A Sensitive LC/MS/MS Method for the Quantification of Telmisartan in Human Plasma

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Introduction

In this study we describe a sensitive LC/MS/MS method for quantification of telmisartan in human plasma. Telmisartan is used for the treatment of hypertension and acts by blocking angiotensin II receptor. The deuterated analog, telmisartan-d3 was used as the internal standard in this study. The structures of the analyte and the internal standard are shown in figure 1. The high sensitivity of the 6460 mass spectrometer with the Agilent Jet Stream ESI source enabled the quantification of 50 pg/mL of telmisartan in human plasma. This makes the method suitable for the analysis of sample from low dose studies.
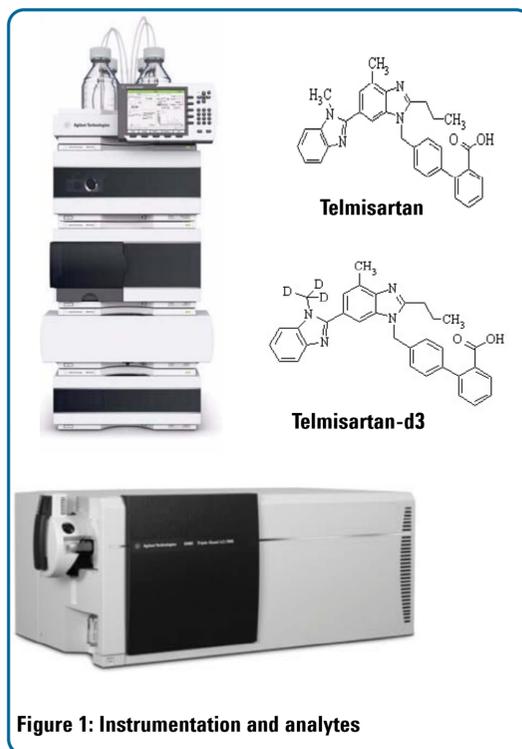

Telmisartan

Telmisartan-d3

Figure 1: Instrumentation and analytes

Experimental

System

G1312B   1260 Infinity binary pump

G1379B   1260 Infinity micro degasser

G1367D   1260 Infinity autosampler

G1330B   1260 Infinity Thermostat

G1316A 1260 Infinity Thermostatted Column Compartment

G6460   Triple Quadrupole Mass Spectrometer with the Agilent Jet Stream Technology

Software MassHunter B.03.01 (Acquisition and Qualitative Analysis)

MassHunter B.04.00 (Quantitative analysis

Column: ZORBAX Eclipse Plus - C8 3.0 X 50 mm, 1.8 μm

Column temperature: 45 °C

Mobile phase A: 10 mM Ammonium acetate

Mobile phase B: Acetonitrile

Flow rate: 0.5 mL/min

Time (min)	% Acetonitrile
0	20
0.5	95
2.5	95
2.6	20
Stop time	5 min

Source: ESI with Jet stream (+)

Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (eV)
515.2 (Telmisartan)	275.6	180	52
518.4 (Telmisartan-d3) (ISTD)	279.2	180	50

Results and Discussion

Tuning of the mass spectrometer was optimized using MassHunter Optimizer software, which identifies the most abundant product ions together with the optimum fragmentor voltages and collision energies. A simple protein precipitation sample preparation method was used to extract telmisartan and the internal standard, telmisartan-d3 from 200 μL of spiked plasma samples.
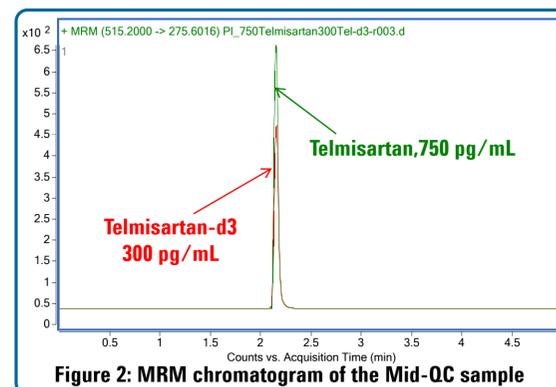

Figure 2: MRM chromatogram of the Mid-QC sample

All plasma samples were treated with 500 μL of cold acetonitrile and then vortexed for 1 min. 675 μL supernatant was transferred to a new microcentrifuge tube and dried by vacuum concentration. The residues were resuspended in 200 μL of 9:1 (v/v) water:methanol, and centrifuged prior to analysis.
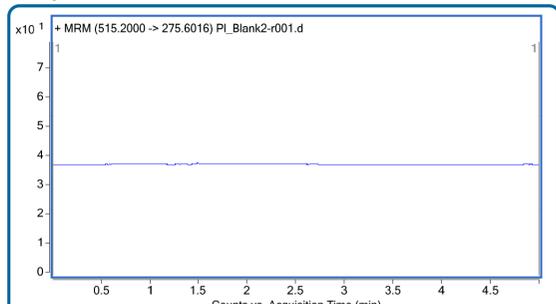

Figure 3: First blank injected after the ULOQ sample injections

No peak was seen for the telmisartan transition in the first plasma blank injected after injecting the ULOQ sample (5000 pg/mL) thrice as can be seen in figure 3.

The figure 4 shows the overlay of the telmisartan peaks in the LLOQ and blank samples. It can be seen that the area of the MRM signal in the blank is less than 20% of the area of analyte peak in the LLOQ sample confirming that the carryover meets the FDA bioanalytical recommended criteria.
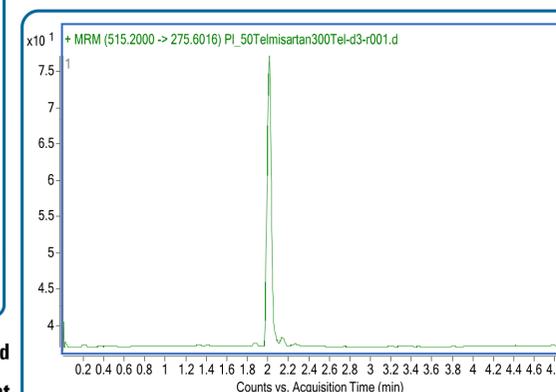

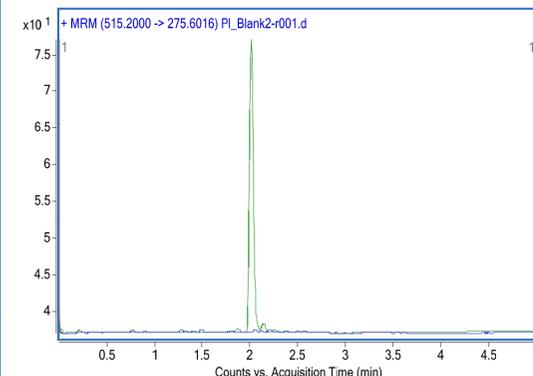Figure 4: Telmisartan peak in the LLOQ (50 pg/mL) sample

Figure 5: Overlay of the telmisartan peaks in the LLOQ and blank samples

Results and Discussion

Each calibration and QC sample was injected three times. The mean response ratios of telmisartan to telmisartan-d3 were plotted against the concentrations of telemisartan to obtain the calibration curve. A linear curve fitting was used and weighted (1/x). A linear dynamic range of 50 - 5000 pg/mL was achieved for telmisartan in human plasma with an R^2 value greater than 0.995. Figure 6 shows a representative calibration curve for telmisartan in plasma using telmisartan-d3 as the internal standard. From Table 1 it can be seen that the calculated concentrations for all calibration standards and the QC samples show good precision and accuracy.
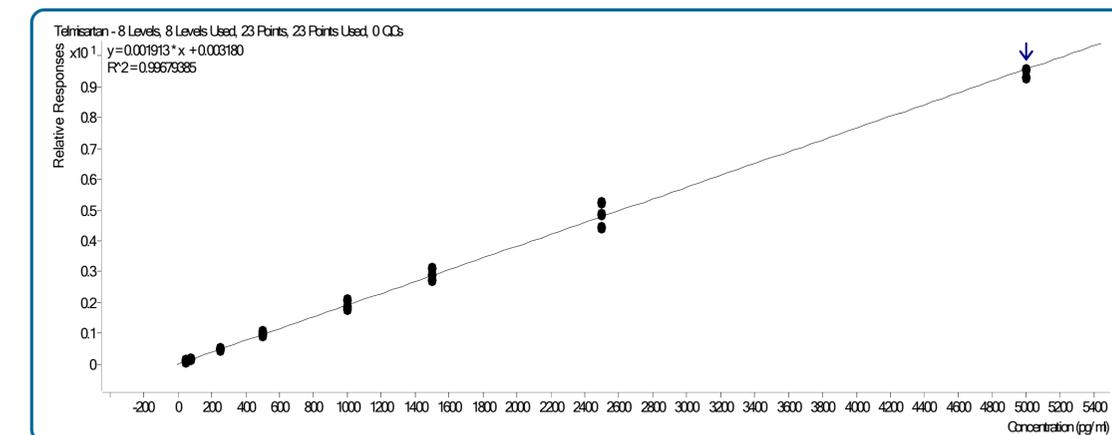

Figure 6:Standard curve in plasma with 3 replicate injections at each calibration level

Concentration (pg/mL)	Mean Calculated Concentration (n=3)	Precision (% R.S.D.)	Accuracy (%)
50	46.93	11.11	93.86
75	77.08	10.40	102.77
250	251.12	2.18	100.45
500	510.31	6.41	102.06
1000	994.50	7.49	99.45
1500	1519.06	6.54	101.27
2500	2531.09	8.34	101.24
5000	4617.22	11.35	92.34
150 (LQC)	130.68	5.80	87.12
750 (MQC)	773.39	4.17	103.12
4000 (HQC)	3746.79	7.95	93.67

Table 1: Response ratios, precision and accuracy at the various concentrations

Conclusions

A sensitive and selective LC/MS/MS method for the quantification of telmisartan in human plasma using an Agilent 1260 Infinity LC system and 6460 Triple Quadrupole Mass Spectrometer with the Agilent Jet Stream ESI source has been described. A simple protein precipitation method was used for extracting the analyte and the internal standard from plasma. The response ratio of the analyte and the internal standard was found to be linear over the range 50 - 5000 pg/mL.