Development of an LC/MS/MS Assay for Atorvastatin 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 demonstrate a case study of successful transfer of an LC/MS/MS assay between laboratories. The transfer of 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 (assuming the instruments have comparable sensitivity performance). In this application note, the transfer of a negative ion mode LC/MS/MS method for the analysis of atorvastatin and its hydroxyl metabolites in human plasma is demonstrated. This method was originally developed on a Shimadzu LC 20A system coupled to an ABI/SCIEX API 4000 System and this work describes its transfer 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 assay results were obtained on both systems for three 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/MS method for the analysis of atorvastatin and its hydroxyl metabolites was transferred from an ABI/Sciex API 4000 System to an Agilent 6460 Triple Quadrupole LC/MS System. Atorvastatin aids in lowering blood cholesterol. The two hydroxyl metabolites of atorvastatin are the ortho-atorvastatin (2-hydroxy) and para-atorvastatin (4-hydroxy) as shown in Figure 1. The Agilent 6460 source parameters were optimized, but the mobile phase, column, and MRM settings were not changed. A 10-point calibration curve was plotted from 0.200 to 200 ng/mL with plasma extracted samples. To demonstrate the effectiveness of the method transfer, linearity, precision, and accuracy obtained from the Agilent system were compared with the accepted criteria for validated bioanalytical methods.[1, 2]

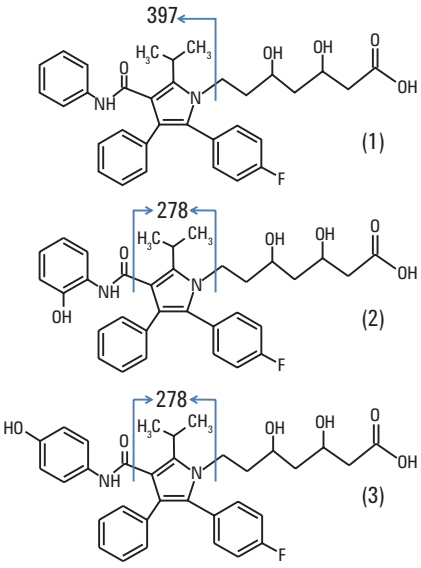

Figure 1. Molecular structure and fragmentation sites of atorvastatin (1), ortho-atorvastatin (2), and para-atorvastatin (3). Deuterated analogs of these three compounds were used as internal standards.

Experimental

Materials

The working standard, atorvastatin calcium and its hydroxyl metabolites, were obtained from Varda Biotech (Mumbai, India). Atorvastatin-D_5, ortho-atorvastatin-D_5, and para-atorvastatin-D_5 were used as internal standards (IS). HPLC grade acetonitrile, methanol, and mass spectrometry grade formic acid were obtained from Merck (Mumbai, India).

Analytical grade ammonium formate was obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). HPLC grade tertiary butyl methyl ether was obtained from Lab Scan. Deionized and purified water from a Milli-Q system (Millipore) was used for the mobile phase and for standard solution preparation. Control human plasma (K_2EDTA anticoagulant), used in 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	Kromosil C18 (100 x 4.60 mm, 5 µ); maintained at 40 °C	
Mobile phase	0.1 % formic acid:acetonitrile (30/70, v/v)	
Flow rate	0.5 mL/min, isocratic with 4.8 min run time	
Autosampler temperature	5 °C	
Injection volume	10 µL	
MRM	Atorvastatin 556.7 → 396.7 Atorvastatin-D_5 (IS) 561.7 → 401.7 Ortho-atorvastatin 572.7 → 277.7 Ortho-atorvastatin-D_5 (IS) 577.7 → 282.7 Para-atorvastatin 572.7 → 277.7 Para-atorvastatin-D_5 (IS) 577.7 → 282.7	
Agilent 6460 Triple Quadrupole LC/MS System	Drying gas flow:	7.0 L/min
	Nebulizer pressure:	50 psig
	Dry gas temperature:	300 °C
	Capillary voltage:	5500 V
	Sheath gas flow:	12.0 L/min
	Sheath gas temperature:	350 °C
	Nozzle voltage:	2000 V
	Ionization source:	Agilent Jet Stream
	Ionization mode:	Negative
	Collision energy:	25 eV (atorvastatin), 25 eV (IS) 35 eV (ortho- and para-atorvastatin and its IS)
	Fragmentor voltage:	135 V (all atorvastatin compounds)
	LC:	Agilent 1290 Infinity LC System

Standard curves

Standard and intermediate stock solutions were prepared in methanol. By adding known amounts of intermediate stock solutions to human plasma, calibration standards and quality control (QC) samples were prepared. The linearity range 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 atorvastatin. One batch refers to one set of linearity and six sets of QC samples. Three such batches were prepared and analyzed.

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

Level	Nominal concentrations (ng/mL)
L1	0.2045
L2	0.4091
L3	1.278
L4	3.995
L5	10.51
L6	19.47
L7	48.67
L8	97.35
L9	162.25
L10	202.8
LQC	0.5887
MQC	86.57
HQC	160.3

Extraction procedure

To 300 µL of atorvastatin spiked calibration plasma standards or QC samples, 50 µL of 10 ng/mL IS solution was added and vortexed for 5 s. Next, 100 µL of ammonium formate (5 mM) was added and the solution was vortexed. To extract atorvastatin, 2 mL of tertiary butyl methyl ether (tBME) was added to the sample. It was then vortexed for 10 min and centrifuged at 4500 rpm, at 4 °C for 5 min. Approximately 1.8 mL of supernatant was collected and evaporated to dryness under nitrogen at 40 ± 5 °C. The residue was reconstituted in 150 µL acetonitrile:mobile phase (50:50) and transferred to a glass vial for LC/MS/MS analysis.

Data acquisition

Data acquisition was performed using Agilent MassHunter Workstation software (B.03.01) and data processing was subsequently performed using MassHunter Quantitative analysis software (B.04.00).

Results and Discussion

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

Level 1 of the calibration curve

The level 1 (L1) concentration was one tenth of the C_{max} concentration of atorvastatin in plasma. Repeatability experiments on the LLOQ, level 1 calibration standard (0.2045 ng/mL) showed an acceptable value of 6.91 % CV. The S/N values for atorvastatin, ortho-atorvastatin, and para-atorvastatin were 162, 660, and 277 respectively (using peak height and RMS X1). A representative chromatogram for L1 is shown in Figure 2. Three separate determinations of the standard curves showed a mean accuracy of 102 % for L1.

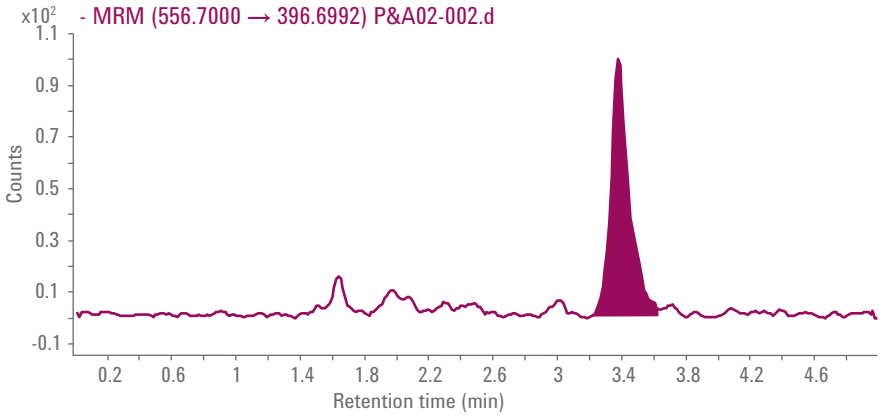

Figure 2. Chromatogram of atorvastatin at the LLOQ level (0.2045 ng/mL).

Linearity, precision, and accuracy

A 10-point calibration curve (in a three batch study) 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 three analytical batch runs, the precision (% CV) of calibration standards ranged from 0.49 % to 11.54 % and the % mean accuracy (back calculated values from linearity equation) ranged from 86.76 % to 108.59 % (Table 2). For the three analytical batches run, the precision (% CV) of QC samples at all concentrations ranged from 6.70 % to 8.91 % and the % mean accuracy of all the QC samples at all concentrations ranged from 90.98 % to 108.42 %. These results are within acceptable bioanalytical regulatory criteria. Therefore, this method can be deemed to have been effectively transferred to the Agilent 6460 Triple Quadrupole LC/MS System.

Speed of method transfer to the Agilent 6460 Triple Quadrupole LC/MS System

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

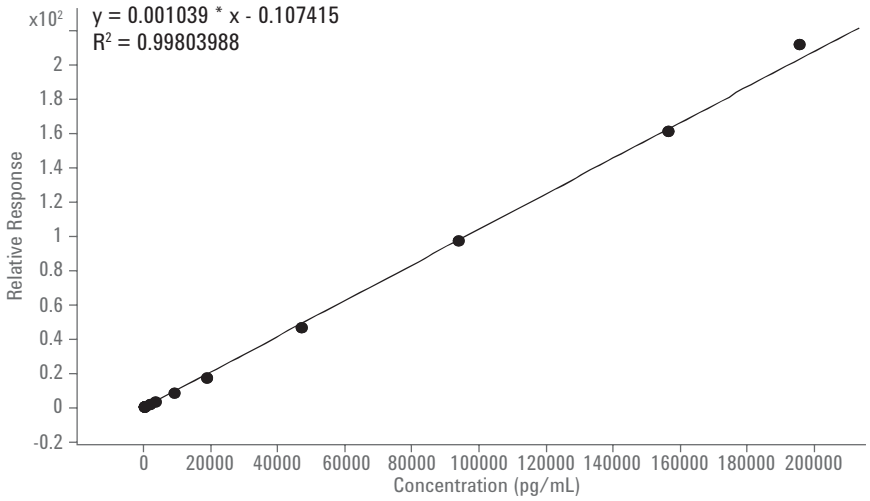

Figure 3. Calibration standard curve of atorvastatin as performed on an Agilent 6460 LC/MS/MS System.

Table 2. Precision and accuracy of atorvastatin calibrations standards and QC samples in human plasma obtained on the Agilent 6460 Triple Quadrupole LC/MS System.

Levels	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	HQC	MQC	LQC
% CV (n = 3)	6.91	11.54	8.65	8.07	6.30	9.42	1.44	0.49	10.19	3.99	8.91	7.80	6.70
% Mean Accuracy (n = 3)	102.67	99.98	106.80	86.76	88.79	89.16	99.71	108.59	100.53	99.37	108.42	103.58	90.98

Similar results were obtained for the other two hydroxyl metabolites of atorvastatin.

Conclusions

An LC/MS method for the quantitation of atorvastatin and its metabolites in human plasma was successfully transferred to an Agilent 6460 Triple Quadrupole system. Keeping the same MRM and LC conditions, only the source tuning parameters required optimization. This resulted in an acceptable method transfer with minimum amount of down time. Three separate batches were analyzed for a repeatability study which showed a linear fit with an R^2 value >0.998. Precision and accuracy for all QC samples, in all batches, meet the bioanalytical acceptance 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.
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