A Sensitive LC/MS/MS Method for the Quantitation of Telmisartan in Human Plasma using the Agilent 6460 Triple Quadrupole LC/MS with Jet Stream Technology

Application Note

Clinical Research

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Abstract

A high sensitivity LC/MS/MS analytical method suitable for the analysis of samples from low dose studies using an Agilent 1260 Infinity LC system coupled to a 6460 Triple Quadrupole LC/MS system is described. A simple protein precipitation sample preparation method was used to extract telmisartan and the internal standard, telmisartan-d3, from plasma samples. The sensitivity of the 6460 Triple Quad LC/MS system enabled with Agilent Jet Stream technology facilitated the quantitation of telmisartan in human plasma over the concentration range of 50 to 5000 pg/mL. This is one order of magnitude below the lower limit of quantitation (LLOQ) reported in the literature, 0.5 ng/mL[1]. Linear calibration curves for telmisartan with R^2 values ≥ 0.99 were achieved over the assay range with good reproducibility.

Introduction

For quantitation of drugs in biofluids, simple sample preparation protocols, and sensitive and specific techniques are preferred. LC/MS/MS is the method of choice for analyzing drugs extracted from biological matrices by protein precipitation.

In this note, we describe how the Agilent 6460 Triple Quad LC/MS system enabled with Agilent Jet Stream technology is suitable for analyzing samples from low dose studies. Telmisartan is used for the treatment of hypertension and acts by blocking the angiotensin II receptor. The deuterated analog, telmisartan-d3, was used as the internal standard in this study (Figure 1). In the literature, the plasma concentrations (Cmax) following a single oral dose of 80 mg telmisartan have been reported to be in the range 300 – 500 ng/mL[1, 2]. Hence, the high sensitivity of the present method makes it suitable for the analysis of samples from low dose pharmacokinetic (PK) studies.

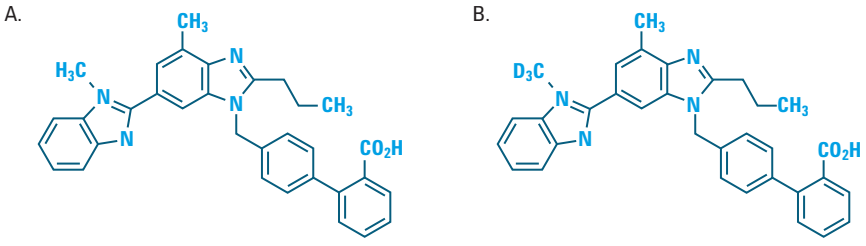

Figure 1. Structures of (A) telmisartan and (B) telmisartan-d3.

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Experimental

Sample Preparation

Materials

Human plasma (lyophilized), telmisartan, and ammonium acetate were obtained from Sigma-Aldrich, Bangalore, India. Telmisartan-d3 was purchased from LGC Promochem Mumbai, India. Acetonitrile and methanol were obtained from Fluka, India.

Preparation of aqueous standards

Stock solutions of telmisartan and telmisartan-d3 were prepared in methanol. The 250 µg/mL telmisartan stock solution was diluted with methanol to prepare 1 µg/mL and 0.5 µg/mL solutions. These two solutions were then serially diluted with water or 9:1 (v/v) water/methanol to obtain aqueous telmisartan standard solutions at concentrations of 0.5. 0.75, 1.5, 2.5, 5.0, 7.5, 10, 15, 25, 40, and 50 ng/mL. The telmisartan-d3 stock solution was serially diluted with 9:1 (v/v) water/methanol to obtain a 3 ng/mL aqueous solution.

Preparation of plasma calibration standards and blanks

Plasma calibration standards: 180 µL of human plasma was spiked with 20 µL of each aqueous telmisartan standard solution and 20 µL of 3 ng/mL aqueous telmisartan-d3 solutions. The concentration of telmisartan in the plasma samples ranged from 50 - 5000 pg/mL. These included 3 QC samples with concentrations of 150, 750, and 4000 pg/mL. The concentration of telmisartan-d3 (internal standard) in all plasma calibration standards was 300 pg/mL.

Plasma blank: 180 µL of plasma was spiked with 40 µL of 9:1 (v/v) water/methanol.

Plasma blank with internal standard: 180 µL of plasma was spiked with 20 µL of 9:1(v/v) water/methanol and 20 µL of 3 ng/mL telmisartan-d3 solutions.

A simple protein precipitation method was used to extract telmisartan and the internal standard. All plasma samples were treated with 500 µL of cold acetonitrile and then vortexed for 1 min. Next, 675 µL of supernatant was transferred to a new microcentrifuge tube and dried by vacuum concentration. The residues were redissolved in 200 µL of 9:1 (v/v) water/methanol and centrifuged prior to analysis.

LC/MS Analysis

Instrumentation

The Agilent 1260 Infinity LC system was coupled to the 6460 Triple Quadrupole LC/MS platform enabled with Agilent Jet Stream technology for LC/MS/MS analyses.

LC Conditions

Flow rate:	0.5 mL/min
Solvents:	10 mM ammonium acetate (A); acetonitrile (B). Flush volume was set at 4 µL
Sample Load:	10 µL
Column:	Agilent ZORBAX RRHT Eclipse Plus-C8, 3.0 x 50 mm, 1.8 µm
Column Temperature:	45 °C
Gradient Program:	2 % B initially; ramp up to 95 % over 0.5 minutes; hold for 2 minutes; bring to 20 %; hold for 2.4 minutes

MS Conditions

Spectra were recorded in positive ion mode using electrospray ionization enabled with Agilent Jet Stream technology.

Drying gas temperature and flow rate:	350 °C, 10 L/min
Nebulizer gas pressure:	40 psi
Capillary voltage:	4000 V
Fragmentor voltage:	180 V (for both the transitions)
Sheath gas temperature and flow rate:	400 °C, 11 L/min
Delta EMV:	300 V
Multiple Reaction Monitoring (MRM):	Telmisartan 515.2 -> 275.6 (Collision Energy= 52 V); telmisartan-d3 internal standard 518.4 -> 279.2 (Collision Energy=50 V)

Data Analysis

The data obtained were analyzed using Agilent MassHunter Quantitative Analysis software.

Results and Discussion

The high sensitivity of the Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream technology enabled the quantitation of 50 pg/mL of telmisartan in plasma (Figure 3B). The chromatogram of telmisartan and telmisartan-d3 in an extracted plasma sample is shown in Figure 2.

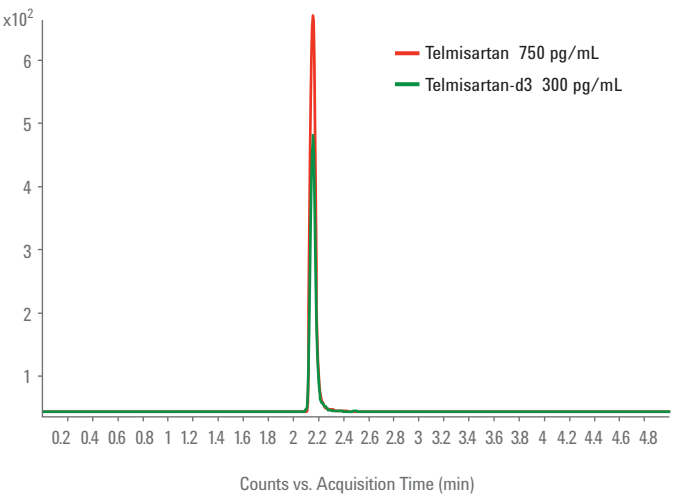

Figure 2. MRM chromatograms for telmisartan and its internal standard telmisartan-d3.

No peak was seen for the telmisartan quantifier transition in the first plasma blank injected after injecting the upper limit of quantitation (ULOQ) sample (5000 pg/mL) three times. Fig. 3C shows the overlay of the telmisartan MRM peak in the lower limit of quantitation (LLOQ) and blank samples. In this case 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 recommended bioanalytical criteria.

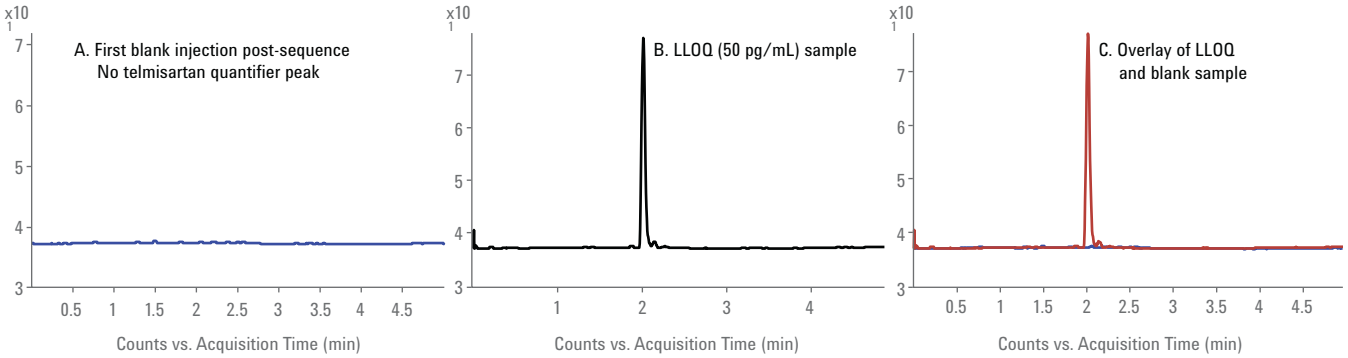

Figure 3. Post injection blank (A) and overlays of telmisartan MRM peak in the LLOQ (B) and blank samples (C).

Each calibration and QC sample was injected three times. The mean response ratios of telmisartan to telmisartan-d3 were plotted against the concentrations of telmisartan to obtain the calibration curve. Linear curve fitting was used and weighted (1/x). A linear dynamic range of 50 - 5000 pg/mL was achieved for telmisartan with an R^2 value of 0.9968. Figure 4 shows a representative calibration curve of telmisartan in plasma using telmisartan-d3 as the internal standard.

The calculated concentrations for all calibration standards and the QC samples showed good precision and accuracy as shown in Table 1. Comparable precision and accuracy values computed for data from 6 replicate injections have been reported for the QC samples in the literature [1, 2].

Concentration (pg/mL)	Mean Calculated Concentration (n+3)	Precision (%R.S.D.)	Accuracy (%)
50	46.93	11.11	93.86
75	77.08	10.4	102.77
250	251.12	2.18	100.45
500	510.31	6.41	102.06
1000	994.5	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 (QC 1)	130.68	5.8	87.12
750 (QC 2)	773.39	4.17	103.12
4000 (QC 3)	3746.79	7.95	93.67

Table 1. Calculated values of calibration standards and QC samples.

Conclusions

A sensitive and selective LC/MS/MS analytical method for the quantitation of telmisartan in human plasma has been demonstrated using an Agilent 1260 Infinity LC and 6460 Triple Quadrupole LC/MS system. The sensitivity of the 6460 Triple Quad LC/MS system enabled with Agilent Jet Stream technology allowed the quantitation of telmisartan in human plasma with a lower limit at 50 pg/mL. The LLOQ of 50 pg/mL reported in this method is one order of magnitude below the lowest LLOQ reported in the literature[1]. Linear calibration curves for telmisartan with R^2 values ≥ 0.99 were achieved over the concentration range of 50 - 5000 pg/mL in human plasma with good reproducibility.

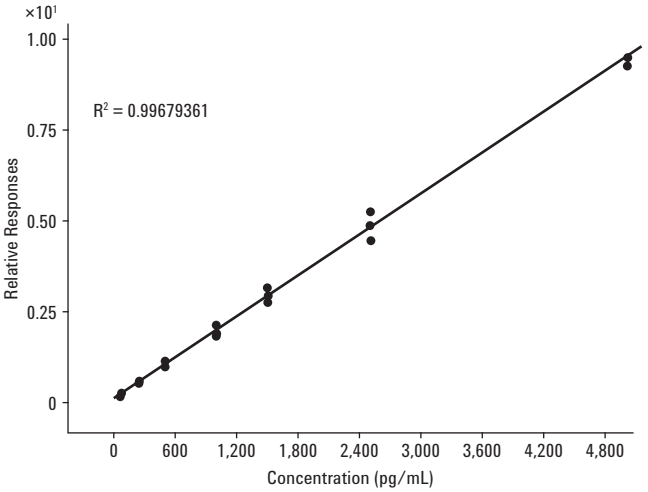

Figure 4. Standard curve in plasma with 3 replicate injections at each calibration level.

References

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