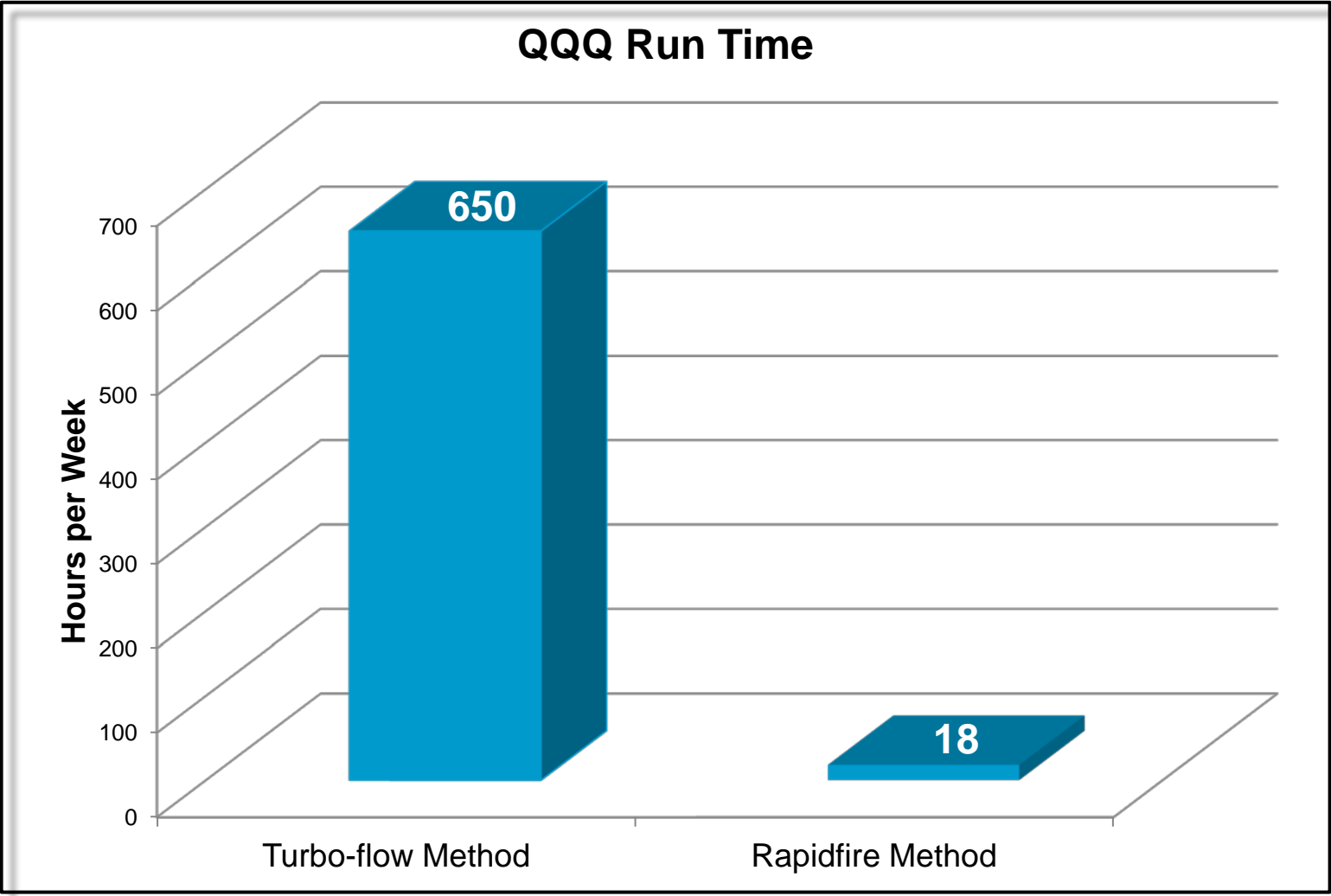
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Between Run Precision (n=20)



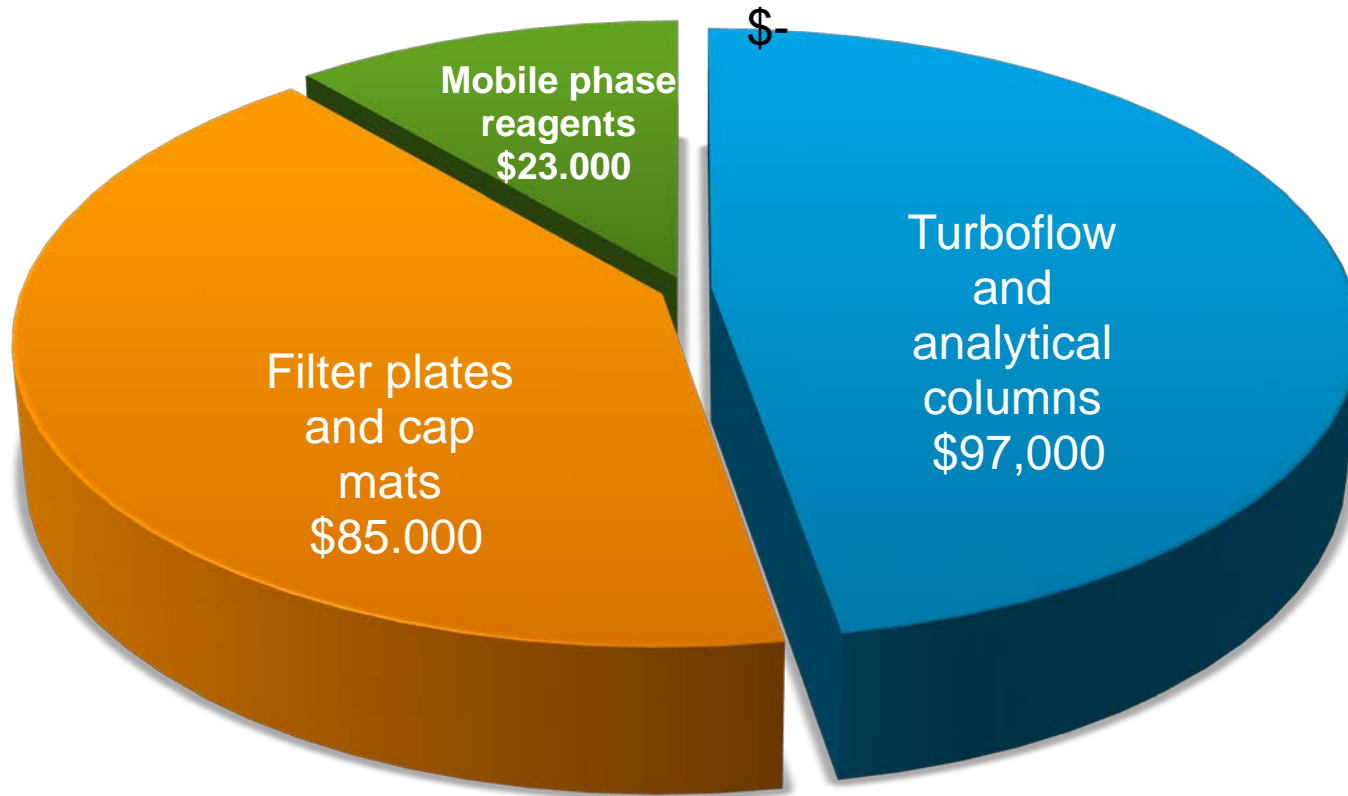
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Mass Spec Time Savings: 97%



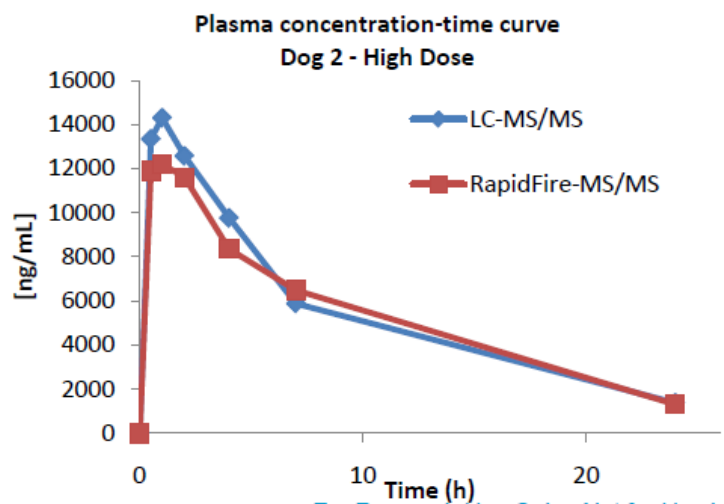
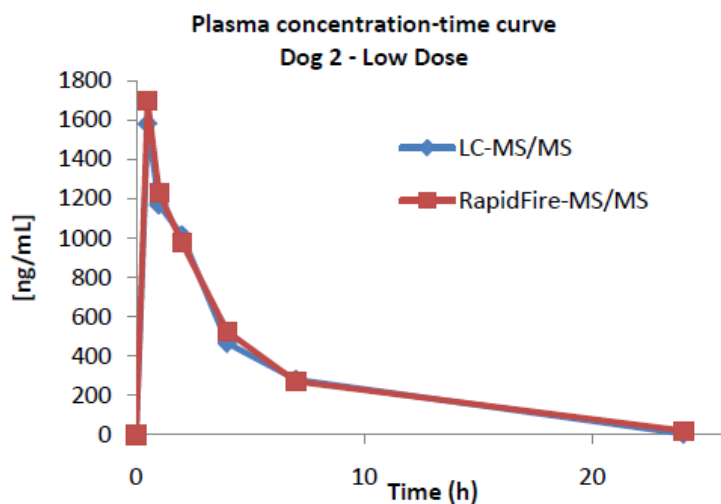
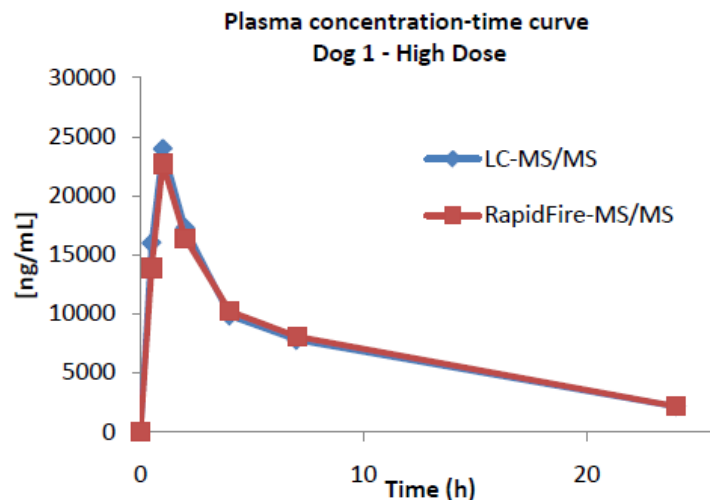
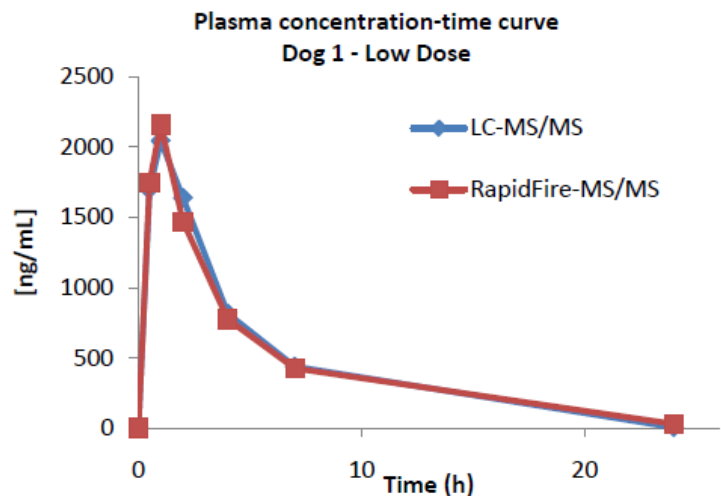
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Estimated consumables annual cost savings: \$205,000



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Proprietary J&J Compound # 1 (incurred dog plasma samples)



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Summary

SPE/MS/MS results are comparable to LC/MS/MS for many applications

- Linearity
- Accuracy & precision
- Reproducibility

Advantages of RapidFire/MS/MS

- Speed to results
 - 7 -16s/sample
- Compatible with standard laboratory workflow
 - Standard multiwell plates & ESI MS
- Capable of ultrafast MS-based screening

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Acknowledgements

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Questions?

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