Highly Selective Bioanalytical Quantitation Method for Analysis of R and S Amlodipine Enantiomers in Human Plasma using LC-MS/MS

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INTRODUCTION

Amlodipine is an angioselective calcium channel blocker used to treat high blood pressure, chest pain and other conditions caused by coronary artery disease. Racemic mixture of amlodipine is used for therapeutic purposes. S amlodipine is the pharmacologically active enantiomer of amlodipine. The use of racemic mixture of amlodipine showed adverse effects like peripheral edema, headache, dizziness, flushing and abdominal pain. Studies have reported these adverse effects are rarely associated with S Amlodipine. R and S enantiomers of the amlodipine exhibit different pharmacokinetics and pharmacodynamics. Here we present a Selective LC-MS/MS method, developed to monitor concentrations of R & S enantiomers of amlodipine in human plasma to evaluate the pharmaceutical equivalence for both racemic and S amlodipine formulations of amlodipine.

MATERIALS AND METHODS

Sample Preparation:
The extraction of Amlodipine enantiomers from human plasma was carried out using a solid phase extraction technique. Phenomenex Strata™-X 33 µm Polymeric Reversed Phase cartridge was first conditioned with 1mL of methanol and equilibrated with 1mL of water. 5µL of internal standard working solution was added to 100µL spiked human plasma and mixed. 500µL of 0.2%v/v ethanolamine in water was added to sample and vortexed. Sample was loaded on conditioned cartridge and allowed to pass through the cartridge at moderate speed. Cartridge was washed with 1mL of water followed by 1mL of 20% methanol in water to remove polar and non-polar interferences. The elution of the analytes was performed by 1mL of 0.1% formic acid in methanol. The eluent was evaporated at 50ºC under nitrogen stream and reconstituted with 100µL of mobile phase.

Calibration and Quality Control samples
Calibration curve and quality control samples for (S)-Amlodipine and (R)-Amlodipine were prepared by spiking 2% of various analyte concentration solutions in human plasma to get the final calibration curve concentrations (0.050, 0.100, 0.200, 1.250, 5.000, 20.000, 40.000, 50.000 ng/mL) and quality control concentrations at 0.050ng/mL (LLOQ QC), 0.150ng/mL (LQC), 20.000ng/mL (MQC), 40.000ng/mL (HQC).

Method Development
A thorough literature review revealed that several LC-MS/MS methods have been published for separation of (S)-Amlodipine and (R)-Amlodipine enantiomers in various biological matrices. The major challenge in developing this method was to have good peak shape, published LC-MS/MS methods using various chiral columns and chromatographic conditions had peak tailing and longer methods. Published methods have used various basic mobile phase additives e.g. ammonium hydroxide, trimethylamine and diethylamine for chiral separations. Various additives were tried for method development, ethanolamine at the concentration of 0.05%v/v showed best peak shapes and signal for amlodipine isomers.

QTRAP® 4500 LC-MS/MS System

LC-MS/MS analysis
Quantitation of (R)-Amlodipine and (S)-Amlodipine was performed using SCIEX LC-MS/MS system. SCIEX ExionLC™ AD HPLC system was coupled with SCIEX QTRAP® 4500 system. Separation of Isomers was achieved on Phenomenex Lux® 3 µm Cellulose-4, LC Column 150 x 2 mm with a flow rate of 0.3mL/min mobile phase. The mobile phase consisted of 0.05% Ethanol amine in Acetonitrile and Isopropyl Alcohol (96:4v/v). Electrospray ionization (ESI) was used in positive acquisition mode at multiple reaction monitoring (MRM) scan type with the transitions 409.3/237.9 for (R)-Amlodipine and (S)-Amlodipine enantiomers and 413.3/237.9 for (R)-Amlodipine-d4 and (S)-Amlodipine-d4 Internal standard enantiomers. The turbo gas temperature was 300ºC and ion spray voltage was set at 5500V.

Table. LC-MS/MS optimized conditions for the quantitation of Amlodipine Enantiomers

Target Compound	Precursor Ion	Daughter Ion	Ionization Mode	Spray Voltage	Collision Energy	Source Temperature
(R)-Amlodipine (S)-Amlodipine	409.3	237.9	ESI Pos	5500V	15V	300ºC
(R)-Amlodipine-d4 (S)-Amlodipine-D4	413.3	237.9				
Injection Volume: 10µL						
Analytical Column: Lux® 3 µm CellµLose-4, LC Column 150 x 2 mm						
Flow Rate: 0.300mL/min						
Isocratic Elution: 0.05% Ethanol amine in Acetonitrile and Isopropyl Alcohol (96:4v/v)						

RESULTS

In the present study total 8 calibration points of different concentrations starting from 50pg/mL to 50ng/mL were used to prepare the calibration curve, which has shown the correlation coefficient more than 0.99 for both the isomers, demonstrated excellent correlation between analyte and IS peak area. The average recovery of Amlodipine in human plasma at three different levels (LQC, MQC and HQC, n=6) was ≈92% and for Internal standard it was ≈96% for both enantiomers. Matrix factor for the Amlodipine enantiomers was evaluated at 3 concentration levels (LQC, MQC and HQC), it was 1.07 and 1.09 for R & S amlodipine respectively. Accuracy and precision for three full batches (inter and intraday) were within the 15% for all concentrations. Autosampler, benchtop and stock stability along with freeze thaw stability was also evaluated using the same method, plasma spiked samples were found stable for 6 hours on bench, extracted samples for 48 hours in autosampler and stock for 3 days in refrigerator. 3 freeze thaw cycle stability was also performed and samples were found stable. The total run time for chromatographic method is 7 min.

Fig. Chromatograms of R and (S)-Amlodipine Analytes and Internal Standards

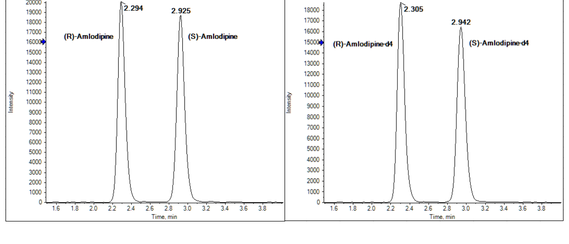


Fig. Chromatograms of extracted plasma blank and plasma LLOQ and Internal standard.

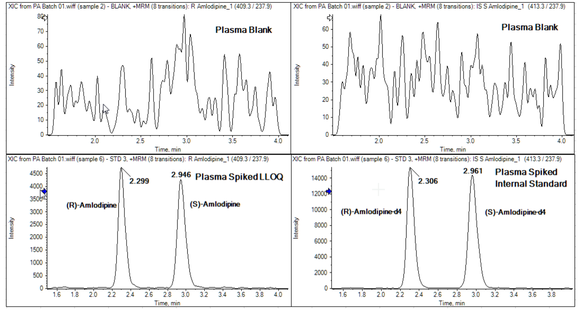
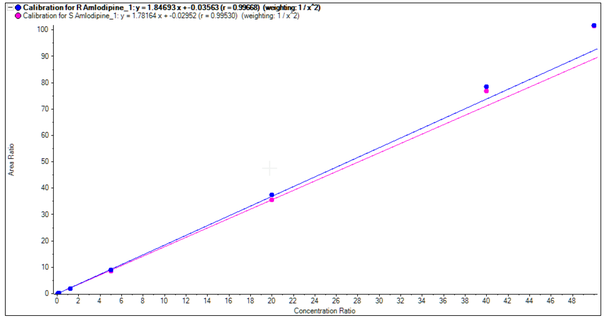


Table. Evaluation of within run and between run accuracy and precision for the quantitation of Amlodipine enantiomers in human plasma

Within run precision and accuracy of QC samples (ng/mL), (n=6)				Between run precision and accuracy of QC samples (ng/mL), (n=18) (3 Batches)		
(R)-Amlodipine						
Nominal Concentration (ng/mL)	Measured Concentration (ng/mL)	Precision (%CV)	Accuracy	Measured Concentration (ng/mL)	Precision (%CV)	Accuracy
0.050	0.048	6.00	96.00	0.047	8.66	94.00
0.150	0.151	4.05	100.67	0.149	3.47	99.33
20	20.399	3.11	102.00	20.576	2.46	102.88
40	41.992	2.79	104.98	42.410	2.74	106.03
(S)-Amlodipine						
0.050	0.048	9.67	96.00	0.047	8.51	94.00
0.150	0.152	3.10	101.33	0.151	4.64	100.67
20	20.812	2.89	104.06	20.693	2.38	103.47
40	42.599	3.19	106.50	42.279	2.98	105.70

Fig.Calibration Curve for the concentration range from 0.050ng/mL to 50.000ng/mL (weighting 1/x², r = 0.99668 for (R)-Amlodipine and r=0.99530 for (S)-Amlodipine)



CONCLUSION

Developed method has the capability to quantify selectively both pharmacologically active and inactive enantiomers of Amlodipine in human plasma.

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Document number:[RUO-MKT-10-7765-A]