Development of an LC/MS/MS Method for Bupropion in Human Plasma Using a 6460 Triple Quadrupole LC/MS System

Application Note

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Abstract

The aim of this work is to present a case study that demonstrates the successful transfer of an LC/MS/MS assay between bioanalytical laboratories. Transferring methods between mass spectrometers from different vendor requires adjusting source and instrument parameters and, in some cases, the mobile phases to achieve comparable levels of sensitivity between instruments (assuming the instruments have comparable sensitivity performance). In this application note, a bupropion LC/MS/MS method originally developed on a Shimadzu LC 20A coupled to an ABI/SCIEX API 4000 LC/MS/MS is transferred to an Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadrupole LC/MS System. The mobile phase, column, and multiple reaction monitoring (MRM) transitions were kept identical on both systems. Linearity, precision, and accuracy of drug-spiked plasma samples were evaluated. Comparable results were obtained on both systems for five batches with minimal down time during the successful method transfer.

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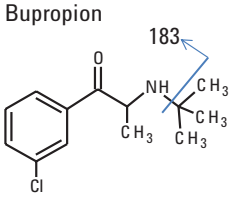
Introduction

The transfer of an LC/MS/MS method to a different instrument requires knowledge of instrument tuning parameters and their effect on the signal response. To determine the ease of method transfer, an LC/MS method for the analysis of bupropion was transferred from an ABI/SCIEX API 4000 System to an Agilent 6460 Triple Quadrupole LC/MS System. Bupropion is an antidepressant which aids in smoking cessation. The Agilent 6460 source parameters were optimized, but without changing the mobile phase, column, and the MRM settings. A 10-point calibration curve was plotted from 1 to 500 ng/mL using plasma extracted samples. Linearity, precision, and accuracy obtained from the Agilent system were compared with the accepted criteria for validated bioanalytical methods[1, 2] in order to demonstrate the success of the transfer.

Bupropion



Risperidone

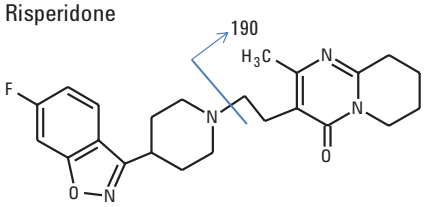


Figure 1. Fragmentation pattern of bupropion and the internal standard risperidone.

Experimental

Materials

The working standard, bupropion hydrochloride, with a HPLC purity of 100 % (anhydrous basis), was obtained from Varda Biotech (Mumbai, India). Risperidone USP (Lot no. F0G288) was used as an internal standard (IS). HPLC grade acetonitrile and methanol were obtained from Merck (Mumbai, India).

Analytical grade ammonium formate was obtained from Loba Chemie Pvt. Ltd (Mumbai, India). Deionized and purified water from a Milli-Q system (Millipore) was used for the mobile phase and for the preparation of standard solutions. Control human plasma (K_2EDTA anticoagulant), used for the preparation of calibration standards and quality control samples, was obtained from a blood bank and stored at -40 °C prior to use.

LC/MS/MS conditions

Experimental Parameters	Details	
Column	Gemini C18 (50 x 3 mm, 5 µ); maintained at 40 ± 5 °C	
Mobile phase	Ammonium formate (10 mM): acetonitrile (20/80, v/v)	
Flow rate	0.8 mL/min, 70 % flow splitting, isocratic with 2 min run time	
Autosampler temperature	5 ± 3 °C	
Injection volume	5 µL	
MRM	Bupropion 239.8 → 183 Risperidone (IS) 411.1 → 190	
Agilent 6460 Triple Quadrupole LC/MS System	Drying gas flow:	13.0 L/min
	Nebulizer pressure:	40 psig
	Dry gas temperature:	350 °C
	Capillary voltage:	3000 V
	Sheath gas flow:	12.0 L/min
	Sheath gas temperature:	350 °C
	Ionization source:	Agilent Jet Stream
	Ionization mode:	Positive
	Collision energy:	7 eV (Bupropion), 25 eV (IS)
	Fragmentor voltage:	135 V (Bupropion), 90 V (IS)
	LC:	Agilent 1290 Infinity LC System

Standard curves

Standard and intermediate stock solutions were prepared in methanol. Known amount of the intermediate stock solutions were added to human plasma to prepare calibration standards and quality controls (QC). The linearity range used to evaluate the Agilent 6460 LC/MS System is shown in Table 1. The spiked low (LQC), middle (MQC), and high (HQC) quality control samples contained bupropion. One batch includes one set of linearity and six sets of QC samples. Five of these batches were prepared and analyzed.

Table 1. Concentrations of bupropion 10-level linearity range and the QC samples used in the study.

Level	Concentration (ng/mL)
L1	1.502
L2	3.005
L3	4.553
L4	8.756
L5	21.89
L6	54.72
L7	109.4
L8	218.9
L9	364.8
L10	456.0
LQC	4.379
MQC	187.1
HQC	346.6

Extraction procedure

Internal standard (IS) solutions (50 µL at 500 ng/mL) were added to 100 µL of calibration standards and QC samples, and the solutions were vortexed for 5 s. Next, 100 µL of ammonium formate (1 mM) was added and each solution was vortexed. To precipitate the proteins, 1 mL of methanol was added and the solution was vortexed for 5 min. Following centrifugation at 4500 rpm (4 °C for 15 min), the supernatant was transferred to a glass vial for LC/MS/MS analysis.

Data acquisition

Data was acquired using Agilent MassHunter Workstation software (B.03.01) and processed using MassHunter Quantitative analysis software (B.04.00).

Results and Discussion

A bioanalytical method for the measurement of bupropion was successfully transferred to an Agilent 6460 Triple Quadrupole LC/MS System without modification to the HPLC conditions. The Agilent 6460 source parameters were optimized using the bupropion standard.

Level 1 of the calibration curve

Level 1 (L1) lower limit of quantitation (LLOQ) calibration standard show an acceptable CV value of 3.80 %. Representative chromatograms for bupropion and IS at L1 level are shown in Figure 2. The S/N value for bupropion at L1, from five different runs, was 1600 (using peak height and RMS X1).

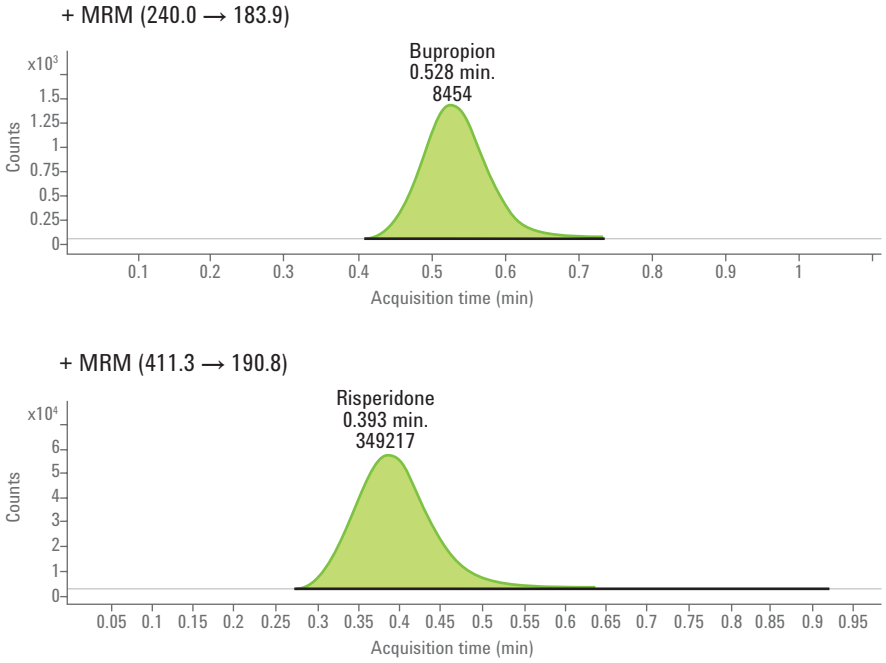


Figure 2. Chromatogram of [A] bupropion and [B] risperidone (IS) at LLOQ (1.502 ng/mL).

Linearity, precision, and accuracy

A 10-point linear calibration curve showed a minimum R^2 value of 0.998 (Figure 3). A linear curve fitting with $1/x^2$ was applied to the curve. For the five analytical batch runs, the precision (% CV) of calibration standards ranged from 2.22 % to 11.90 % and the % mean accuracy (back calculated values from linearity equation) ranged from 94.17 % to 108.32 % (Table 2). The correlation coefficients (R^2) for these analytical batches of bupropion were ≥ 0.99. The precision and accuracy at all concentrations are given in Table 2. For the five analytical batch runs, the precision (% CV) of QC samples at all concentrations ranged from 6.72 % to 7.69 % and the % mean accuracy of all the QC samples at all concentrations ranged from 98.21 % to 103.92 %.

These results are within acceptable bioanalytical regulatory criteria[2], indicating that this method has been successfully transferred to the Agilent 6460 Triple Quadrupole LC/MS System.

Speed of method transfer

For the Agilent 6460 Triple Quadrupole LC/MS System, the compound dependent tuning parameters are the capillary voltage, fragmentor voltage, sheath gas temperature, nozzle voltage, drying gas temperature, and collision energy. The LC flow dependent parameters are nebulizer pressure, drying gas pressure, and sheath gas flow. Both sets of parameters were easily optimized within one day with the combination of Optimizer software and infusion experiments. The LC method was directly transferred from the Shimadzu LC-20A without any modification.

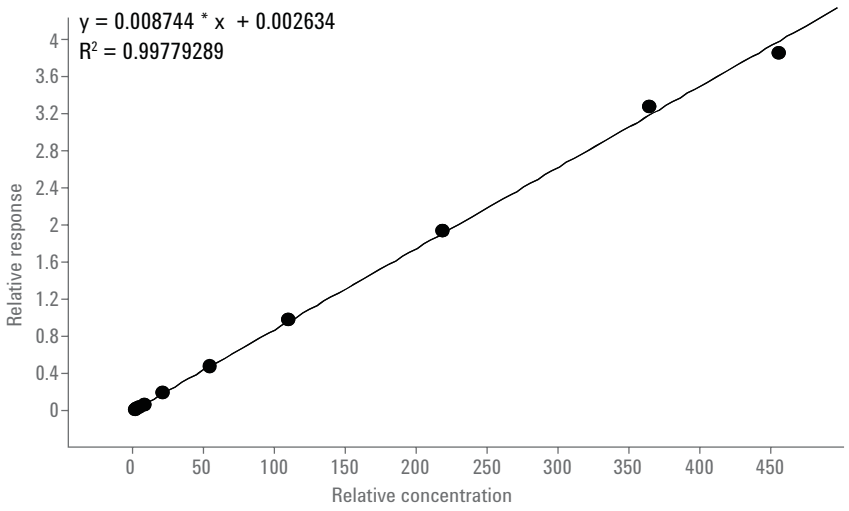


Figure 3. Calibration standard curve of bupropion as performed on an Agilent 6460 LC/MS System.

Table 2. Precision and accuracy of bupropion calibration standards and QC samples acquired using an Agilent 6460 Triple Quadrupole LC/MS System.

Levels	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	HQC	MQC	LQC
% CV (n = 5)	3.80	6.60	3.83	6.42	4.49	2.22	11.90	3.50	3.60	5.85	6.72	6.81	7.69
% Mean Accuracy (n = 5)	98.98	102.19	99.51	100.84	98.71	99.94	108.32	102.02	101.57	94.17	103.92	103.66	98.21

Conclusions

An LC/MS method for the quantitation of bupropion in human plasma was successfully transferred to an Agilent 6460 Triple Quadrupole LC/MS System. Since the MRM and LC conditions were kept the same, only a minimum amount of down time was necessary to optimize the source tuning parameters. This resulted in a successful method transfer with minimal down time. Five separate batches were analyzed for this repeatability study, with a linear fit R^2 value > 0.998. The precision and accuracy results for the QC samples in all batches meet the bioanalytical acceptable criteria.

References

1. Zhou, S., *et al*. Critical Review of Development, Validation, and Transfer for High Throughput Bioanalytical LC-MS/MS Methods. *Current Pharmaceutical Analysis*, **2005**, 1:3-14.
2. Bansal, S., and DeStefano, A. Key Elements of Bioanalytical Method Validation for Small Molecules. *The AAPS Journal*, **2007**, 9(1), Article 11.

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