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Application News

No. AD-0077

Quantitative Bioanalysis / LCMS-8050

A High Sensitivity LC/MS/MS Method for Quantitative Determination of Telmisartan in Human Plasma

❑ Introduction

Telmisartan is an angiotensin II receptor antagonist commonly used in the treatment of hypertension and heart failure. Bioanalysis of telmisartan has been reported for human pharmacokinetics and bioequivalence studies, which is carried out mostly on LC/MS/MS with solid phase extraction (SPE) method for high sensitivity and selectivity. The reliability of a bioassay depends on the performance of LC/MS/MS system employed and the method of sample pre-treatment. With rapid progress of interface and triple quadrupole MS techniques, simpler sample pre-treatment without SPE may be adopted to achieve not only reliable quantitative results, but also higher throughput and lower running cost. In this issue of Application News, a high sensitivity LC/MS/MS method for quantitative determination of telmisartan in human plasma is described. The method was developed on LCMS-8050, a tandem mass spectrometer with a heated ESI interface. The high sensitivity and robust interface design of the system allow the use of protein-precipitation only in plasma pre-treatment and achieve a LLOQ of 4 pg/mL in plasma.

❑ Experimental

Preparation of calibrants and Samples

Pooled human plasma obtained from i-DNA Biotechnology was used as bio-matrix in this work. Stock solutions of telmisartan and irbesartan, the latter as internal standard (IS), were prepared from high purity solid chemicals in the diluent (acetonitrile and 0.1% formic acid - water, 60/40, vol/vol). The sample pre-treatment without SPE or other cleanup method is shown in Figure 1. Pure acetonitrile was added to the plasma, with or without spiked telmisartan and IS, in a ratio of 3:1 for protein precipitation.

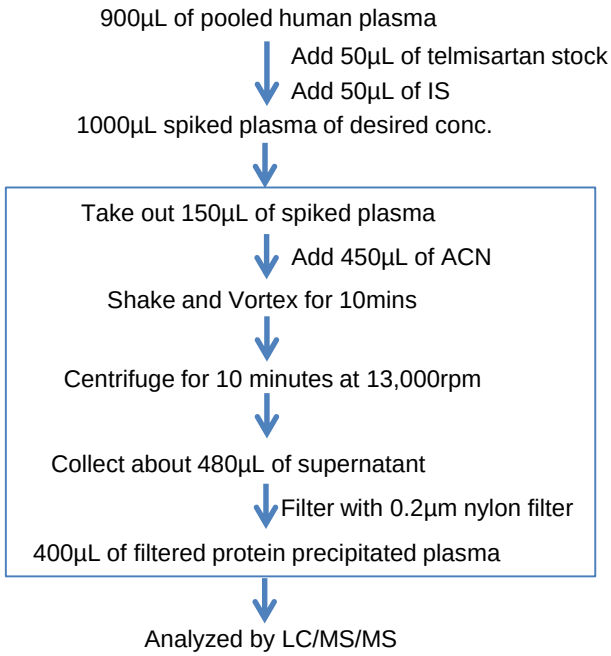

Figure 1: Flow chart of protein-precipitation (steps in the box) of a pre-spiked plasma sample.

Analytical system and conditions

Shimadzu's LCMS-8050 with a heated ESI interface coupled with a Nexera UHPLC system was employed. A ZORBAX Eclipse Plus C18 column (3.0mmID, 100mmL, 1.8 µm) was used. An isocratic elution method was adopted with a mobile phase of 40% water - 60% acetonitrile with 0.04% formic acid, a flow rate of 0.4 mL/min and oven temperature of 50°C. The MS interface conditions were: ESI in positive mode, block Temp at 400°C, DL Temp. at 200°C, nebulizing gas (N_2) at 2 L/min, drying gas (N_2) at 6 L/min and heating gas (purified air) at 14 L/min. The injection volume of sample was 5 µL.

❑ Results and Discussion

MRM method with internal standard (IS)

A MRM method was established on the LCMS-8050 with the optimized MRM parameters as shown in Table 1. Telmisartan and IS were eluted as sharp peaks under the isocratic LC conditions as shown in Figure 2. The first MRMs of both compounds were used to establish internal standard (IS) calibration method. The second MRMs of them were used as reference ions for identification.

Table 1: Retention times, MRMs and optimized CID voltages for analysis of telmisartan with IS on LCMS-8050

Name	RT (min)	Transition (m/z)	Voltage (V)		
			Q1 Pre Bias	CE	Q3 Pre Bias
Telmisar-tan	1.127	515.2 > 276.1	-26	-49	-30
		515.2 > 497.2	-26	-35	-25
Irbesartan (IS)	1.448	429.2 > 207.0	-22	-25	-22
		429.2 > 195.1	-22	-23	-21

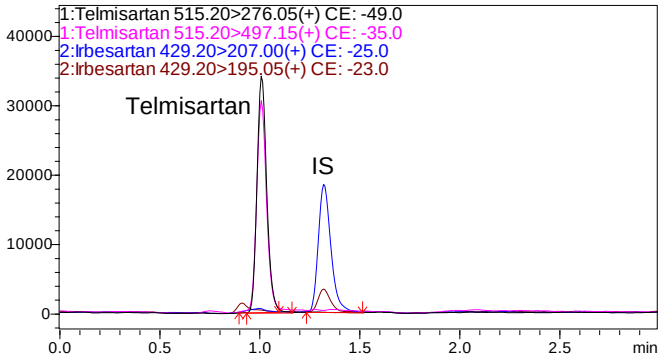

Figure 2: MRM chromatograms of telmisartan (50 pg/mL) and IS (50 pg/mL) post-spiked in plasma.

Calibration curve and linearity

A quantification method for telmisartan in plasma was set up based on the first MRM transitions of telmisartan and IS in Table 1. Linear calibration curves were established by IS method for plasma samples prepared by pre-spiked and post-spiked procedures, the latter is displayed in Figure 3. Excellent linearity was obtained with R^2 coefficient greater than 0.999 across the range from 1.0 pg/mL to 2000 pg/mL. The details of the calibrant series, accuracy and recovery in duplicate measurements are shown in Table 2.

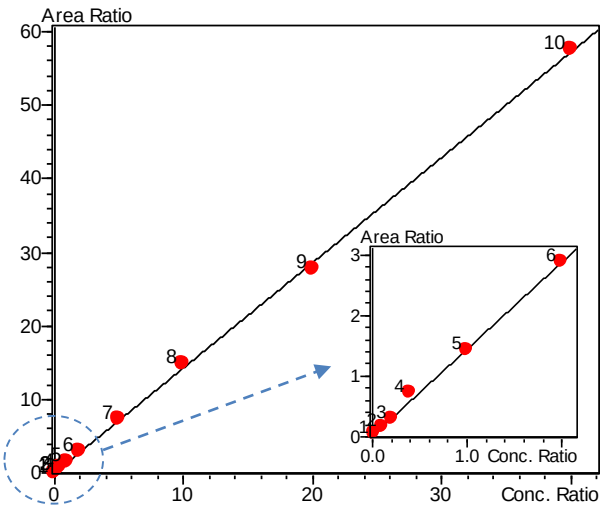

Figure 3: IS calibration curve of telmisartan post-spiked in plasma, ranging from 1 pg/mL ~ 2000 pg/mL.

Evaluation of method performance

The LLOQ of the method was determined with post-spiked plasma sample at 1.0 pg/mL. The average S/N ratio of the telmisartan peak was 11, which resulted in LOQ of 0.8 pg/mL. The repeatability of this level obtained was RSD = 13.3% (see Table 3). With QC1 sample (5.0 pg/mL post-spiked in plasma), the average S/N obtained was 40 and the LOQ calculated from the LabSolutions was 1.1 pg/mL (RSD = 3.2%, see Table 3). Based on the above results, it can be confirmed that the LLOQ of the method is at 1 pg/mL in plasma solution and 4 pg/mL in plasma before adding three volumes of acetonitrile for protein precipitation (dilution factor of sample preparation: 4.0).

Table 2: Calibrant series and performance of IS calibration method for quantitation of telmisartan in plasma

Level[1]	Conc. Calc. (pg/mL)	Tel/IS. Ratio[2]	Conc. Mea. (pg/mL)	Accuracy (%)	Recovery (%)
1	1	0.02	0.85	85.2	132.1
2	5	0.1	4.69	93.8	107.4
3	10	0.2	9.67	96.7	95.4
4	20	0.4	24.7	123.5	91.7
5	50	1	49.6	99.3	90.2
6	100	2	99.8	99.8	89.4
7	250	5	252.0	100.8	93.7
8	500	10	518.2	103.7	95.2
9	1000	20	970.9	97.1	94.1
10	2000	40	2008.2	100.4	96.4

[1] Duplicate injections of each level; [2] IS: 50 pg/mL

Table 3: Performance evaluation of quantitation method for telmisartan in plasma with LLOQ and low QC samples

Sample	Conc. Cal. (pg/mL)[1]	Ave. Conc. (pg/mL)	Accuracy (%)	RSD (%)
LLOQ	1	0.83	82.8	13.3
QC1	5	4.65	93.8	3.2
QC2	20	23.91	123.5	2.3

[1] IS: 50 pg/mL

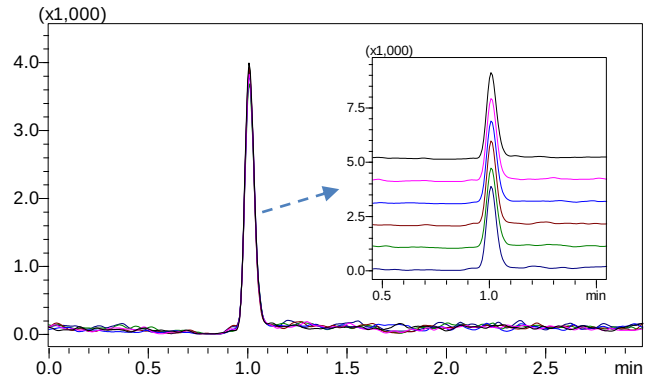

Figure 4: Overlay of six chromatograms of QC1 (5.0 pg/mL telmisartan post-spiked in plasma)

The accuracy and precision of the method were evaluated with low QC samples. The results are compiled into Table 3. The six MRM chromatograms of consecutive injections of QC1 are plotted into Figure 4, which shows impressively the excellent reproducibility of the analysis on LCMS-8050.

It is worth to note that the method exhibited a certain level of ion enhancement at all concentrations. In addition, a small interference peak was found from the plasma due to the simple sample pretreatment and fast elution of telmisartan peak. Validation of the method is needed for applying it in bioanalysis of actual samples.

❑ Conclusions

A high sensitivity bioanalytical method for determination of telmisartan in human plasma was established on LCMS-8050 with a heated ESI. The method allows the use of simple sample pre-treatment, achieving high sensitivity and reliable performance required for bioanalysis.

Note: The data shown in this Application News are for Research Use Only. Not used for clinical diagnostic purposes.

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