High-Throughput Analysis of Levetiracetam in Serum Using Ultra-fast SPE-MS/MS

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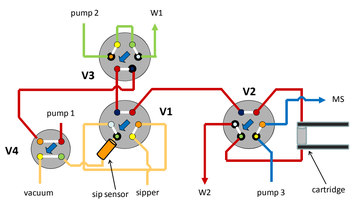
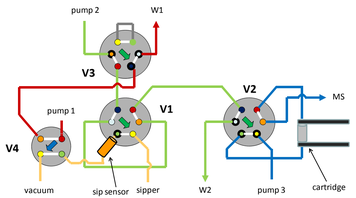
Introduction

Mass spectrometry-based assays have emerged as a viable analytical method due to their sensitivity, specificity, and robustness. We evaluated the ability of an ultra-fast SPE-MS system (Agilent RapidFire High-throughput Mass Spectrometry System) to analyze levetiracetam in human serum with much faster sample cycle times and similar analytical results compared to HPLC or LC/MS/MS assays.

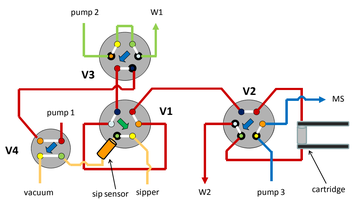
States 1&4: Aspirate & Re-equlibrate



State 3: Elute



State 2: Load/Wash



RapidFire Conditions		
Cycle durations (ms)	State #1 aspirate	600
	State #2 load/wash	3500
	State # 3 elute	3000
	State # 4 re-equilibrate	500
Solvents	Solvent A: water + 10mM ammonium acetate + 0.1% formic acid	
	Solvenet B: methanol + 0.1% formic acid	
Column	C18	

Experimental

MS methods for levetiracetam and its internal standard (d3-levetiracetam) were optimized on an Agilent 6460 triple quadrupole mass spectrometer for analysis. Calibration standards were prepared by spiking human serum with levetiracetam to final concentrations ranging from 1ug/ml to 100ug/ml. Commercially available quality control standards made in human serum were also analyzed. The serum samples were precipitated with acetonitrile containing d3-levetiracetam. The precipitated samples were centrifuged, subsequently, the supernatant was removed and transferred to a 96-well plate for analysis. Sample analysis was performed at a rate of <10 seconds per sample using a RapidFire 300 system coupled to an Agilent 6460 triple quadrupole mass spectrometer with an Agilent JetStream source. Data analysis was performed using RapidFire Integrator software. This methodology is capable of throughputs >370 samples per hour.

Agilent 6460 Settings

Source Parameters

Ionization mode	ESI + Agilent Jet Stream
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	9 L/min
Nebulizer pressure	45 psi
Nozzle voltage	500 V
Capillary voltage	3000 V

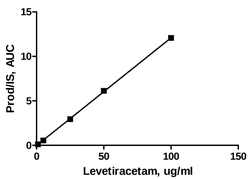
Acquisition Parameters (Positive Mode)

Transition	Precursor Ion	Product Ion	Dwell (ms)	Frag. (V)	CE (V)	CAV
IS	174.01	129.1	100	70	9	1
Quantifier	171.01	126.1	100	70	9	1
Qualifier	171.01	69.1	100	70	15	1

Results and Discussion

Prepared calibration standards and commercially available quality controls were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Levetiracetam (both the quantifier and qualifier ions) had intra- and inter-day accuracies within 15% and coefficient of variation values less than 10% for all concentrations within the linear range. This method had excellent linearity within the measured range of 1-100 µg/ml with an R^2 value greater than 0.99. There was no carryover detected. Signal to noise ratios were calculated looking at peak to peak height and found to be greater than 20:1.

Representative Standard Curve



Levetiracetam Conc (ug/ml)	Accuracy (%) Interday (n=4)	Precision (%) Interday (n=4)	Accuracy (%) Intraday (n=3)	Precision (%) Intraday (n=3)	Quant/Qual AUC
1	106.9	2.8	106.5	1.3	19.1
5	92.9	1.3	93.8	0.9	18.2
25	98.4	3.4	98.1	0.9	18.3
50	102.3	1.8	101.9	0.3	17.9
100	99.5	1.1	99.8	0.2	17.1
UTAK 1 (15.5)	93.1	3.4	94.6	0.7	17.8
UTAK 2 (39.7)	102.8	3.8	102.6	0.8	17.3
UTAK 3 (73.7)	105.1	3.5	104.1	0.6	17.3

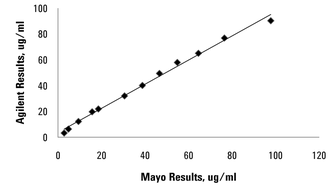
* 1/x weighing factor was applied.

Results and Discussion

Blinded Spiked Samples

Levetiracetam was spiked into bovine serum at the Mayo Clinic. The samples were processed and run immediately at the Mayo Clinic, while identical samples were frozen and shipped to Agilent. The values determined at Agilent were then compared to the values obtained at the Mayo Clinic. The R^2 value was greater than 0.99 and the slope was within 1.0 ± 0.1

Correlation between RapidFire-MS and LC-MS



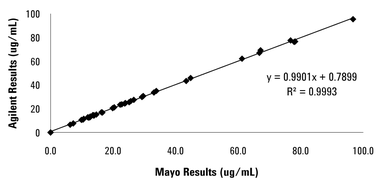
Blinded Human Samples

Blinded human samples were processed and run immediately at the Mayo Clinic, while identical samples were frozen and shipped to Agilent. Samples were run in the following order: Standards, QC's, P1-P20, QC's, P21-40, QC's. The methods had a good correlation with an R^2 value greater than 0.99.

Levetiracetam	Mayo ug/mL	Agilent ug/mL	% Difference
P1	12.8	11.9	-7.0%
P2	25.5	25.1	-1.6%
P3	33.7	33.1	-1.8%
P4	24.4	23.7	-2.9%
P5	76.3	77.9	2.1%
P6	14.2	13.8	-2.8%
P7	10.6	9.9	-6.6%
P8	45.9	44.8	-2.4%
P9	13.0	12.6	-3.1%
P10	76.7	78.2	2.0%
P11	16.8	16.6	-1.2%
P12	26.4	25.6	-3.0%
P13	13.4	12.7	-5.2%
P14	0.0	0.0	0.0%
P15	35.0	33.8	-3.4%
P16	23.4	22.3	-4.7%
P17	24.8	24.0	-3.2%
P18	0.0	0.0	0.0%
P19	62.2	61.2	-1.6%
P20	11.5	10.6	-7.8%

Levetiracetam	Mayo ug/mL	Agilent ug/mL	% Difference
P21	17.0	16.3	-4.1%
P22	12.9	12.6	-2.3%
P23	6.5	6.3	-3.1%
P24	20.5	19.9	-2.9%
P25	15.1	14.6	-3.3%
P26	69.3	67.2	-3.0%
P27	14.7	13.5	-8.2%
P28	10.9	10.4	-4.6%
P29	77.6	76.8	-1.0%
P30	23.7	22.8	-3.8%
P31	27.5	26.6	-3.3%
P32	21.2	20.4	-3.8%
P33	95.5	96.7	1.3%
P34	7.6	7.3	-3.9%
P35	30.7	29.6	-3.6%
P36	12.5	12.2	-2.5%
P37	43.4	43.4	0.0%
P38	14.4	13.9	-3.5%
P39	67.1	66.8	-0.4%
P40	29.9	29.2	-2.3%

Correlation between RapidFire-MS and LC-MS



Conclusions

Based on these results, levetiracetam can be accurately and precisely measured in human serum using the Agilent RapidFire High-throughput Mass Spectrometry System at rates of 9.5 seconds per sample. While the analytical results of blinded human samples were comparable to LC-MS/MS, the analysis time was approximately 20 times faster. RapidFire-MS may be useful for the fast and efficient analysis of similar assays.