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Estimation of Telmisartan in Human Plasma by Reversed Phase Liquid Chromatography Coupled with Tandem Mass Spectrometry - A Bioequivalence Study Application

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ABSTRACT

A sensitive, accurate and rapid reverse phase Liquid chromatography coupled tandem mass spectrometry (LCMS/MS) method was described to estimate Telmisartan in human plasma. Detection was made at m/z 513.2/469.3 for Telmisartan and 344/193.8 for internal standard using ESI Negative ion spray ionization mode. Analyst 1.5.1 software was used for the quantification. The stationary phase was Hypurity Advance C18, 50 X 4.6 mm, 5µm. The separation method developed produce recovery of 84.67%. Acceptable intra-day and inter-day precision (<15%) and accuracy (<10% diff.) were observed over the linear range of 2.901 to 330.015 ng/mL. The absence of any matrix effects was displayed. The retention time of analyte and internal standard was 1.38 and 1.41 minutes. The developed and validated method was successfully applied for bioequivalence and pharmacokinetic studies.

Keywords: Human plasma; Telmisartan; Protein Precipitate Method; LCMS/MS.

INTRODUCTION

Telmisartan is a non-peptide angiotensin II receptor (type AT^1) antagonist. Telmisartan is chemically described as 4'-[(1, 4'-dimethyl-2'-propyl [2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid. Its empirical formula is $C_{33}H_{30}N_4O_2$, its molecular weight is 514.63, and for structural formula refer Figure No: 1.

Telmisartan is a white to slightly yellowish solid. It is practically insoluble in water and in the pH range of 3 to 9, sparingly soluble in strong acid (except insoluble in hydrochloric acid), and soluble in strong base (1).

Several LCMS methods have been reported for the quantitative analysis of Telmisartan in human plasma (2-13). Compared to the reported methods, present estimation performed with a simple mobile phase consisting of Acetonitrile and ammonium formate and this method reports a sensitive, cost effective and simple method for the determination of Telmisartan in human by

single step precipitation, also faster and simpler than other methods. The injected samples were diluted two times to avoid possible contamination of mass spectrometer. Additionally stability parameters like wet extract, wet extract bench top, extended batch verification, blood stability, hemolytic and lipemic effect, concomitant drug effect were also performed which are not reported in other available methods. We have applied this method for the bioequivalence study of Telmisartan.

MATERIALS AND METHODS

Chemicals

Acetonitrile and methanol of HPLC grade was procured from JT Becker. Water HPLC grade was obtained from a Milli-Q water purification system. Ammonium Formate was procured from CDH. A reference standard of Telmisartan & omeprazole internal standard was provided by Strides Arco lab Limited and SeQuent Research Limited, India.

Instrumentation and Chromatographic Conditions

Ultra flow liquid chromatography Coupled with Mass Spectrometry was used for the method development and validation. Mass Spectrometry Model API 4000, UFLC model UFLC XR equipped with a model LC-20ADXR a binary pump, SIL-20ACXR auto sampler was used to keep temperature at 5°C, CTO-20AC column oven used to keep temperature at 35° C and CBM-20Alite system controller. Detection was made at m/z 513.2/469.3 for Telmisartan and 344/193.8 for internal standard using ESI negative ion spray ionization mode. Analyst 1.5.1 software was used for the quantification. The stationary phase was Hypurity Advance C18, 50 X 4.6 mm, 5µm.

LCMS Method Development

The procedure was developed to validate a method for the estimation for Telmisartan in human plasma using K_3EDTA as an anti coagulant. The standard stock solution was diluted with methanol to 50 ng/mL before injecting into the Hypurity Advance C18 column with Different ratios of Acetonitrile and Ammonium formate buffer. The optimum mobile phase was found to be Acetonitrile: Ammonium formate buffer 2 mM (70:30 v/v). The separation was carried out at ambient temperature with a flow rate of 0.5 mL by using split. The injection volume was 5 µL and run time was 2 minutes. The RT of analyte and internal standard was 1.38 and 1.41 minutes.

Sample Processing (Protein Precipitate Method)

Aliquoted 100 µL of plasma from the pre-labelled polypropylene tubes into RIA vials and added 20 µL of Omeprazole internal standard (approx.300 ng/mL) and vortexed. Plasma was precipitated with 500 µL Acetonitrile, vortexed and centrifuged for 10 minutes at 4000 rpm and 4°C. Supernatant was diluted to 1 ml with dilution solvent.

20 µL of Omeprazole internal standard was added to 100 µL of plasma vortexed and Precipitated with 500 µL Acetonitrile, centrifuged for 10 minutes at 4000 rpm and 4°C. Supernatant was diluted to 1 ml with dilution solvent.

Calibration and Quality Control Samples:

Stock solutions of Telmisartan were made up in methanol at approximately 0.5 mg/mL and these stock solutions were refrigerated. Working standard solutions of varying concentrations of Telmisartan were prepared on the day of analysis by diluting the stocks with dilution solution.

Calibration curve standards consisting of a set of eight non-zero concentrations ranging from 2.901 ng/mL to 330.015 ng/mL for Telmisartan were prepared. Prepared quality control samples

consisted of Telmisartan concentrations of 2.905 ng/mL (QCLLQ), 11.250 ng/mL (QCL), 130.750 ng/mL (QCM) and 235.258 ng/mL (QCH). These samples were stored below -50 °C until used. Twelve sets of QCL and QCH were stored to below -20 °C freezer for generation of below -20 °C stability.

System Suitability

The system suitability was performed before starting each day's activity according to in-house and it was within acceptance criteria less than or equal to 2 % for area ratio and less than or equal to 4 % for RT.

Method Validation Parameters

The method was validated over a concentration range of 2.901 ng/mL to 330.015 ng/mL for Telmisartan. This validation provides the results of specificity and selectivity, carryover, matrix effect, calibration standards and quality control samples data, precision and accuracy data, the results of various stabilities, dilution integrity, reinjection reproducibility, ruggedness, Extended Batch verification, concomitant drug effect, effect of haemolysed and lipemic plasma and blood stability.

RESULTS AND DISCUSSION

Specificity / Selectivity

Specificity and selectivity were performed in plasma obtained from nine different lots. No interference was observed at the RT of Telmisartan and Internal standard.

Matrix Effect

Blank samples (plasma) from six independent sources of matrix were processed in duplicate and then spiked with analyte at QCL and QCH level and internal standard at the concentration used in the method being validated just before injection into the LC-MS/MS. An aqueous solution of analyte was prepared at QCL and QCH with internal standard in diluent (Reference solution). Peak area ratios of the plasma samples were compared with that of reference solution to ensure that the matrix factor was consistent for different sources of matrix.

The IS-normalized matrix factor was found to be 0.9420 for QCL and 0.9840 for QCH (close to unity) for six different matrix lots for Telmisartan and the % CV was 0.95 for QCL and 0.99 for QCH.

Signal to Noise Ratio

Signal to Noise ratio was obtained at the lower limit of quantification (LLOQ) from the chromatogram by comparing the area obtained at LLOQ for each lot used in the specificity / selectivity experiment with area obtained in respective blank samples. The signal-to-noise ratio obtained for the samples was greater than 5 for all the plasma lots tested.

Carry Over

Processed and injected Blank, 2LLOQ and 2ULOQ samples and re-injected blank samples to check carry over. The % carry over was found to be 0.00 for analyte and 0.00 for internal standard.

Linearity

A regression equation with a weighting factor of $1/x^2$ of drug to IS concentration was judged to produce the best fit for the concentration-detector response relationship for Telmisartan in human plasma. The representative calibration curves for regression analysis are illustrated in Figure 4.

Correlation coefficient (r^2) was greater than 0.9985 in the concentration range of 2.901 ng/mL to 330.015 ng/mL for Telmisartan.

Precision and Accuracy

The precision of the assay was measured by the percent coefficient of variation over the concentration range of QCLLOQ, QCL, QCM and QCH samples respectively during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LLOQ, low, middle and high quality control samples to their respective nominal values, expressed in percentage.

Within-Batch Precision and Accuracy

PA 1: Within batch precision ranged from 0.68 % - 2.94% (QCH - QCL) and accuracy ranged from103.60 % - 105.85% (QCH - QCLLQ).

PA2: Within batch precision ranged from 1.81 % - 3.13% (QCM - QCLLQ) and accuracy ranged from 102.11 % - 107.80% (QCH - QCL).

PA3: Within batch precision ranged from 2.42 % - 4.61 % (QCM - QCLLQ) and accuracy ranged from 103.35 % - 108.77 % (QCLLQ -QCM).

Intraday Batch Precision and Accuracy

1st day: Intraday precision ranged from 1.65 % - 2.93 % (QCM - QCL) and accuracy ranged from 102.85 % - 106.45% (QCH - QCL).

2nd day: Intraday precision ranged from 2.42 % - 4.61 % (QCL - QCLLQ) and accuracy ranged from 103.35 % - 108.77 % (QCLLQ - QCH).

Between Batch Precision and Accuracy

The between batch precision ranged from 2.17 % - 3.41 % (QCM - QCLLQ) and the within batch accuracy ranged from 103.56 % - 107.17 % (QCH - QCM).

Recovery

Prepared 6 sets of recovery comparison samples by spiking 5 µL of dilution of quality control samples (QCL, QCM, QCH) of Telmisartan, 20 µL of internal standard dilution (approx. 300 ng/mL) and 975 µL of diluent, representing 100 % extraction and injected. The recovery comparison samples of Telmisartan were compared against extracted samples of QCL, QCM and QCH samples of PA1 batch.

The mean overall recovery of Telmisartan was 84.67 % with a precision of 2.60 %. The mean recovery of internal standard was 79.52 %. Recovery of Internal Standard was similar to the analyte henceforth omeprazole was preferred as an internal standard.

Dilution Integrity

Twelve dilution integrity samples were prepared by spiking approximately 1.7 times (561.235 ng/mL) of the highest standard concentration (330.015 ng/mL). Six dilution integrity samples were processed by diluting them twice and another six samples by diluting them four times using pooled plasma. These samples were analyzed along with a PA2 batch .The sample concentrations were calculated using appropriate dilution factor. Results demonstrated acceptable dilution integrity for two times and four times dilution.

The within batch precision and accuracy, for a dilution factor of 2 was 1.29 % and 97.46 %. The within batch precision and accuracy, for a dilution factor of 4 was 4.52 % and 107.38 %.

Re-injection Reproducibility

One precision and accuracy batch (PA1) was retained in the auto sampler at 5°C for 45 hours to test the re-injection reproducibility of the method. The results demonstrate that the reinjection of the sample was reproducible for 45 hours

The mean accuracy ranged from 101.79 % (QCLLQ) to 106.74 % (QCL) and the precision ranged from 2.36 % (QCH) to 5.88 % (QCLLQ).

Stabilities

Freeze-Thaw Stability Three Cycles

The stability in human plasma was determined for three freeze-thaw cycles. Six replicates of QCL and QCH were analyzed after undergoing three freeze-thaw cycles. The freeze-thaw quality control samples were quantified against the freshly spiked calibration curve standards and % change calculated against fresh quality control samples.

The % nominal ranged from 100.71 % to 101.76 % and precision ranged from 0.89 % to 2.41 % and % change ranged from 0.69 to 0.91 respectively.

Bench Top Stability

Bench top stability was determined for 24 hours, using six sets each of QCL and QCH. The quality control samples were quantified against the freshly spiked calibration curve standards and % change calculated against fresh quality control samples. Telmisartan was found to be stable for 24 hours.

The % nominal ranged from 100.56 % to 102.08 % and the precision ranged from 1.65 % to 1.65 %, and % change ranged from 0.76 to 1.13.

Long Term Stability at below -20° C and -50°C

To assess the stability of the analytes in the biological matrix under the same conditions of storage as that of the study samples the following test was performed.

Six samples of each quality control samples at low and high concentrations were stored below -20°C and -50°C in the freezer for 61 days. These samples were quantified against the freshly spiked calibration curve standards and % change calculated against fresh quality control samples. The % nominal ranged from 100.15 % to 100.87 % and precision ranged from 1.71% to 2.70 % and % change ranged from 0.19 to 0.36 respectively for samples stored at -20°C.

The % nominal ranged from 100.33 % to 101.92 % and precision ranged from 1.04 % to 2.81 % and % change ranged 0.53 to 0.86 from respectively for samples stored at-50°C.

Wet Extract Stability

To assess the wet extract stability, six sets of QCL and QCH samples were extracted and retained in the auto sampler to prove wet extract / auto sampler stability. These samples were injected after a period of 34 hours and were quantified against freshly spiked calibration curve standards and % change calculated against fresh quality control samples. The results demonstrate that the processed samples were stable for 34 hours in auto injector.

The % nominal at around 34 hours ranged from 101.03 % to 102.02, precision ranged from 1.20 % to 4.59 % and % change ranged from 0.95 to 1.24 respectively.

Wet Extract Bench Top Stability

To assess the wet extract stability on bench top at room temperature, six sets of QCL and QCH were processed, reconstituted and kept on bench top at room temperature. These samples were injected after a period of 3 hours and were quantified against freshly spiked calibration curve standards and % change calculated against fresh quality control samples. The results demonstrate that the processed samples were stable for 3 hours on bench top at room temperature.

The % nominal at around 3 hours ranged from 101.44 % to 102.21, precision ranged from 1.47 % to 4.18 % and % change ranged from 1.14 to 1.64 respectively.

Stock Dilution Stability

The stability of stock dilutions of analytes and the internal standard was evaluated at room temperature. Aqueous stock dilutions of the analytes and the internal standard were prepared. One portion of the stock dilution was placed in the refrigerator between 2-8°C, while the other portion was placed at room temperature for 24 hours.

The percent change for Telmisartan was 1.41 % and for Omeprazole is 0.51 %, respectively.

Stock Solution Stability

Stock solution stability was carried out for 23 days by injecting six replicates of stock dilution of stability standards (analyte and internal standard which prepared and stored in the refrigerator between 2 - 8° C) and freshly prepared stock dilutions of Comparison standard (analyte and internal standard). The response of stability sample was corrected by multiplying with correction factor.

The percent change for Telmisartan was 0.12 % and for Omeprazole is 0.08 %, respectively.

Hemolysed and Lipemic Effect

To assess the effect of hemolysed and lipemic biological matrix on analyte the following test was performed. Six samples of each quality control samples at low and high concentrations were freshly prepared in hemolysed and lipemic matrix. These samples were quantified against the freshly spiked calibration curve standards. The stability of the analytes was evaluated by comparing each of the back calculated concentrations of stability QCs with the nominal concentration of QCs.

The percent nominal ranged from 101.03 % to 102.02 % and precision ranged from 1.20 % (QCH) to 4.59 % (QCL) and percent change ranged 0.95 to 1.24 from respectively.

Extended Batch Verification

To check the batch size of the run during the study sample analysis following experiments was preformed. Processed one set of CC and six sets of QCL, QCM and QCH along with 102 blank samples. These QC samples were interspersed with the blank samples and processed.

The % nominal for calibration curve standards ranged from 96.46 to 104.63%. The accuracy of QCL, QCM and QCH was found to be 94.91, 100.87 and100.58 respectively. The precision of QCL, QCM and QCH was found to be 3.90, 2.07, and 2.79 respectively.

Ruggedness

One precision and accuracy batch was processed and analyzed by different analyst using different column.

The mean accuracy ranged from 101.79 % (QCLLQ) to 106.74 % (QCL) and the precision ranged from 2.36 % (QCH) to 5.88 % (QCLLQ).

Blood Stability

To assess the stability of the analyte in the blood at room temperature the following test was performed.

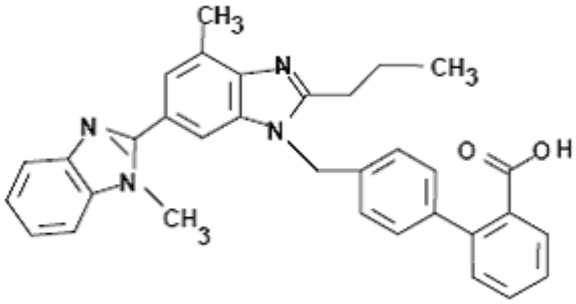
Three sets of each quality control samples at low and high concentrations were spiked in to whole blood and kept on bench top at room temperature for 2 hrs. The results were compared with freshly spiked quality control samples at low and high concentrations.

The % difference for QCL and QCH were 6.55 and 1.68.

Table No. 1: Precision and Accuracy for Telmisartan

Within Batch Precision	PA1	0.68 % - 2.94 % (QCH-QCL)
	PA2	1.81 % - 3.13 % (QCM-QCLLQ)
	PA3	2.42 % - 4.61 % (QCL-QCLLQ)
Within Batch Accuracy	PA1	103.60 % - 105.85 % (QCH-QCLLQ)
	PA2	102.11 % - 107.80 % (QCH-QCL)
	PA3	103.35 % - 108.77 % (QCLLQ-QCH)
Intraday Batch Precision	Day-1	1.65 % - 2.93 % (QCM - QCL)
	Day-2	2.42 % - 4.61 % (QCL-QCLLQ)
Intraday Batch Accuracy	Day-1	102.85 % - 106.45% (QCH - QCL)
	Day-2	103.35 % - 108.77 % (QCLLQ-QCH)
Between Batch Precision		2.17 % - 3.41 % (QCM - QCLLQ)
Between Batch Accuracy		103.56 % - 107.17 % (QCH - QCM)

Figure 1: Chemical Structure of Telmisartan



Concomitant Drug Effect

To check the interference of concomitant medication on the anlayte, spiked 5µL of caffeine (997.754 µg/mL) in triplicates into QCM samples and 5µL of aspirin (999.091 µg/mL) is spiked into another set of triplicates of QCM samples. These samples were quantified against the freshly spiked calibration curve standards. The effect of the concomitant drug was evaluated by comparing each of the back calculated concentrations of stability QCs with the nominal concentration of QCs. Two blank samples were also spiked separately with 5µL of caffeine and 5µL of aspirin.

The % nominal of the QCM sample ranged from 104.99 – 105.95. No interference was observed at RT of analyte and IS in the blank samples.

Figure 2 - A Representative Chromatogram of Telmisartan for Blank

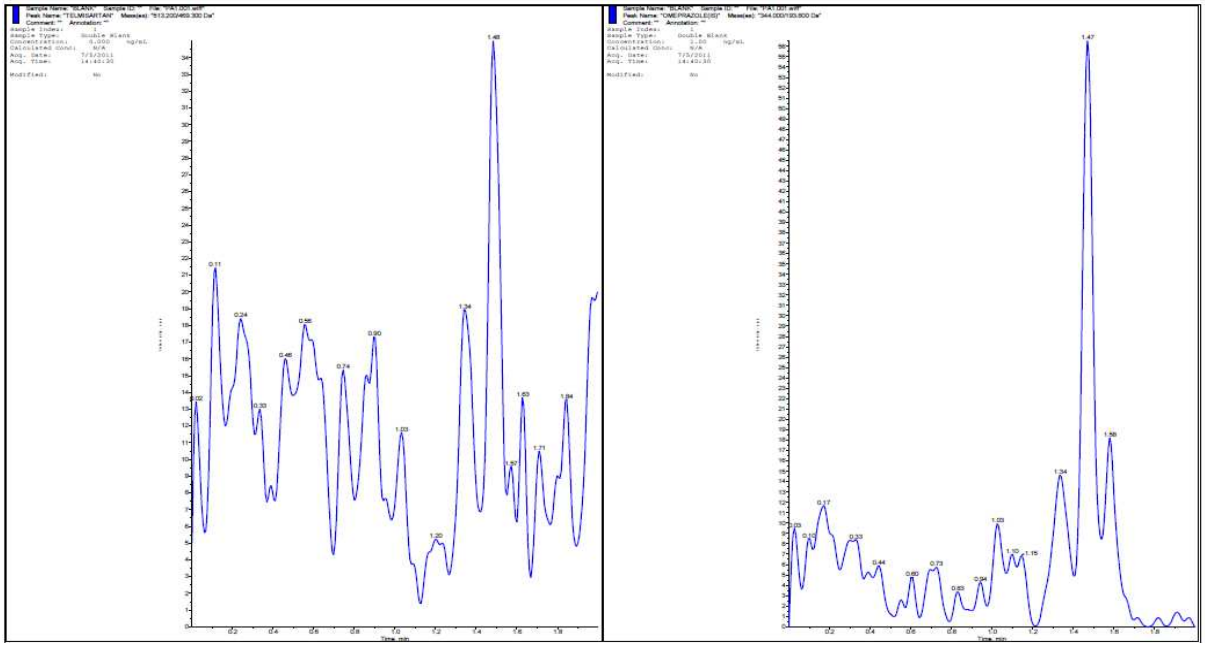


Figure 3 - A Representative Chromatogram of Telmisartan for QCL

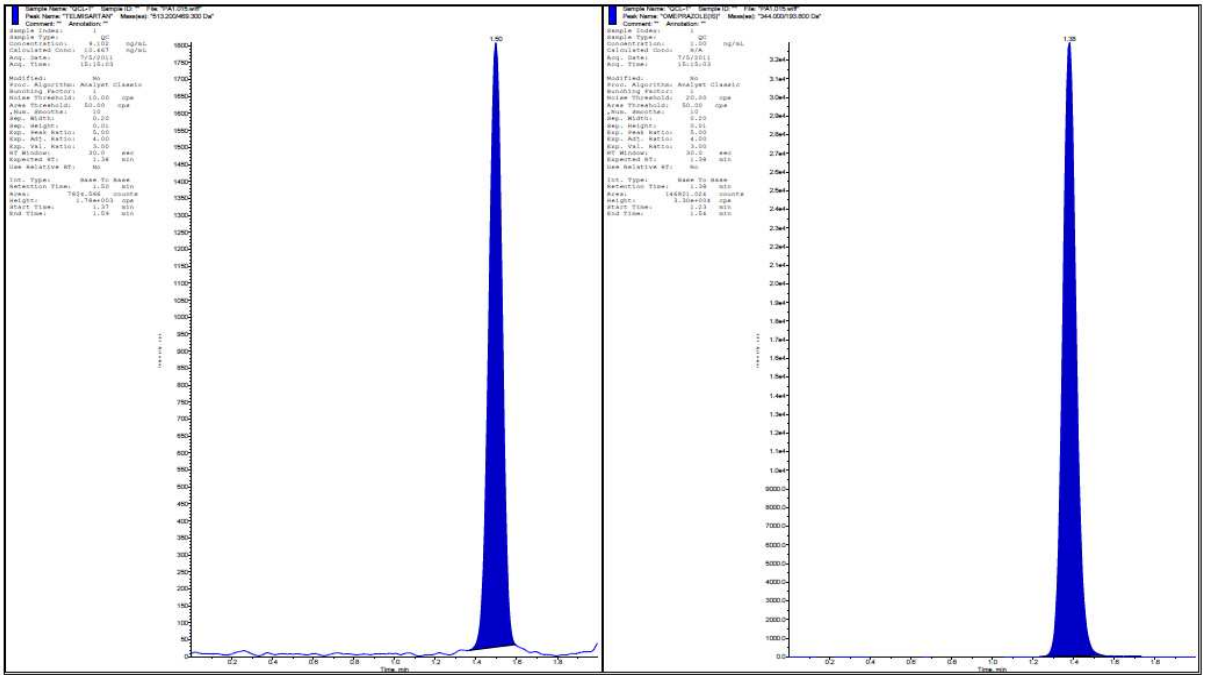
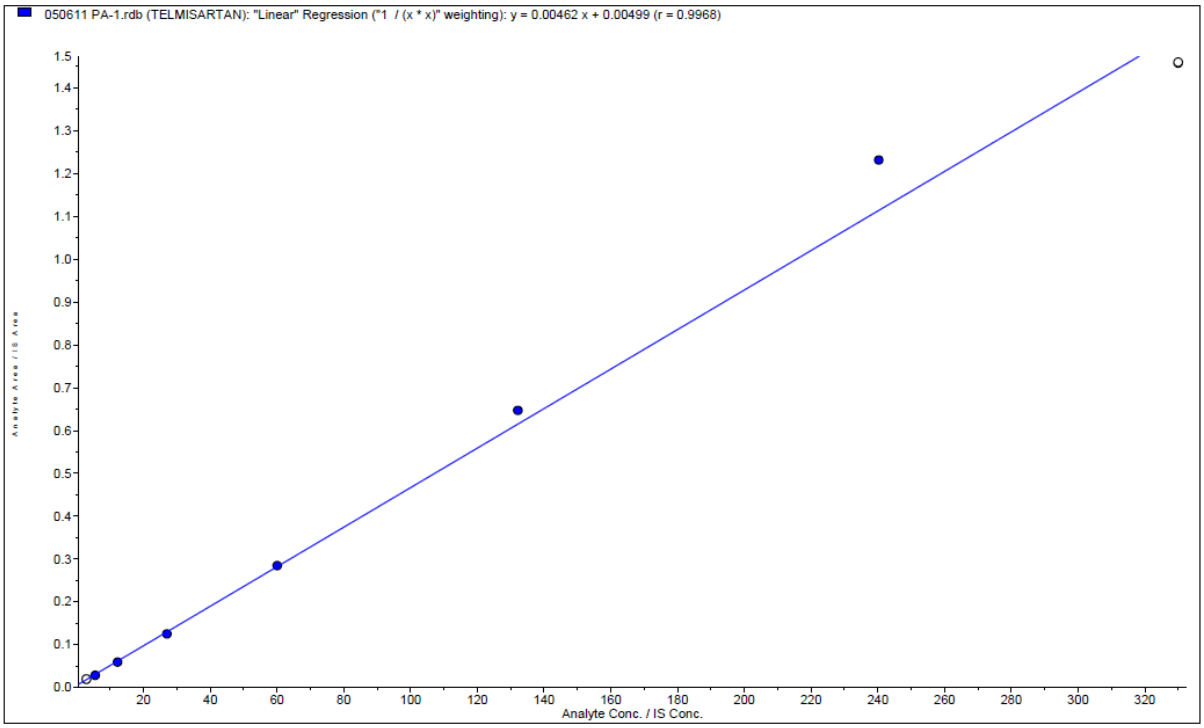


Figure 4 - A Representative Calibration Curve for Telmisartan



CONCLUSION

A rapid, specific isocratic LCMS-MS method has been developed for the estimation determination of Telmisartan. The method was validated for precision, specificity, linearity, robustness and stability and stability. No interference was observed. The method uses a simple mobile phase composition easy to prepare with little or no variation. The rapid run time of 2 min and the relatively low flow rate (0.5 mL/min) allows the analysis of a large number of samples with less mobile phase that proves to be cost-effective. This method was successfully applied for the bioequivalence study of Telmisartan.

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