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A SENSITIVE LC/MS/MS METHOD FOR THE ANALYSIS OF BARNIDIPINE AT SUB-PICOGRAM LEVEL IN HUMAN PLASMA

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ABSTRACT

Purpose : To represent an assay performance utilizing TSQ-Altis high sensitive MS system for the quantitation of Barnidipine at sub-picogram concentration levels in Human plasma .

Methods : Barnidipine was analyzed in the presence of human plasma sample matrix using Selected Reaction Monitoring in TSQ Altis Mass spectrometer and data analysis was done by Thermo Scientific LCquan 3.0 software.

Results : The lower limit of quantitation (LLOQ) for Barnidipine was determined to be 0.5 pg/mL after extraction from human plasma.

INTRODUCTION

Barnidipine belongs to the dihydropyridine group of Calcium channel blockers and it is used in the treatment of hypertension. Barnidipine is effective in very low concentrations in human plasma and in fact, being a highly lipophilic molecule, barnidipine is expected to accumulate in the cell membrane and consequently, gains access to its target receptor in a slow manner. Following a single, modified-release dose of 10mg barnidipine, the peak plasma concentration is approximately 0.48 ng/mL i.e., picogram concentration levels and thus there is a requirement of a highly selective and sensitive quantitative analysis which could produce reproducible and reliable concentration data for pharmacokinetic analysis and support in development of better formulations in future.

Here we evaluated the advantage of highly sensitive TSQ Altis mass spectrometer in achieving sub-picogram (femtogram) concentrations of Barnidipine in human plasma. A rational approach was investigated to evaluate the high sensitivity of the instrument and overall assay performance of this LC-MS/MS analysis. The main challenges observed were interferences by keeping high volume of plasma as well as addition to the back pressures which was eliminated by reducing plasma volume that in turn reduced matrix factor significantly.

MATERIALS AND METHODS

Sample Preparation

Barnidipine hydrochloride working standard was dissolved in Methanol at a concentration of 1mg/mL. This standard was used to prepare calibration curve and Quality control (QC) dilutions. These dilutions were prepared in Methanol:Water:: 50:50 diluent to achieve a concentration range of 0.5 pg/mL to 250.0 pg/mL. Filtered human plasma was used to generate subsequent Calibration curves and QC samples.

Spiked Calibration curve standards and quality control samples were prepared after adding appropriate amount of Barnidipine dilutions in human plasma at each calibration curve and quality control concentrations. 200 µL aliquot from each spiked sample was taken for sample processing. Added extraction buffer to each sample and then Liquid-liquid extraction was performed after adding Extraction solution. Later, the supernatant from each sample was transferred to pre-labelled Polypropylene tubes and evaporated to dryness under a gentle stream of Nitrogen. Resulting residue was reconstituted using 150 µL of Reconstitution solution & vortexed. 10 µL of the samples was injected for analysis on LC-MS/MS system.

Liquid Chromatography

Chromatographic separation was achieved using a Thermo Scientific Vanquish UHPLC system. Samples were injected at 10 µL onto a 3 x 100 mm, 5 µ Thermo Scientific Syncronis C18 UHPLC column. Gradient elution was accomplished using 0.1% Formic acid in Water (A) and 0.1% Formic acid in Methanol (B) at a flow rate of 0.450 mL/minute. Total runtime was 8 minutes.

Mass Spectrometry

Barnidipine was analyzed using Thermo Scientific TSQ Altis MS with Heated Electrospray Ionization (Optamax NG Ion Source). Appropriate source conditions suitable for a 0.450 mL/minute LC flow rate were applied for all data collection (Table 1) Data were acquired in SRM mode with an external mass calibration.

Data Analysis

All data were acquired and processed utilizing Thermo Scientific LCquan 3.0 Software. All chromatographic integrations were accomplished using method defined processing settings. No manual integration was applied to any chromatographic data.

RESULTS

Figure 1. Barnidipine - Chemical structure & Formula

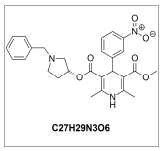
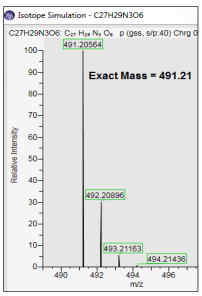


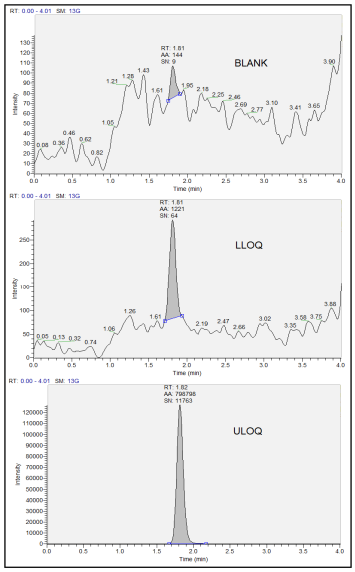
Figure 2. Exact mass from Isotopic distribution



The LC-MS/MS analysis of Barnidipine was performed on a Vanquish UHPLC system coupled with TSQ Altis MS in SRM mode. This hyphenated system is easy to handle and enables highly selective and sensitive determination of compounds from complex matrices.

Barnidipine was tuned using exact mass (as in Figure 2) given by Thermo Scientific Freestyle software. After fine tuning, the final SRM transition as well as the best suitable ion source parameters were selected for this analysis that are given in Table 1.

Figure 3.
Chromatograms of Plasma samples – Blank, LLOQ (0.5 pg/mL) and ULOQ (250.0 pg/mL)

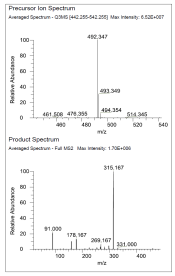


Chromatographic separation was performed to clear maximum interfering peaks and achieved the final chromatography as represented in Figure 3 for Extracted Plasma Blank, LLOQ (Lower limit of quantification) and ULOQ (Upper limit of quantification). The LC-MS method established here was found to be best suitable to achieve good sensitivity, selectivity and reproducibility and have improved column cleanup within each sample run.

Table 1. SRM & Ion source parameters

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	Polarity
Barnidipine	492.182	315	24.52	93	Positive
Source settings					
Spray Voltage (V)				3650	
Vaporizer Temperature (°C)				420	
Ion Transfer Tube Temperature (°C)				350	
Sheath gas pressure (Arb)				50	
Aux gas pressure (Arb)				10	
Sweep gas pressure (Arb)				0	

Figure 4. Spectrum of Precursor and Products



MS Spectrum

Automatic compound optimization was also performed to obtain full scan spectra and found the precursor and product scans as conspicuous in Figure 4. The Final ion source parameters manifested in Table 1 are as a result of a process of method development where these parameters were set according to LC flow rate, better ionization and reproducibility.

SRM Analysis

The gradient setup used here benefits in good separation of interferences from the RT of analyte that was achieved during development phase of this method where the effect of matrix from one sample to another in long run was found to be nil. During this study the main focus was targeted as Good precision and accuracy, No matrix factor and Good extraction recovery at all the low, middle and high quality control concentrations.

The chromatography of the method was found to be stable in terms of RT, Response and Reproducibility and this is very much self-explanatory from the chromatograms visible in Figure 3. Further the results present in Table 2 show the precise and accurate quantitation with good extraction recovery and no matrix factor. Lower limit of quantitation gives excellent S/N ratio and further explains the sensitivity of the Mass spectrometer and excellent performance of Vanquish UHPLC at low flow rate.

Precursor scan as shown in Figure 4 was obtained from a tuning solution of 500 ng/mL that gave a good sensitivity in Precursor and with same solution very good and intense product scan was also obtained.

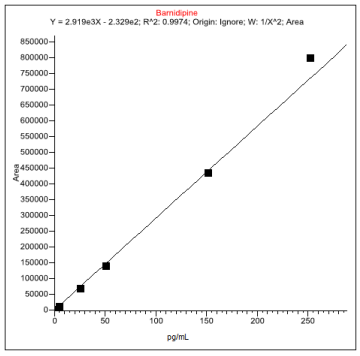
Assay Performance

To determine the assay performance after completion of the method development, it was planned to perform the most vital validation activities viz. Reproducibility exercise, Intraday precision and accuracy, Matrix factor evaluation and Recovery. The results of these experiments are tabulated and presented here in Table 2 and Calibration curve is shown in Figure 5. The values obtained as a result of these experiments are well within all acceptance limits of regulatory guidelines and the standard of acceptance limits has been selected to match with the best Clinical standards where the most critical biological matrix handling is involved and due to variability in pharmacokinetics from person to person ethical as well as regulatory norms are mandatory with certain criteria and ways of performing validation and clinical studies.

Table 2.
Assay performance results

Precision & Accuracy (6 replicates)				
QC	LOQQC	LQC	MQC	HQC
Concentration (pg/mL)	0.506	1.511	75.532	201.419
Mean Accuracy (%)	110.0	97.7	98.7	100.2
Precision (%)	6.7	4.9	1.9	2.3
Matrix Factor				
QC	LQC	-	HQC	
Matrix factor (%CV)	1.6	-	1.9	
Recovery (6 replicates)				
QC	LQC	MQC	HQC	
Recovery (%)	78.2	76.4	75.5	

Figure 5. Calibration curve



The overall assay performance fits the purpose and determines the Lower limit of quantification to be 0.5 pg/mL which is as low as sub-picogram or femtogram level of sensitivity for Barnidipine in human plasma and looks suffice to prove that the LC-MS system used here is an excellent combination that facilitates a selective as well as sensitive sample analysis.

CONCLUSIONS

SRM analysis on TSQ Altis MS provide a highly selective, sensitive and reproducible determination. The LLOQ for Barnidipine was determined to be 0.5 pg/mL and was found reproducible and appropriate for a quantitative analysis with linear response over a range of approx. 0.5 to 250 pg/mL.

The assay performance was good and acceptable from the regulatory point of view as well as the ethical requirement of using low sample volume for analysis.

This system configuration viz. Vanquish UHPLC coupled with TSQ Altis MS proves here its robustness and very high level of assay selectivity with complete removal of matrix interferences even at low flow rates.

Detector sensitivity and SRM method selectivity coupled with an efficient and reproducible Liquid-liquid extraction provide rugged and appropriate assay for the low level quantitative analysis of Barnidipine.

REFERENCES

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2. A Simple, Isocratic and Ultra-Fast Liquid Chromatography / Mass Spectrometry Method for the Estimation of Barnidipine in Human Plasma. Napa Delhiraj1* and Sockalingam Anbazhagan2. 1Department of Pharmaceutical analysis, A.S.N Pharmacy College, Tenali, Guntur (Dt), Andhra Pradesh, India. 2Department of Pharmaceutical analysis, Surya School of pharmacy, Vikravandi, Villupuram (Dt), Tamil Nadu, India

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