



Ultrafast SPE/MS Analysis of TCAs in Serum

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RapidFire Applications
Manager

Today's Agenda

RapidFire/MS features and benefits

Demonstration data

- Tricyclic antidepressants in serum

Summary

Follow-up information

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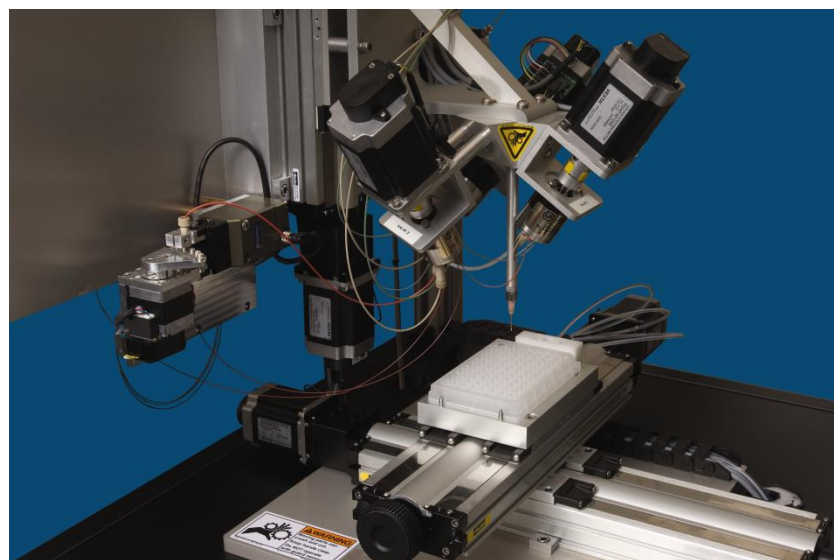
What is RapidFire/MS?

Ultrafast autosampler & online SPE system

- Replaces LC in LC/MS
- Reusable SPE cartridge
- Integrates with standard ESI MS instruments (QQQ & TOF)
- **Cycle time = 8-15 s/sample**

Compatible with biological matrices

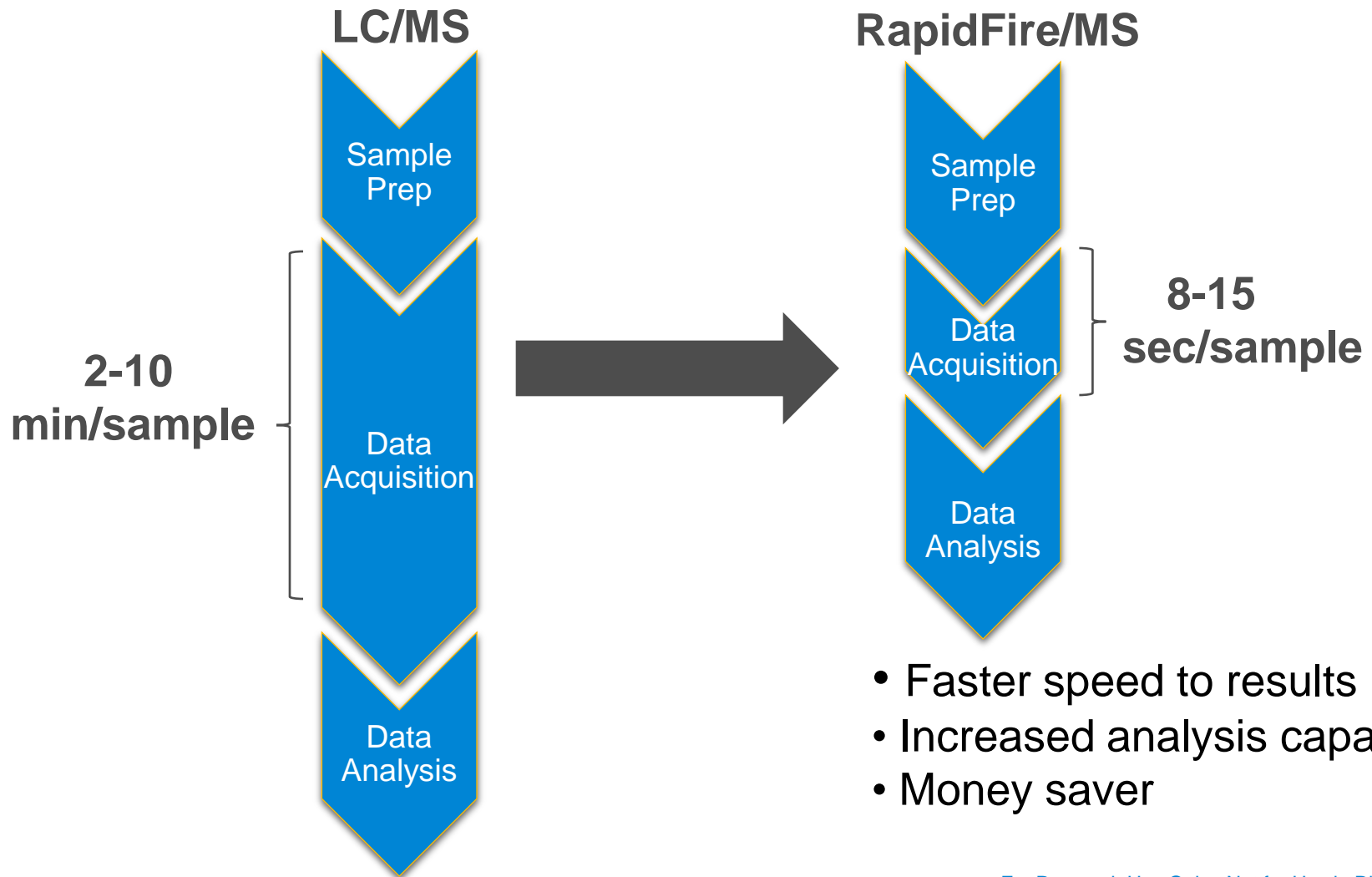
- Microsomal incubations
- Cell culture media
- Serum, plasma or whole blood
- Urine



[Agilent Web Link](#)

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RapidFire Decreases Data Acquisition Time



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

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Demonstration Data

Tricyclic Antidepressant (TCA) Drug Panel in Serum

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Tricyclic Antidepressant Drug Panel in Serum

8 Analytes, 20 MRMs

Sample preparation

- MeOH/ZnSO₄ crash
- 1:10 dilution (water)

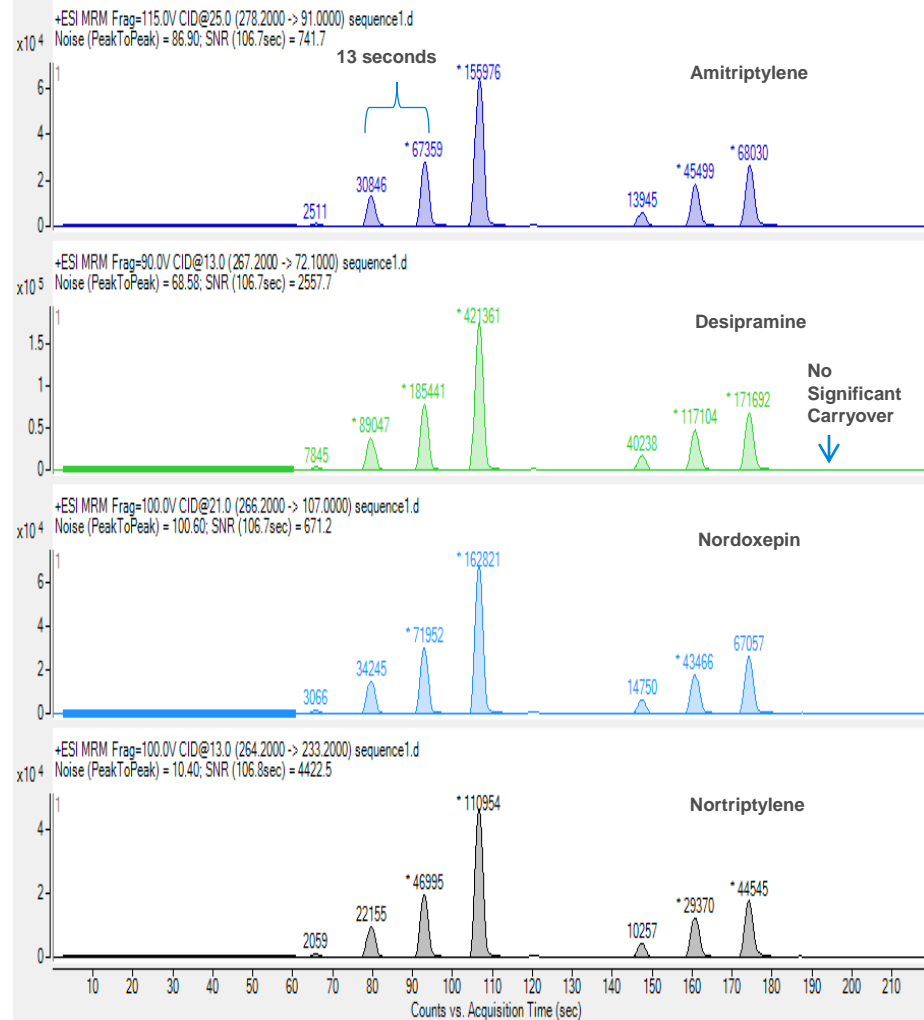
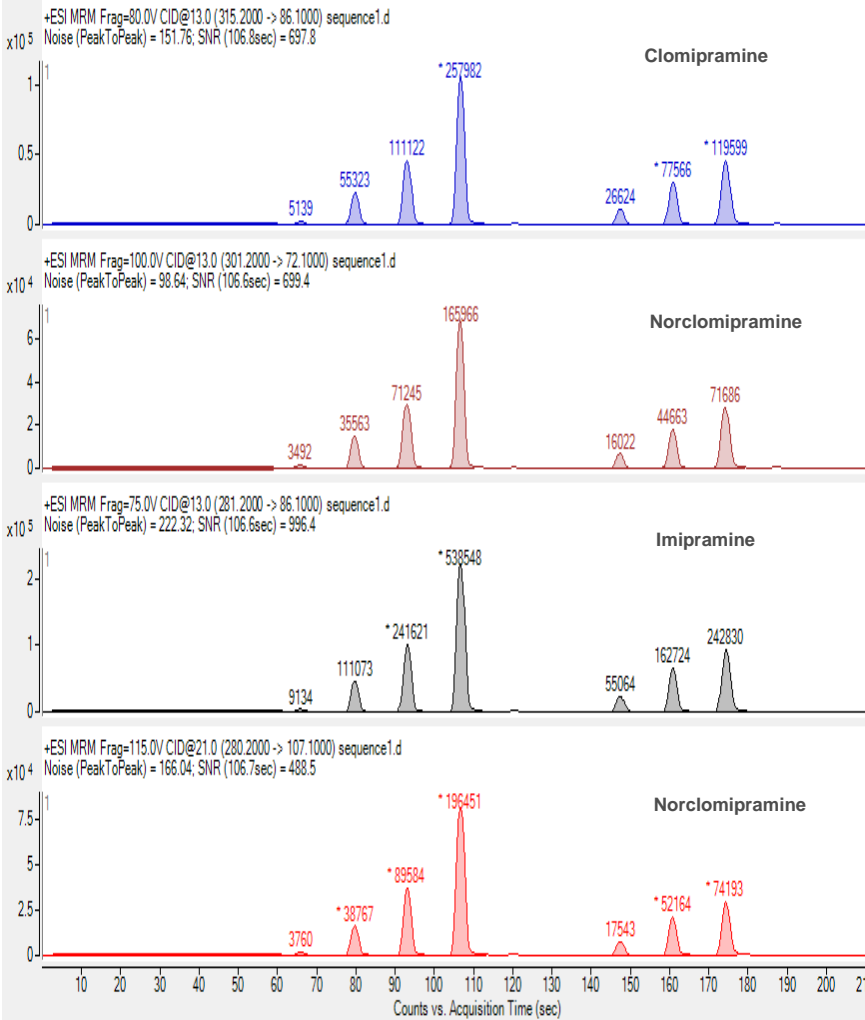
RapidFire analysis

- RapidFire 300 + Agilent 6460 Triple quad
 - Solvent A: 0.1% formic acid in water; 1.5 mL/min
- Solvent B + C: 0.1 % formic acid in methanol;
1.25 and 0.8 mL/min
- C18
- Total sample cycle time = 13 sec
- LOQ = 10 ng/ml

Compound Name	Precursor Ion	Product Ion
Clomipramine_d3	318.2	89.1
Clomipramine Q	315.2	86.1
Clomipramine	315.2	58.1
Norclomipramine Q	301.2	72.1
Norclomipramine	301.2	44.1
Imipramine-d3	284.2	89.1
Doxepin_d3	283.2	107.1
Amitriptylene-d3	281.2	91.1
Imipramine Q	281.2	86.1
Imipramine	281.2	58.1
Doxepin	280.2	115
Doxepin Q	280.2	107.1
Amitriptylene	278.2	117.1
Amitriptylene Q	278.2	91
Desipramine Q	267.2	72.1
Desipramine	267.2	44.1
Nordoxipin	266.2	235.1
Nordoxipin Q	266.2	107
Nortriptylene Q	264.2	233.2
Nortriptylene	264.2	91.1

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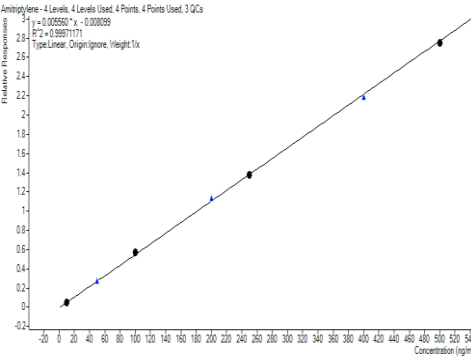
Analysis Speed and Carryover



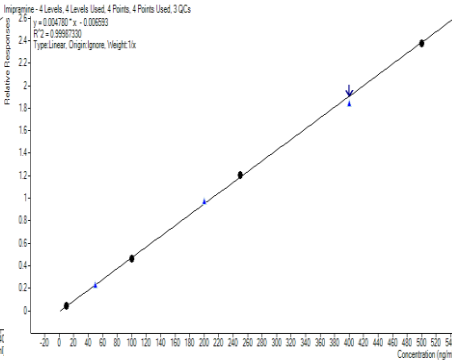
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Calibration Curves

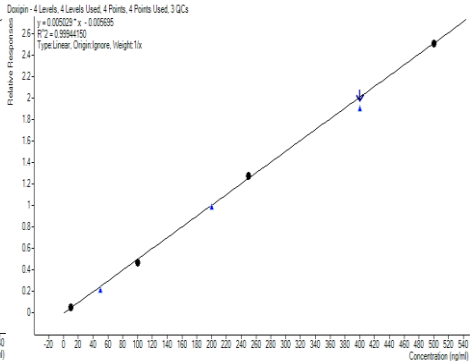
Linear Range from 10-500 ng/mL



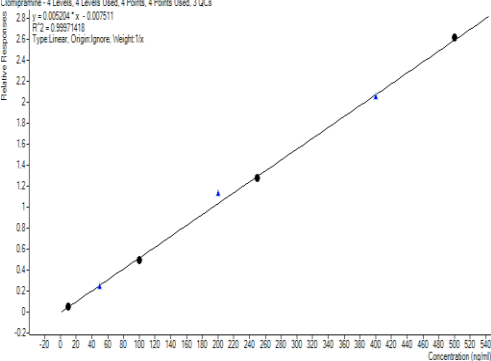
Amitriptylene



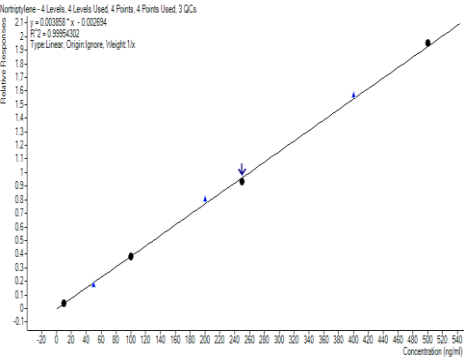
Imipramine



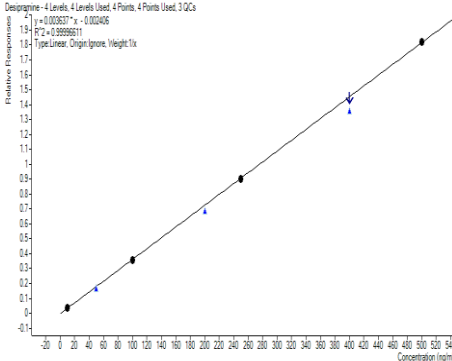
Doxepin



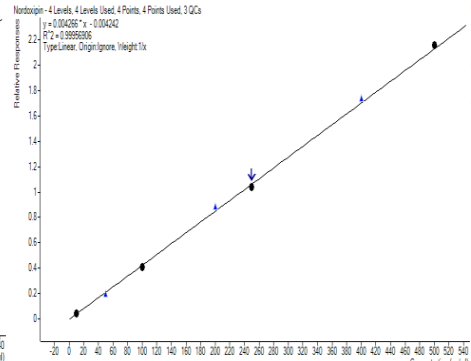
Clomipramine



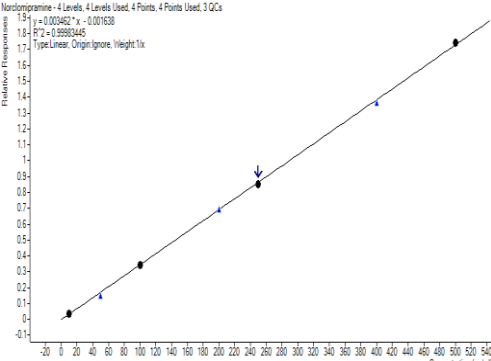
Nortriptylene



Desipramine



Nordoxepin



Norclomipramine

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Inter and Intraday Accuracy & Precision

Amitriptylene (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	99.1	3.3	98.7	5.4
200	107.6	1.5	104.8	2.2
400	96.9	2.9	102.1	1.9
Nortriptylene (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	97.3	2.3	97.3	2.7
200	105.9	3.9	101.9	0.4
400	96.2	2.9	98.7	2.8
Imipramine (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	103.7	2.9	102.4	2.6
200	103.9	0.8	98.9	2.2
400	94.6	2.3	98.5	3.9
Desipramine (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	98.1	1.6	97.3	2.3
200	97.6	1.4	96.6	0.8
400	91.8	1.5	92.9	1.5

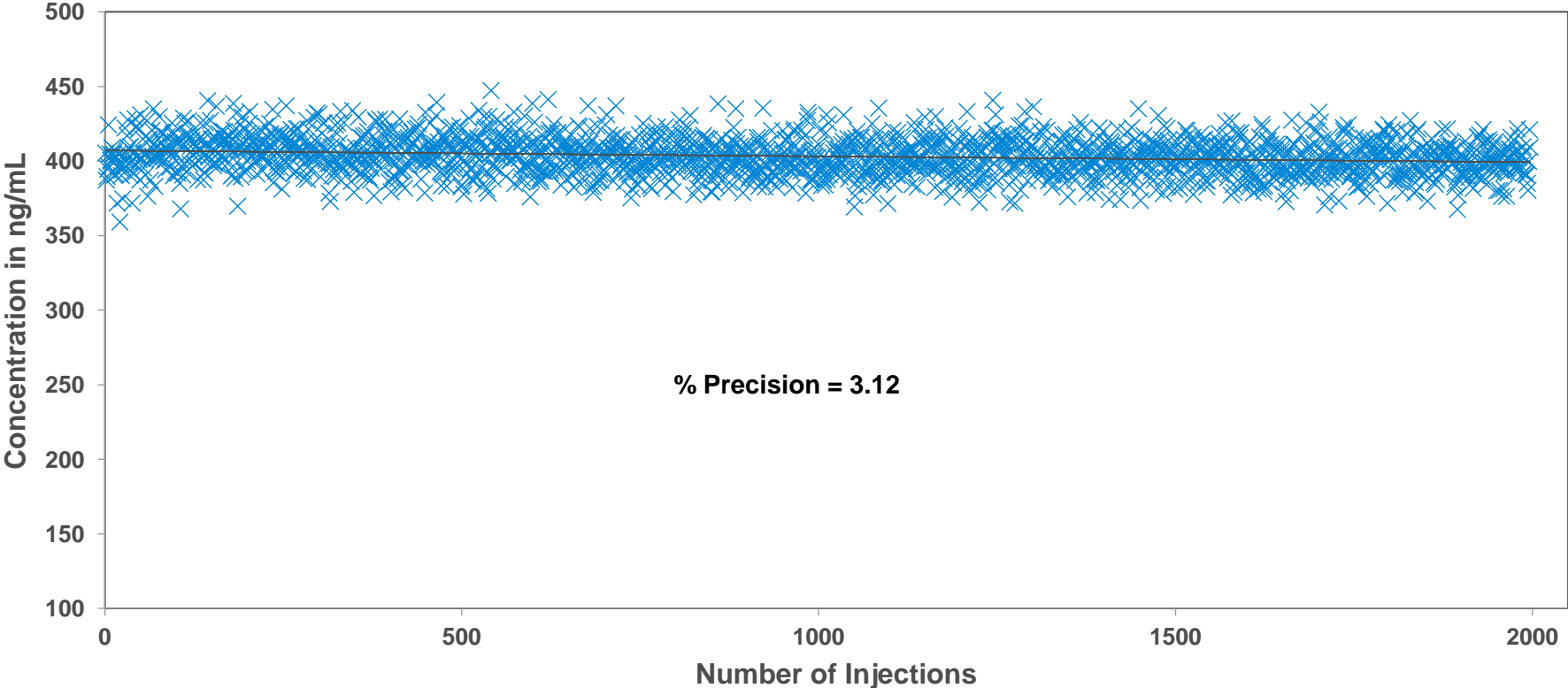
Doxepin (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	92.2	2.0	99.8	3.3
200	102.6	2.2	100.4	1.5
400	97.2	2.3	96.4	1.5
Nordoxepin (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	97.4	4.8	99.4	4.9
200	102.9	2.9	103.1	1.7
400	97.6	1.3	101.7	1.4
Clomipramine (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	97.6	2.6	97.0	3.6
200	107.2	2.5	102.7	4.8
400	104.2	1.4	100.9	5.0
Norclomipramine (ng/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
50	91.5	1.5	97.8	4.0
200	98.1	1.6	99.9	2.4
400	97.5	1.7	97.3	3.5

- Utak Laboratories QC standards
- Coefficient of variation values were all < 8%

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Reproducibility Study

Norclomipramine



2000 injections of the same sample showing robustness of the RapidFire system, SPE cartridge lifetime and consistency in area counts and quantitation for the drugs in the panel.

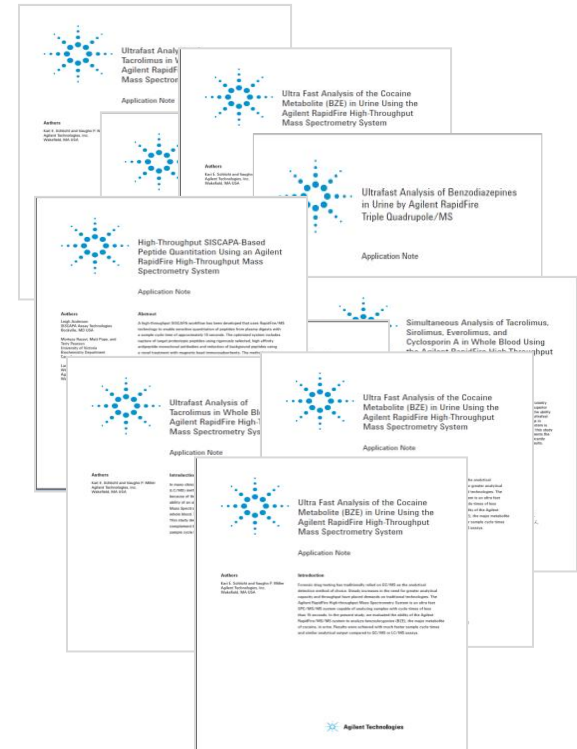
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RapidFire/MS Clinical Research Methods

Serum or whole blood matrix, <16 seconds/sample

Quantitative Analysis

- Antiepileptic panel
- Tricyclic antidepressant panel
- SSRI panel
- Clozapine/norclozapine
- Antifungal panel
- SISCAPA peptide analysis
- AssayMAP protein analysis



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RapidFire/MS Forensic Toxicology Methods

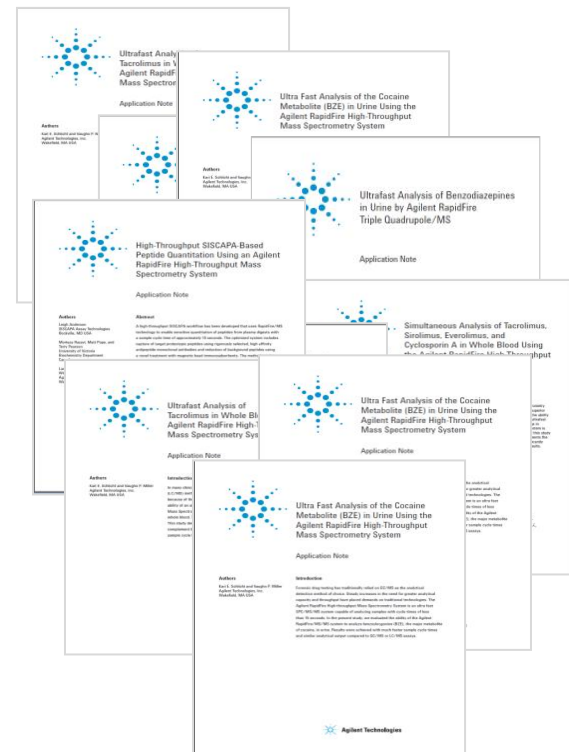
Urine matrix, <16 seconds/sample

Quantitative Analysis

- Methadone & EDDP
- Benzodiazepine panel
- THCCOOH
- BZE
- Z-drugs panel
- Buprenorphine & Norbuprenorphine
- Gabapentin & Pregabalin
- Cotinine in urine or serum

Qualitative Analysis

- Amphetamine panel
- Bath salts panel
- Synthetic cannabinoids panel
- Barbiturates panel
- TOF panels – coming soon



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The Value of RapidFire/MS

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

- Linearity
- Accuracy & precision
- Reproducibility

Advantages of RapidFire/MS

- **Fastest Time to Result**
 - < 20 seconds per sample
 - > 250 samples/hour
 - > 5,000 samples/day
- **Increased Capacity**
 - Able to analyze 1000's of samples per day on a single system
- **Cost efficient**
 - Lower operating cost and smaller lab footprint than multiple MS systems
 - Least expensive way of analyzing hundreds of samples (or more) per day
 - Direct cost < \$0.1/sample
- **Excellent tool for development of new drug analyte panels**
 - More sensitivity and specificity without the interference found in traditional methods
 - Ability to create new panels quickly (i.e. designer drugs)

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Questions?

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Agilent Web Site

- RapidFire 365 home page ([link](#))
- RapidFire clinical research video ([link](#))
- Productivity calculator ([link](#))
 - Compare LC/MS to RapidFire/MS: capital cost & turn around time
- Brochure
 - Pharma discovery focused, but specs of instrument ([link](#))

Review article: SPE/MS technology

- Bioanalysis, 2012
 - [SPE-MS analysis of absorption, distribution, metabolism and excretion assays: a tool to increase throughput and streamline workflow](#)



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