Ultrafast Analysis of Gabapentin and Pregabalin in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Authors

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Abstract

The measurement of gabapentin and pregabalin in urine requires a reliable, efficient, and accurate method. We developed an analytical method using a SPE/MS/MS system to measure these analytes in urine with much faster sample cycle times, and similar analytical results compared to traditional methods. A simple dilute-and-shoot based analysis by the Agilent RapidFire/MS/MS permits accurate and precise measurement of these analytes over a linear range of 0.5 to 100 mcg/mL. Samples were analyzed at 25 seconds per sample, providing a much higher throughput method of analysis compared to traditional protocols.

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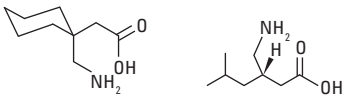
Introduction

Several chromatographic methods are used to measure gabapentin and pregabalin (Figure1) in urine, such as GC/MS, HPLC, and recently LC/MS/MS. Some of these methods use complicated extraction instruments, long and tedious extraction procedures, and large amounts of solvents or biological fluids. The main objective of this work was to develop a rapid, selective, and sensitive online SPE/MS/MS method that has short and simple extraction procedures, consumes small amounts of solvent and biological fluid for extraction, and has an ultrafast turn-around time. We developed a method using the Agilent RapidFire/MS/MS system to measure gabapentin and pregabalin in urine with much faster sample cycle times and similar analytical results compared to traditional analytical methods.



Gabapentin Pregabalin

Figure 1. Chemical structures of the analytes.

The use of a 96-well plate based method (dilute-and-shoot) allows for automation of the entire sample analysis process to increase efficiency and laboratory throughput. The Agilent RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times of less than 25 seconds. This new method allows for the rapid, accurate, and precise measurement of gabapentin and pregabalin in urine over a linear range of 0.5–100 mcg/mL.

Experimental

The Agilent RapidFire/MS/MS system consisted of the following modules: Agilent RapidFire 360, Agilent 6460 Triple Quadrupole Mass Spectrometer using Agilent MassHunter Triple Quadrupole Acquisition Software (B.05.01) with Qualitative Analysis (B.05.00), Quantitative Analysis (B.05.00), and RapidFire Acquisition Software.

Samples were analyzed at a rate of 14 seconds per sample. Quantitative and qualitative ions for gabapentin, pregabalin, and the internal standard gabapentin-d10 were monitored simultaneously in all experiments (Table 1). Agilent MassHunter Quantitative Software automatically calculated qualifier ion ratios.

Chemicals and reagents

All of the analytes and stable-labeled isotopic internal standards were purchased from Cerilliant, Round Rock, TX. The quality controls were obtained from Utak Laboratories, Valencia, CA. All other solvents and reagents were purchased from Thermo Fisher Scientific.

Agilent RapidFire/MS/MS conditions

Parameter	Value
Buffer A (Pump 1)	LC/MS grade water + 0.1 % formic acid; 1.5 mL/min flow rate
Buffers B and C (Pumps 2 and 3)	50 % methanol, 25 % isopropanol, 25 % acetonitrile, 0.1 % formic acid; 1.25 and 1.0 mL/min flow rate respectively
Aqueous wash	HPLC grade water
Organic wash	50 % methanol, 25 % isopropanol, 25 % acetonitrile
SPE cartridge	Agilent RapidFire cartridge E (reversed-phase C18 chemistry, G9205A)
RF State 1	600 ms
RF State 2	2,500 ms
RF State 3	0 ms
RF State 4	7,000 ms
RF State 5	1,000 ms
Agilent 6460 Triple Quadrupole Mass Spectrometer conditions	
Gas temperature	325 °C
Gas flow	12 L/min
Nebulizer	45 psi
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Capillary voltage	3,000 V
Nozzle voltage	500 V

Table 1. MRM transitions.

Compound	Q1	Q3	Dwell	Fragmentor	CE	CAV
Gabapentin-d10	182.2	164.1	10	90	9	2
Gabapentin Quant	172.1	137	10	80	13	2
Gabapentin Qual	172.1	95	10	80	25	2
Pregabalin Quant	160.1	142.1	10	80	10	2
Pregabalin Qual	160.1	97	10	90	10	2

Sample preparation

The samples, calibrators (0.5, 2.0, 25, 50, and 100 mcg/mL), and QC standards (10 and 24 for gabapentin and 10 and 27 for pregabalin) were prepared using the following procedure. First, 5 µL of sample was added to a deep-well plate containing 1.0 mL of internal standard (gabapentin_d10) at 200 ng/mL prepared in LC/MS grade water (1–200 dilution). Next, the plate was sealed with an Agilent PlateLoc Thermal Microplate Sealer and mixed gently prior to RapidFire/MS/MS analysis.

Data analysis

System control and data acquisition were performed by MassHunter Triple Quadrupole Data Acquisition Software. Data analysis was performed using MassHunter Triple Quadrupole Quantitative Analysis Software. Calibration curves were constructed using linear least squares regression with $1/X^2$ weighting for the multiple reactions monitoring (MRM). The quantitation was performed by comparing spectral peak area ratio to a known concentration of the internal standards.

Results and Discussion

Samples were prepared by spiking gabapentin and pregabalin into drug-free human urine, then diluting samples 200-fold with water. Samples were then analyzed through SPE/MS/MS using the RapidFire/MS/MS system and a hydrophobic C18 cartridge at 25 seconds per sample inclusive of a blank injection following the analyte injection (Figure 2). This RapidFire/MS/MS methodology is capable of throughputs greater than 140 samples per hour providing a high-throughput and very efficient mode of analysis. Gabapentin and pregabalin standard curves in urine had excellent linearity within the measured range (0.5–100 mcg/mL) with an R^2 value greater than 0.995 (Figure 3). Intra- and interday accuracies were within 5 %, and coefficient of variation values were all less than 5 % for concentrations within the measured range (Table 2).

Carryover was assessed by analyzing the AUC of the blank calculated as % of the mean peak area of the 0.5 mcg/mL samples. No significant carryover was determined for gabapentin and pregabalin (Figure 2). Since these are sticky compounds, we recommended using one blank injection between samples, using a strong organic solution. Addition of a blank injection raises the total injection-to-injection interval to 25 seconds, which is several times faster than traditional LC/MS/MS methods for these same analytes.

The reproducibility of the method was evaluated by measuring > 2,000 sequential injections of both analytes spiked into blank human urine. The instrument response was stable for both analytes with a coefficient of variation of 1.67 and 2.65 %, showing the robustness of the RapidFire system, SPE cartridge lifetime, and consistency of quantitation for the analytes in the panel. As an example, the data for gabapentin can be found in Figure 4, where the coefficient of variation over > 2,000 injections was 1.67 %.

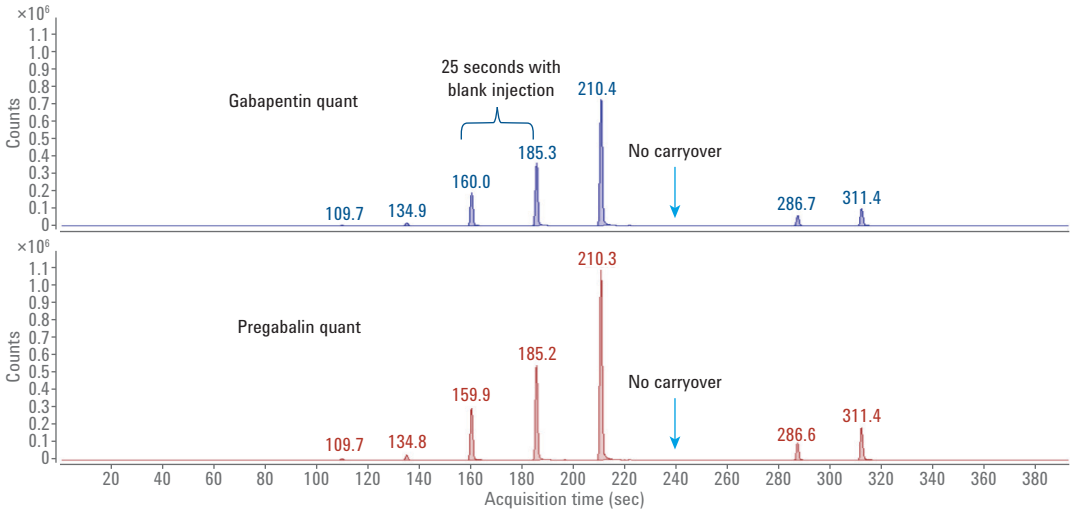


Figure 2. Representative calibration curve data for each of the analytes showing the injection to injection interval of 25 seconds including a blank injection. Carryover assessment using a matrix blank immediately after the highest calibrator for both analytes shows no significant carryover was observed for any of the analytes.

Conclusions

The analytes gabapentin and pregabalin were rapidly, accurately, and precisely measured in urine using a simple (dilute-and-shoot) methodology and the Agilent RapidFire/MS/MS System. Samples were analyzed with injection-to-injection cycle times of 25 seconds, providing a high-throughput method of analysis for these analytes. This methodology is capable of throughputs greater than 140 samples per hour. This methodology provides comparable results to LC/MS/MS, but at > 5x the speed and efficiency of LC/MS/MS methods. The new method described here provides a very efficient mode for measuring gabapentin and pregabalin in urine compared to traditional analytical methods.

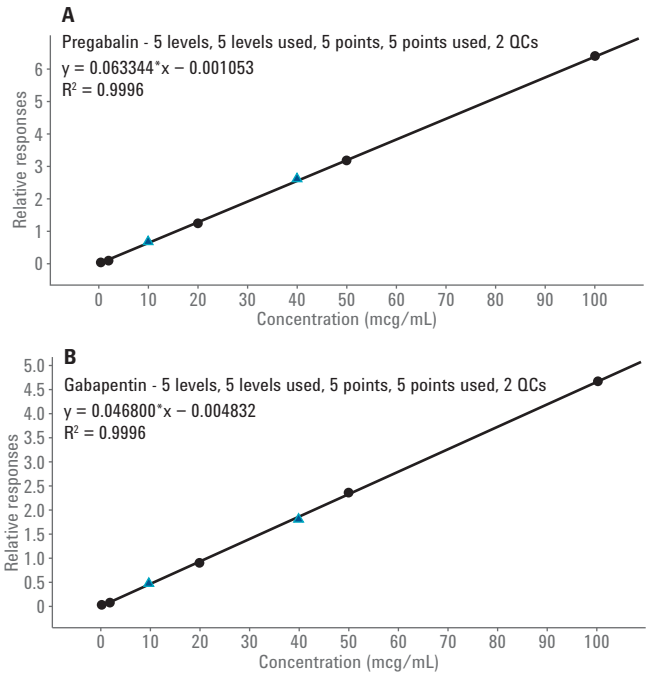


Figure 3. Representative calibration curves showing the linear range from 0.5–100 mcg/mL for each analyte. Dark circles are calibrators and triangles are QC standards.

Table 2. Intraday and interday accuracy and precision data for the QC standards.

Gabapentin (mcg/mL)	Intraday % accuracy (n = 6)	Intraday % precision (n = 6)	Interday % accuracy (n = 6)	Interday % precision (n = 6)
10	98.3	2.7	99.6	1.3
24	101.7	4.1	97.8	0.5
Pregabalin (mcg/mL)	**Intraday % accuracy (n = 6)**	**Intraday % precision (n = 6)**	**Interday % accuracy (n = 6)**	**Interday % precision (n = 6)**
10	106.6	2.8	106.5	1.5
27	101.3	4.1	101.5	0.9

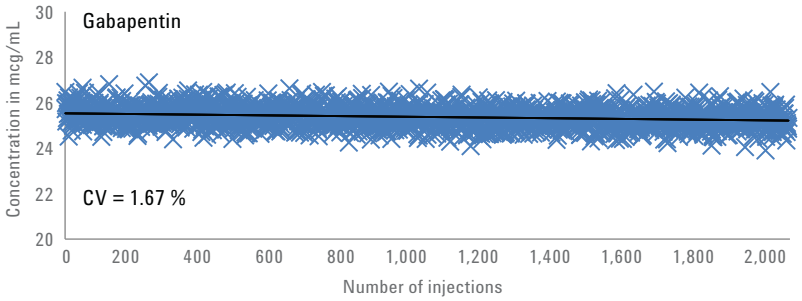


Figure 4. Reproducibility evaluation using sequential injections of the gabapentin high quality control.

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