Highly Sensitive LC-MS/MS Method for Quantification of Barnidipine in Human Plasma

Using the SCIEX QTRAP® 6500+ System with ExionLC™ AD System

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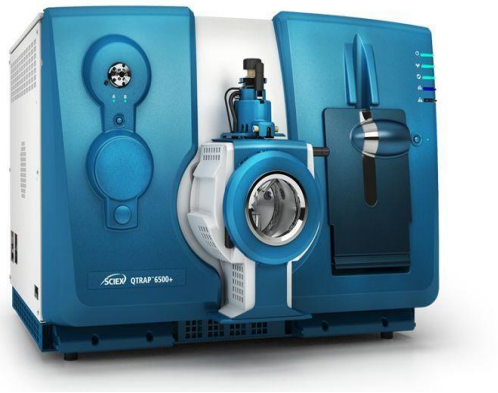

Barnidipine is a long-acting novel calcium antagonist that belongs to the dihydropyridine (DHP) group of calcium channel blockers. Barnidipine is used to treat hypertension and displays high affinity for smooth muscle cell calcium channels in vascular walls and selectivity against cardiovascular L-type calcium channels. Plasma concentrations of Barnidipine in therapeutic dose ranges are extremely low and require sensitive assays to determine the pharmacokinetic parameters, which necessitates the use of a sensitive analytical method that can quantify at picogram per mL levels in plasma.

A few analytical methods have been developed for pharmacokinetic studies or clinical trials of barnidipine (1) however, to achieve the necessary sensitivity, most of the methods use a large plasma sample aliquot and a low reconstitution volume which limits the feasibility of performing reinjection reproducibility or repeat analysis in a GLP regulated bioanalytical laboratory.

The main objective of this work is to develop a picogram level LC-MS/MS quantitation method for Barnidipine in plasma samples using Barnidipine D5 as Internal standard on a SCIEX QTRAP 6500+ System.

Key Features of the QTRAP 6500+ System for Bioanalytical Studies

- QTRAP 6500 system with IonDrive™ technology for high sensitivity assays in bioanalysis
- Optimized geometry of large diameter heaters in the IonDrive™ Turbo V source result in improved ionization efficiency at high flows and more robust source conditions
- Efficiency gains in ion sampling with the IonDrive™ QJet ion guide increase sensitivity without compromising robustness
- IonDrive™ High Energy Detector for up to 6 orders of magnitude linear dynamic range.

Methods

Sample Preparation: Aliquoted 500 µL plasma sample and mixed with 50 µL internal standard Barnidipine D5. Vortex followed by add 500 µL 2% ortho-phosphoric acid solution in water. Condition cartridge Bond Elute Plexa (1cc / 30mg), with methanol followed by water, then load the prepared plasma sample. Wash the cartridge with 1mL of 1% glacial acetic acid in 5% methanol in water, followed by two times with 1mL of 100% water. Elute sample with 1 mL of Acetonitrile and evaporate with N_2 steam to dryness. Reconstitute with 200 µL mobile phase and transfer to an HPLC vial for LC-MS/MS analysis.

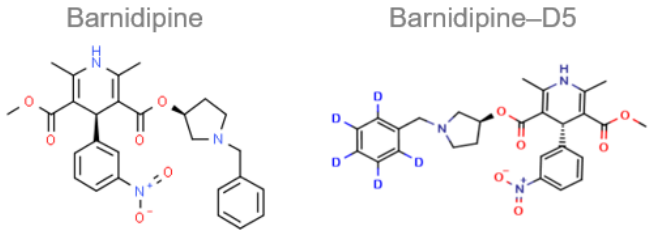

Figure 1. Structure of Barnidipine and Barnidipine-D5.

Chromatography: Separation was performed using the ExionLC AD System with a Phenomenex Luna HILIC (150mm * 4.6mm, 3.0 µm). Using a flow rate of 500 µL/min and a mobile phase of 2 mM Ammonium Formate (pH 4 with Formic Acid) in 80%, an isocratic elution was performed. The column temperature was 40°C and the injection volume was 10 µL.

Mass Spectrometric Conditions: The SCIEX QTRAP 6500+ LC-MS/MS system was operated in positive electrospray ionization mode. The MS conditions were as follows: scan type positive MRM, Q1 and Q3 at unit resolution; curtain gas set at 30; ion source temperature 600°C, ion source gas (GS1) at 60 and drying gas (GS2) at 60; ion spray voltage at 3200 V; and dwell time 200 ms for all transitions. The compound dependent parameters for analyte and internal standard were as follows:

Data Processing: Analyst® software 1.6.3 was used for mass spectrometer data acquisition and processing. A $1/x^2$ weighted linear regression was used to calculate the concentrations.

Results

This method was partially validated as per the USFDA guidelines. The Barnidipine standard was spiked into blank plasma matrix to check the linearity, accuracy, precision and recovery, which was found to be within limits set by USFDA. Linearity was plotted in the range from 5.0 pg/mL to 8000 pg/mL in the plasma samples. LOD was 0.1pg/mL in a neat solution. The regression coefficient of r > 0.99 was observed using weighing factor of $1/x^2$ (Figure 2).

Recovery was performed using LQC, MQC and HQC concentration, results were found to be more than 80%. Precision was evaluated at different QC levels and all were within the acceptance criteria of %CV ±20% at LLOQ level and ±15% at other levels. Table 3 shows the accuracy and precision data at different QC levels of Barnidipine. All are within the acceptance criteria of %CV ±20% at LLOQ level and ±15% at other levels. Example chromatograms of the blank, and calibration standards are shown in Figure 3.

Table 2. Mass Spectrometry Compound Parameters Conditions.

Compound	Q1	Q3	DP	EP	CE	CXP
Barnidipine	492.2	315.1	100	10	*34*	*12*
Barnidipine D5	497.2	315.1	100	10	*34*	*12*

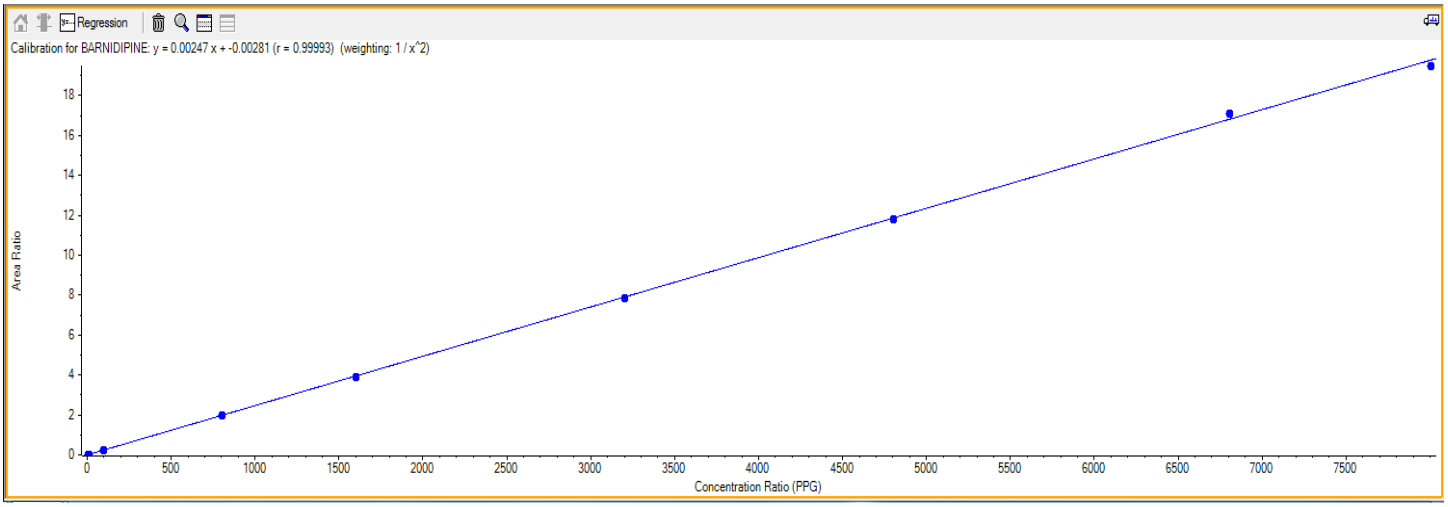

Figure 2: Linearity Plot for Barnidipine. Concentration range of 5 to 8005.88pg/mL.

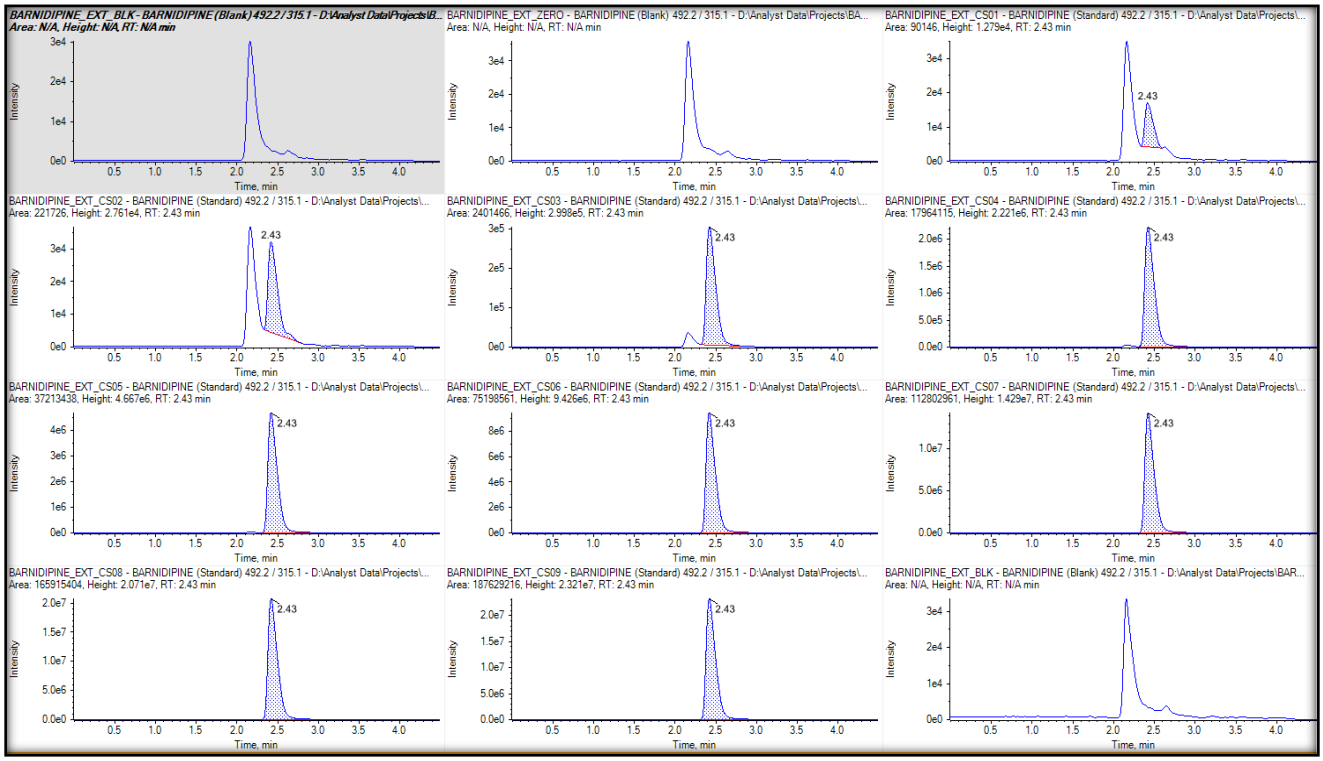

Figure 3: Chromatograms of Blank Plasma, and Calibration Curve Samples from the Barnidipine Method.

Table 3. Accuracy and Precision Data at Different QC Levels.

Sample Number	LQC (pg/mL)	MQC (pg/mL)	HQC (pg/mL)
1	*15.352*	*4104.794*	*6142.144*
2	*14.789*	*4035.982*	*6103.927*
3	*14.607*	*3994.908*	*6123.278*
4	*14.547*	*3991.117*	*6096.62*
5	*14.378*	*4022.044*	*6110.604*
6	*14.507*	*3977.872*	*5919.455*
Mean	***14.697***	***4021.120***	***6082.671***
S.D (+/-)	***0.348***	***46.218***	***81.550***
C.V (%)	***2.37***	***1.15***	***1.34***
% Nominal	***14.970***	***3998.25***	***6003.38***
Accuracy (%)	***99.18***	***100.57***	***101.32***

Conclusions

A highly selective, sensitive and reproducible bioanalytical method was developed for the detection of Barnidipine with an LLOQ 5 pg/mL in human plasma and 0.1pg/mL in neat solution on the SCIEX QTRAP 6500+ LC-MS/MS system.

Lower plasma volume of 500µl and final reconstitution volume of 200µl makes this method amenable for reinjection of samples or repeat analysis in a regulated bioanalytical laboratory.

References

1. Talari GP, Kumar VK, (2019) Simultaneous Estimation and Validation of LC-MS Method for Determination of Barnidipine in Human Plasma: *RASAYAN J. Chem.* **12(1)** 389-401

2. Delhira N, Anbazhagan S. (2015) A Simple, Isocratic and Ultra-Fast Liquid Chromatography / Mass Spectrometry Method for the Estimation of Barnidipine in Human Plasma. *Pharm Anal Acta*, **6:7.**

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