

An Ultra-Sensitive Method for the Determination of Nisoldipine in K₂-EDTA Human Plasma by LC-MS-MS

Authors:

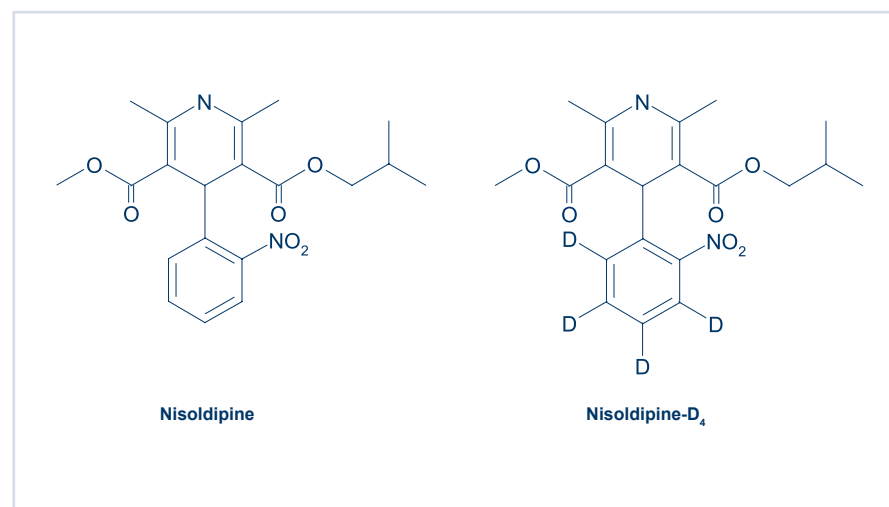
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Introduction:

Nisoldipine is a light-sensitive second-generation dihydropyridine calcium antagonist that is used in the treatment of arterial hypertension. This presentation provides details for the determination of nisoldipine in K₂-EDTA human plasma by LC-MS-MS.

Objective:

Develop a very sensitive, rapid and robust LC-MS-MS assay to measure nisoldipine in human plasma with a range of 1.00 to 100 pg/mL.



Methodology:

Chemicals

Nisoldipine was obtained from Synfine. Nisoldipine-D₄ was purchased from CDN Isotopes. All other chemicals were AR grade and solvents were HPLC grade or better.

Sample Preparation

Stock solutions of nisoldipine were prepared in acetonitrile/water 1:1, as were intermediate, calibration spiking and internal standard solutions. Quality Control (QC) samples were prepared in human K₂-EDTA plasma and stored in 1.0-mL aliquots at -20° C. QC samples were prepared from different weighings of drugs than the calibration spiking standards. Due to the light sensitivity of nisoldipine, solutions and samples had to be prepared and stored protected from light.

Extraction

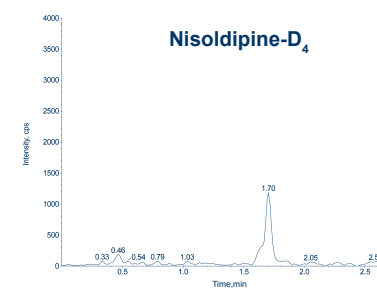
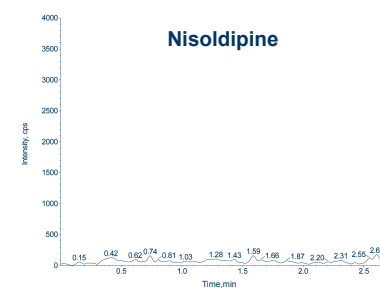
To all samples (K₂-EDTA plasma, 1.0 mL) except blank-blank samples, nisoldipine-D₄ internal standard solution was added. The samples were extracted with 2.0 mL of ethyl acetate/cyclohexane, 9:1. After centrifuging the samples, the organic layer was removed and dried with a gentle stream of nitrogen at 40 °C. The samples were reconstituted in 150 µL of mobile phase.

Sample Analysis by LC-MS-MS

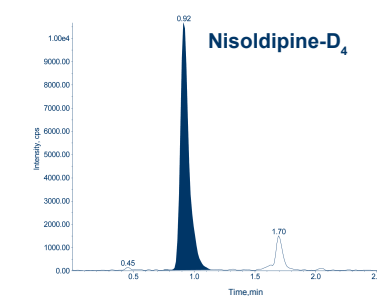
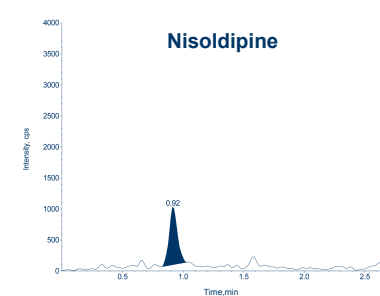
Extracts were injected onto a Luna C18, 50 x 2 mm HPLC column and separated isocratically with 95 % acetonitrile/water/ammonium acetate/acetic acid (150:50:0.1:0.01 v/v/w/v) : 5% acetonitrile at 0.3 ml/min followed by a step gradient (95% acetonitrile). The eluant was analyzed by a Sciex API 5000 LC-MS-MS in negative ion ESI mode. Mass spectra were acquired in multiple reaction monitoring mode using the mass transition of m/z 387→122.

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Mean of Three Validation Runs

Amount Added, pg/mL	1.00	2.00	4.00	8.00	20.0	50.0	90.0	100
Mean Found, pg/mL	0.987	2.09	3.88	7.79	20.2	49.0	92.7	100
CV (%)	3.2	8.6	5.0	7.6	3.2	6.1	1.6	1.5
% Bias	-1.3	4.5	-3.0	-2.6	1.0	-2.0	3.0	0.0

LLOQ and QC Precision and Accuracy

Mean of Three Validation Runs

Amount Added, pg/mL	1.00	Low	Med	High	VH (dil 1:10)
Mean Found, pg/mL	1.03	3.00	15.0	75.0	500
CV (%)	9.7	7.3	3.6	4.2	1.8
% Bias	3.0	-2.3	-3.3	-0.8	-3.6
n	18	18	18	18	6

Stability

Concentration, pg/mL	75.0	3.0
Mean % Change		
BTS, 120 hrs @ 22°C	1.8	-3.6
FTS, 10 cycles	-1.4	-2.2
XTS, 46 hrs @ 22°C	4.8	5.2
LTS, 109 days @ -20°C	3.3	-2.5

The linear quantitative range was established from 1.00 to 100 pg/mL using freshly fortified calibration samples ($R = 0.9962$, $1/x^2$ weighting). Precision and accuracy was determined to be acceptable after evaluating LLOQ samples and QC samples over three days of validation. Stability of QC samples in plasma was determined to be acceptable (less than 15% change) after stressing with ten freeze-thaw cycles, 120 h room temperature exposure and 109 days in -20°C frozen storage. The average extraction recovery was 84.4 %. There were no matrix effects or chromatographic interferences observed from six lots of plasma, or from over-the-counter drugs.

Conclusion:

This method demonstrates good ruggedness coupled with excellent sensitivity. A large number of clinical study samples has been analyzed with this method.