Poster Reprint

ASMS 2018
WP-101

Small but Powerful: Antiepileptic Drugs in Human Serum Analyzed with a Miniature Triple Quadrupole Mass Spectrometer in Research

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Introduction

Liquid chromatography-mass spectrometry (LC/MS/MS) has long demonstrated its value in analytical research laboratories due to its specificity and analytical sensitivity when analyzing multiple compounds in a single injection. Despite this convenience, many large clinical research laboratories find space to be at a premium, driving the need for creative solutions. As such, minimizing the instrument footprint while maintaining the power of a traditionally sized mass spectrometer may provide an answer for such laboratories. To test the capabilities of the miniature Agilent Ultivo LC/TQ, a panel of antiepileptic drugs in human serum was analyzed and compared to the same sample set run on a larger footprint Agilent 6470 LC/TQ [1]. Compounds included in the panel were acetylretigabine, carbamazepine-10,11-epoxide, carbamazepine, 10,11-dihydro-10-hydroxy-carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, retigabine, rufinamide, tiagabine, and vigabatrin. The method further tested the ability of the instruments to detect compounds over varying concentration ranges simultaneously, as the calibration concentrations ranged from 0.59 ng/mL to 20,000 ng/mL for the various analytes. Top concentrations ranged from 0.15 to 20 µg/mL.

Experimental

Sample Prep

Drug-free human serum was spiked with drug standards of the 15 compounds to achieve the top concentration, while eight lower concentrations were created by a serial dilution into drug-free serum. Each sample was combined with an internal standard solution and extracted by protein precipitation using methanol. Samples were vortexed and centrifuged, and an aliquot of supernatant was diluted 10-fold with water prior to introduction into the LC system.

LC/TQ Analytical Method

The LC/TQ system consisted of a 1290 binary pump, a thermostatted autosampler, a temperature-controlled column compartment, and an Ultivo triple quadrupole mass spectrometer. A performance comparison was also undertaken using the identical HPLC with an Agilent 6470 triple quadrupole mass spectrometer. Separation conditions are given in Table 1, while MS conditions are in Table 2.

Experimental

LC Conditions	
Guard Column	Agilent Poroshell 120 EC-C18, 2.1x5mm, 2.7 µm
Analytical Column	Agilent Poroshell 120 EC-C18, 2.1x100mm, 2.7 µm
Injection Volume	4 µL
Mobile Phase A	Water + 2 mM ammonium acetate
Mobile Phase B	Methanol + 2 mM ammonium acetate
Needle Wash	50:50 Isopropanol:Methanol
Autosampler Temp	4 °C
Column Temp	50 °C
Flow Rate	0.4 mL/min
Stop Time	8 min
Post Time	1 min

Table 1. LC conditions.

MS Conditions	
Gas Temp	350 °C
Gas Flow	12 L/min
Nebulizer Pressure	50 psi
Sheath Gas Temp	350 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	3500 V
Nozzle Voltage	0 V
Delta EMV	0 V

Table 2. AJS ESI MS source conditions.

Figure 1. Agilent Ultivo LC/TQ.

Chromatography

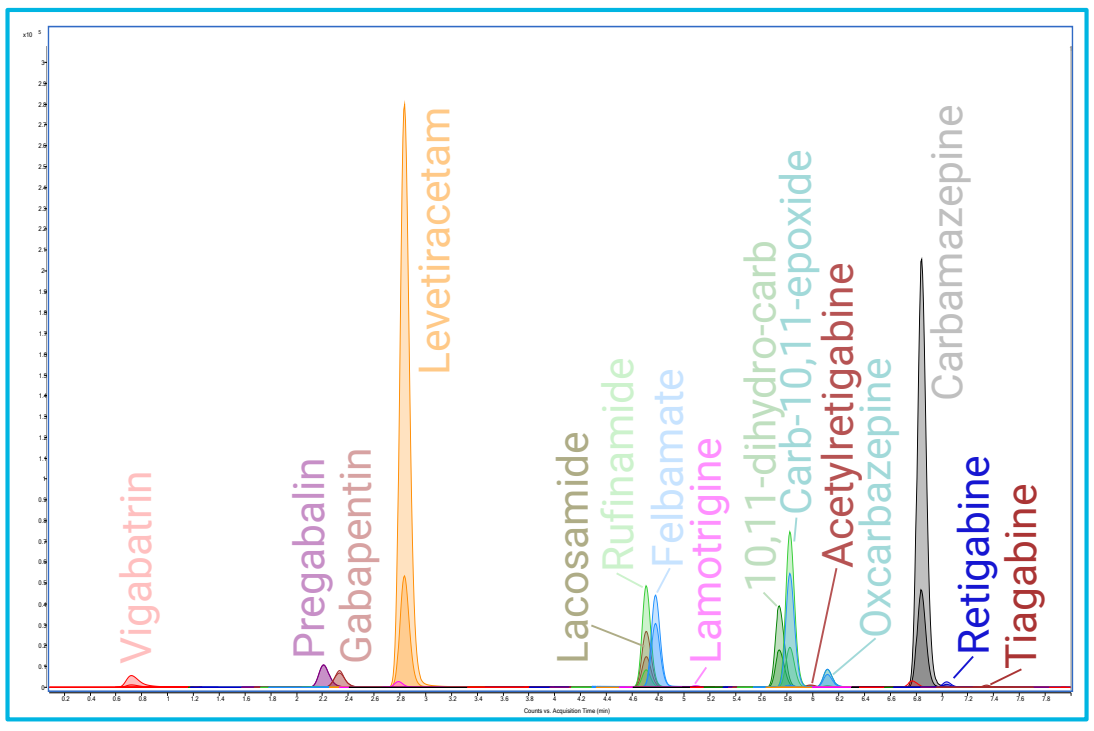
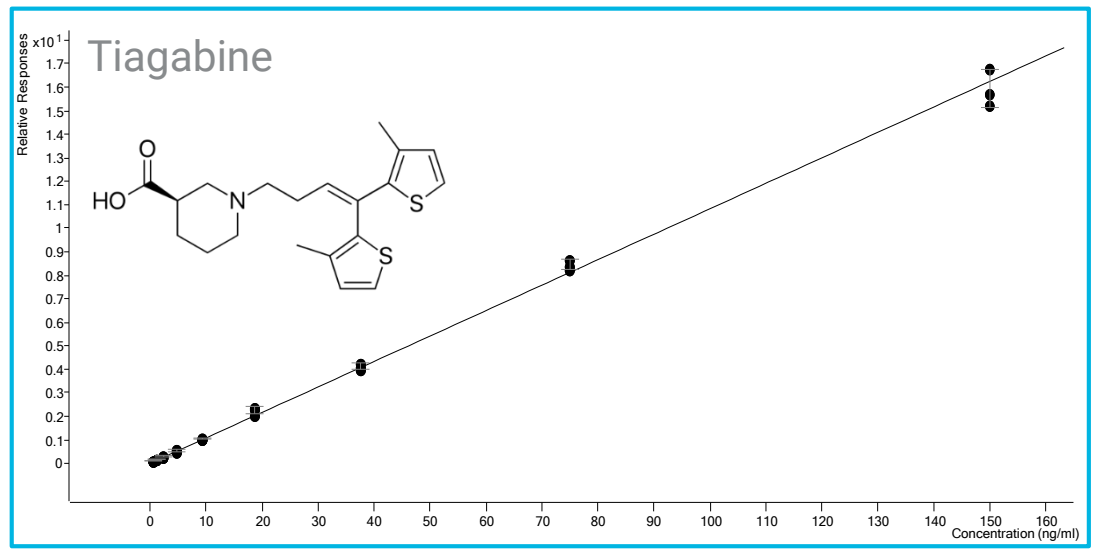


Figure 2. Overlaid dMRM chromatograph showing 15 compounds.

Calibration

All calibration curves utilized a 1/x weighting factor.



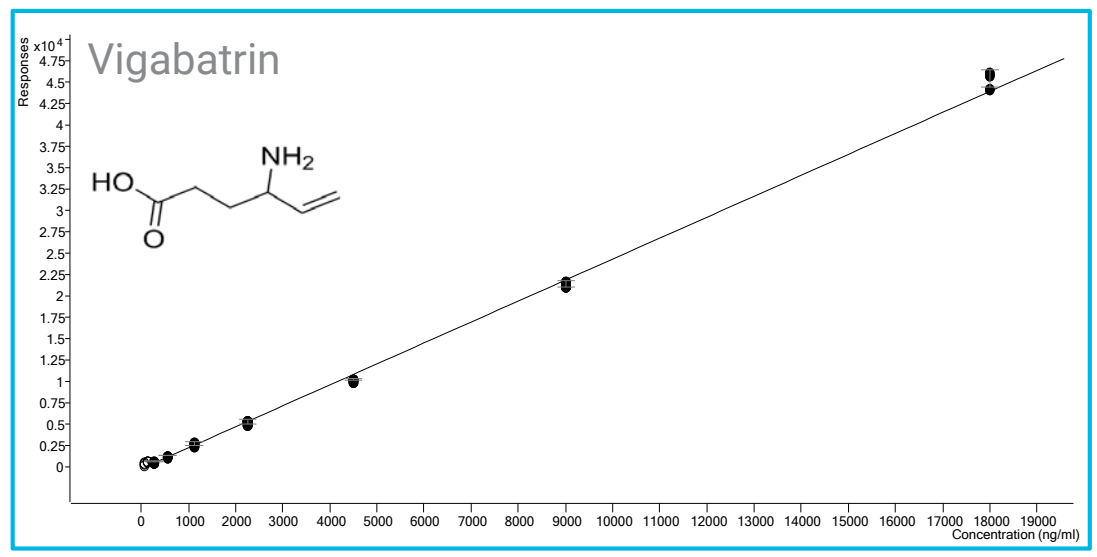


Figure 3. Representative calibration curves, showing the most (tiagabine) and least (vigabatrin) sensitive compounds.

The calibration concentrations ranged from 0.59 ng/mL to 20,000 ng/mL for the various analytes. Top concentrations ranged from 0.15 to 20 µg/mL. R^2 values were greater than 0.994 for all analytes, with ten of the compounds displaying linear responses throughout the concentration range, and five requiring quadratic fits to cover the complete concentration range.

Accuracy and Reproducibility

Calibration curves for each of the 15 compounds demonstrated accuracies within 20% of the expected concentration at the lowest calibration level, and reproducibility across all levels was acceptable with CVs less than 15%. A comparison of accuracies and reproducibility between the Ultivo and the 6470 are shown in Tables 3 and 4. Representative compounds at the LOQ and ULOQ demonstrate the reproducibility in Figure 4.

Results and Discussion

	Viga		Pregaba		Gaba		Levet		Lacos		Rufin		Felbam		Lamot		10,11-dihydro-carb		Carb epox		Acetyl-retig		Oxcarb		Carb		Retig		Tiaga	
Level	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV
1			87.4	11.8	83.2	8.0	103.5	1.5	88.6	2.4	88.8	1.8	84.6	2.5			87.6	4.2	101.6	1.7	96.2	6.7	103.2	5.5	83.8	1.6	92.5	7.4	103.9	13.5
2			95.9	8.8	98.6	4.2	99.6	1.4	93.9	1.4	101.2	2.2	100.0	1.3	99.9	6.3	100.4	3.1	98.8	3.9	104.1	13.3	97.2	1.3	95.1	1.4	94.5	9.8	92.4	6.2
3	104.2	8.5	99.8	0.3	101.8	3.2	98.7	1.6	101.8	1.8	103.3	2.4	103.4	2.1	92.4	1.9	102.5	1.2	99.0	0.4	103.1	3.1	95.9	1.1	99.8	0.7	98.2	1.2	98.1	13.1
4	100.6	4.7	102.4	1.4	104.1	2.1	100.6	1.2	102.8	1.7	106.0	0.2	108.1	1.3	101.9	7.5	104.9	3.0	98.6	1.4	109.3	1.8	98.5	3.5	105.0	1.6	104.3	6.9	98.4	5.8
5	104.0	8.2	102.9	1.1	102.9	3.7	96.7	0.8	101.3	2.0	102.2	1.9	105.4	1.7	101.3	7.0	104.0	1.2	98.4	1.3	102.7	4.5	101.0	3.2	104.2	1.5	107.2	3.8	98.7	5.6
6	97.7	5.9	108.7	0.8	106.5	1.5	101.8	1.7	109.1	1.4	101.2	0.7	102.0	0.8	102.1	1.4	104.3	3.2	102.8	1.1	102.1	2.2	104	2.2	109.2	2.3	105.4	5.9	107.9	1.8
7	93.3	1.3	102.4	1.2	101.5	5.5	97.5	1.0	102.0	1.6	96.7	1.0	97.3	1.8	100.7	2.4	97.7	1.9	99.1	1.1	93.8	3.0	100.1	0.5	102.0	0.5	96.1	3.3	99.6	2.6
8	97.1	1.6	104.5	1.1	105.6	1.2	102.0	1.8	104.4	1.9	100.5	2.3	98.4	2.2	99.9	0.8	97.9	1.0	103.5	0.2	101.9	1.7	101.0	1.0	105.6	1.6	104.2	3.0	103.3	3.5
9	103.1	2.3	95.9	1.4	95.8	2.1	99.6	1.5	96.1	1.6	100.1	1.2	100.9	2.6	99.5	1.9	100.7	0.9	98.3	0.8	99.8	0.6	99.0	1.6	95.2	1.1	97.7	4.0	97.7	5.7

Table 3. Accuracy and reproducibility results for curves analyzed on the Ultivo, n=3.

	Viga		Pregaba		Gaba		Levet		Lacos		Rufin		Felbam		Lamot		10,11-dihydro-carb		Carb epox		Acetyl-retig		Oxcarb		Carb		Retig		Tiaga	
Level	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV	Avg	CV
1	119.7	14.4	87.6	4.7	90.7	1.2	103.9	1.3	99.2	3.3			77.3	2.5	103.9	6.3	82.4	3.6	102.2	3.4	87.8	6.7	101.2	2.7	83.7	1.9	109.5	0.7	100.8	2.6
2	105.3	12.1	91.8	0.8	92.9	1.4	97.6	1.3	94.5	0.4	97.6	0.5	94.3	1.2	96.5	6.8	94.4	1.4	96.9	1.5	100.0	3.5	97.0	5.8	91.3	0.5	110.4	12.5	97.8	3.0
3	96.8	3.2	100.0	2.7	99.3	0.6	98.9	0.3	98.0	0.8	120.4	2.2	108.0	1.6	99.6	4.5	107.1	0.9	97.9	1.7	104.2	3.6	99.9	5.0	99.7	0.4	87.6	2.7	96.8	4.0
4	95.1	6.7	104.4	2.6	103.5	2.0	100.3	1.4	101.2	1.5	122.2	3.9	113.3	1.8	97.1	2.2	110.3	2.2	100.3	1.3	105.8	1.8	98.6	2.5	104.7	0.9	86.4	8.9	102.7	2.4
5	92.5	6.3	103.0	1.4	102.3	1.2	98.2	0.4	100.3	0.7	111.6	3.5	109.4	0.7	98.5	3.2	107.3	1.6	98.7	0.8	104	1.6	98.3	1.8	105.7	0.8	99.2	14.9	97.8	0.8
6	96.5	0.1	109.7	2.0	108.5	1.4	102.5	1.4	105.3	0.9	103.0	3.3	103.6	1.5	13.4	1.4	103.1	0.8	103.7	1.1	100.8	3.9	103.3	1.7	110.9	0.7	101.0	6.6	102.5	1.1
7	92.3	3.8	103.0	1.5	102.3	1.0	97.2	1.2	100.1	0.6	94.9	2.1	96.9	1.2	100.0	1.6	97.4	1.3	99.0	0.9	97.3	2.2	98.5	1.9	104.0	0.9	97.8	6.7	98.9	0.4
8	97.6	0.7	105.2	1.4	104.7	1.2	101.6	0.9	104.2	1.0	94.6	2.4	95.3	1.4	102.7	2.2	96.5	1.7	102.9	1.0	99.8	1.5	106.0	2.0	105.5	0.7	104.1	5.5	105.3	0.8
9	104.1	1.2	95.2	2.1	95.9	2.6	99.7	1.7	97.2	1.1	103.6	3.0	102.9	2.5	98.4	1.2	101.8	1.6	98.5	1.8	100.2	1.9	97.1	1.9	94.5	1.2	98.8	5.6	97.4	0.7

Table 4. Accuracy and reproducibility results for curves analyzed on the 6470, n=3.

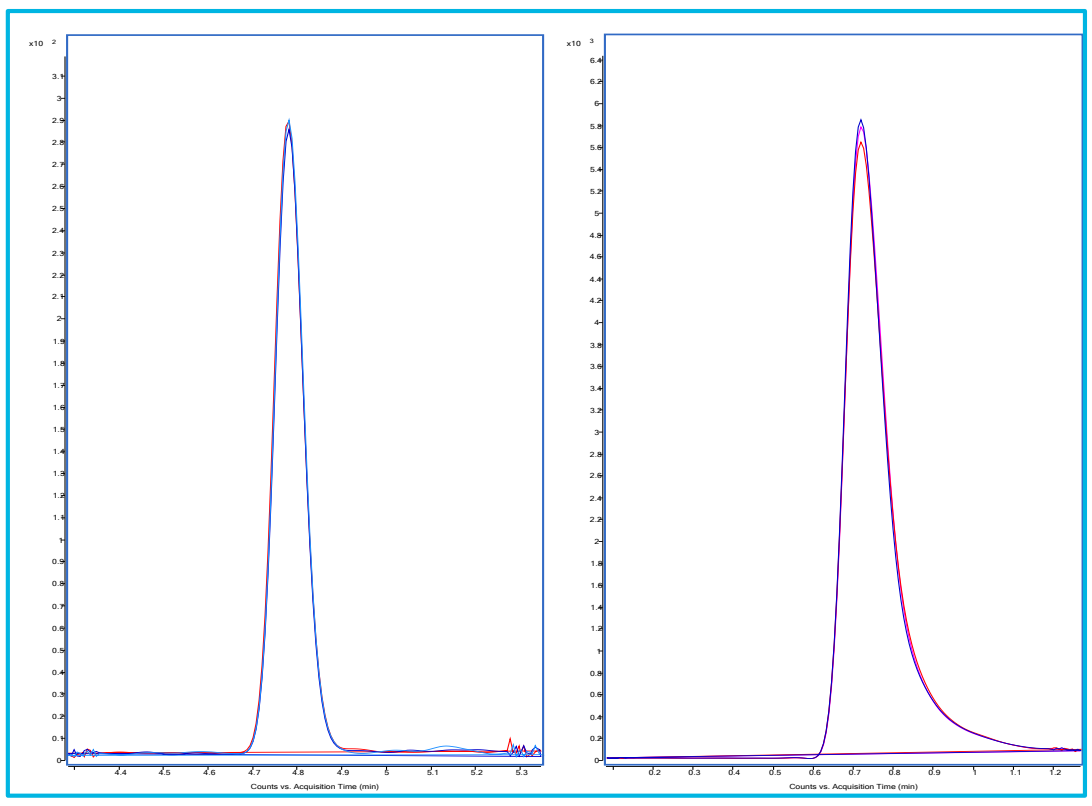


Figure 4. The reproducibility at the LOQ (left) and ULOQ (right) are shown in these overlaid chromatographs.

Conclusions

The new miniature Agilent Ultivo LC/TQ delivers results comparable to those achieved on the Agilent 6470, demonstrating that downsizing the instrument does not compromise analytical sensitivity and dynamic range, as determined from a head-to-head comparison. Future work is required to assess potential matrix interferences and to include additional anti-epileptic drugs creating a comprehensive method

References

[1]Frick LE, Miller VP. Simultaneous LC/MS/MS Quantitation of 20 Antiepileptic Drugs in Human Serum. Poster presented at MSACL January 23-26, 2017; Palm Springs, CA.