Ultrafast Analysis of Levetiracetam in Serum Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Clinical Research

Authors

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Abstract

Mass spectrometry-based analyses have emerged as a viable analytical method due to their sensitivity, specificity, and robustness. We evaluated the ability of an ultrafast SPE/MS/MS system (Agilent RapidFire High-throughput Mass Spectrometry System) which is capable of analysis times of < 10 seconds per sample to analyze levetiracetam in human serum. The Agilent RapidFire/MS System had comparable accuracy, precision, linearity, and sensitivity to LC/MS/MS, but with a 10-fold faster sample analysis cycle time.

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Experimental

The RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360, an Agilent 6460 Triple Quadrupole LC/MS System, Agilent MassHunter Triple Quadrupole Acquisition Software B.04.01 with Qualitative Analysis B.04.00, and RapidFire Integrator Software.

RapidFire-triple quadrupole conditions

Samples were analyzed at a rate of 9.5 seconds per sample using the conditions shown in Table 1. Levetiracetam and the internal standard were monitored simultaneously in all experiments (Table 1).

Chemicals and reagents

The analyte levetiracetam and its stable-labeled isotope internal standard levetiracetam-[D3] were purchased from Cerilliant Round Rock, TX. Quality control samples were purchased from UTAK Laboratories, Inc. Valencia, CA.

Sample preparation

Calibration standards were prepared by spiking human serum with levetiracetam to final concentrations ranging from 1 µg/mL to 100 µg/mL. Commercially available quality control standards made in human serum were also analyzed. The serum samples were precipitated with acetonitrile containing internal standard. The precipitated samples were centrifuged, and the supernatant was removed and transferred to a 96-well plate for analysis.

Data analysis

RapidFire Integrator software was used for peak integration. The quantifier ion AUC of levetiracetam was normalized by the AUC of the internal standard. The data was subjected to linear regression with 1/x weighting.

Table 1. RapidFire/MS/MS conditions.

RapidFire conditions						
Buffer A	Water with 10 mM ammonium acetate, 0.1 % formic acid; 1.5 mL/min flow rate					
Buffer B	Methanol with 0.1 % formic acid; 1.25 mL/min flow rate					
Injection volume	10 µL					
SPE cartridge	Agilent RapidFire cartridge C (reversed-phase C18 chemistry, p/n: G9203E)					
RF state 1	sip sensor					
RF state 2	3,500 ms					
RF state 3	3,000 ms					
RF state 4	500 ms					
Triple quadrupole conditions						
Gas temperature	350 °C					
Gas flow	8 L/min					
Nebulizer	45 psi					
Sheath gas temperature	400 °C					
Sheath gas flow	9 L/min					
Nozzle voltage	500 V					
Capillary voltage	3,000 V					
	Q1	Q3	Dwell	Fragmentor	CE	CAV
IS	174.01	129.1	50	70	9	2
Quantifier	171.01	126.1	50	70	9	2
Qualifier	171.01	69.1	50	70	15	2

Results and Discussion

Prepared calibration standards and commercially available quality controls were analyzed using an Agilent RapidFire/MS system 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 (Table 2). This method had excellent linearity within the measured range of 1–100 µg/mL with an R^2 value greater than 0.995 (Figure 1). Carryover was assessed by analyzing the AUC of a blank injection immediately following the highest standard curve concentration and calculated as a % of the mean peak area of the 1 µg/mL standard. No significant carryover (< 1 %) was seen using this method. Signal-to-noise ratios were calculated looking at peak-to-peak height and found to be greater than 20:1 at 1 µg/mL.

Levetiracetam was spiked into bovine serum, processed, and run immediately at the Mayo Clinic, while identical samples were frozen and shipped to Agilent Technologies, Inc. The values determined at Agilent using RapidFire/MS were then compared to the values obtained by LC/MS/MS at the Mayo Clinic. The correlation between the two analytical methodologies was very good, R^2 value greater than 0.99 and slope within 1.0 ± 0.1 (Figure 2).

Table 2. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of leviteracetam in serum.

Leviteracetam (ng/mL)	Intraday % accuracy (n=3)	Intraday % precision (n=3)	Interday % accuracy (n=4)	Interday % precision (n=4)
1	104.3	2.5	105.9	2.9
5	93.5	0.5	91.8	2.4
25	100.9	2.3	100.9	2.9
50	102.4	1.5	102.6	2.3
100	98.8	1.3	98.8	1.6
UTAK1 (15.5)	95.9	1.4	95.2	4.3
UTAK2 (39.7)	16.2	0.5	15.5	3.1
UTAK3 (73.7)	104.3	0.6	105.1	2.9

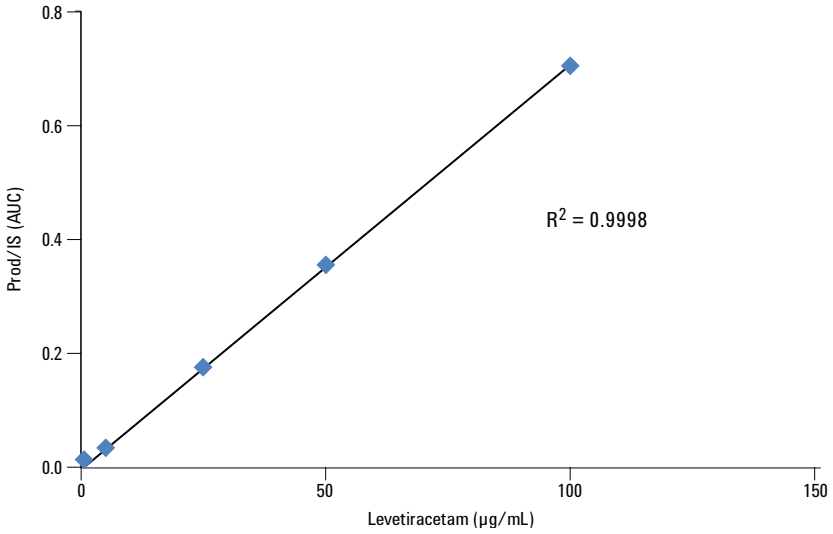

Figure 1. Representative standard curve for levetiracetam spiked into serum.

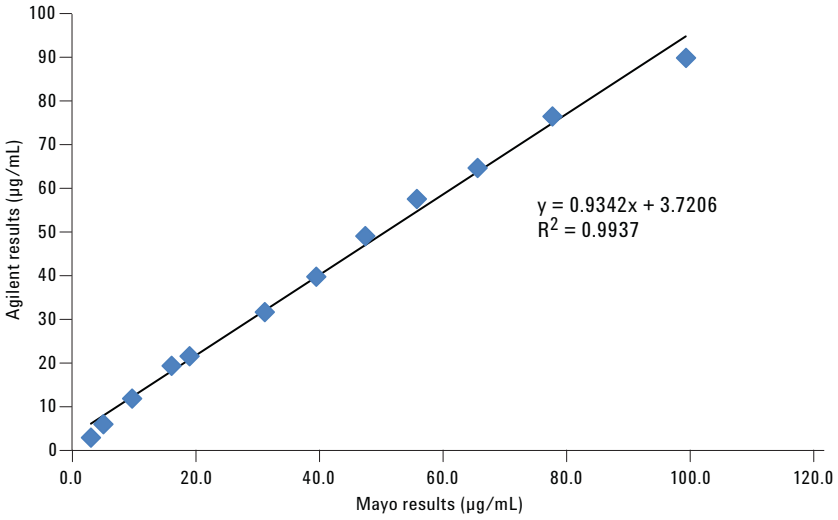

Figure 2. Correlation between RapidFire/MS/MS and LC/MS/MS for spiked levetiracetam samples.

Blinded human samples were processed and run immediately at the Mayo Clinic using LC/MS/MS, while identical samples were frozen and shipped to Agilent for RapidFire/MS analysis. The two methods had a very good correlation with an R^2 value greater than 0.995 and a slope within 1.0 ± 0.1 (Figure 3).[1]

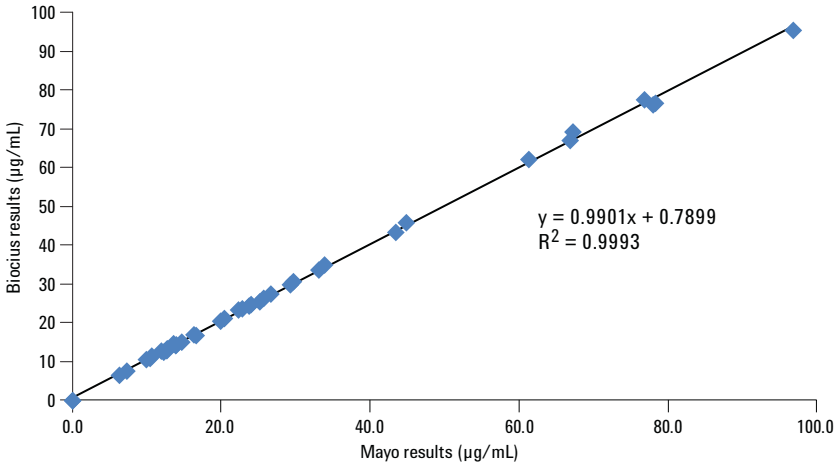

Figure 3. Correlation between RapidFire/MS/MS and LC/MS/MS for blinded human samples.

Conclusions

Based on these results, levetiracetam can be accurately and precisely measured in human serum using the Agilent RapidFire/MS System at rates of 9.5 seconds per sample. While the analytical results of human samples were comparable to LC/MS/MS, the analysis time was approximately 10 times faster. RapidFire/MS may be useful for the fast and efficient analysis of similar targets of clinical research.

Acknowledgements

The authors gratefully acknowledge Frank Crow, Eric Korman, Christine Snozek and Loralie Langman at the Mayo Clinic, Rochester, Minnesota for proving technical assistance and the blinded human samples used in this study.

Reference

1. Romm, M.V., *et al.* High-Throughput Analysis of Levetiracetam in Serum Using Ultrafast SPE/MS/MS. *Poster #161 presented at the 59th ASMS Conference on Mass Spectrometry and Allied Topics,* June 7th **2011**, Denver, CO.

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Published in the USA, August 12, 2014
5990-9995EN

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