

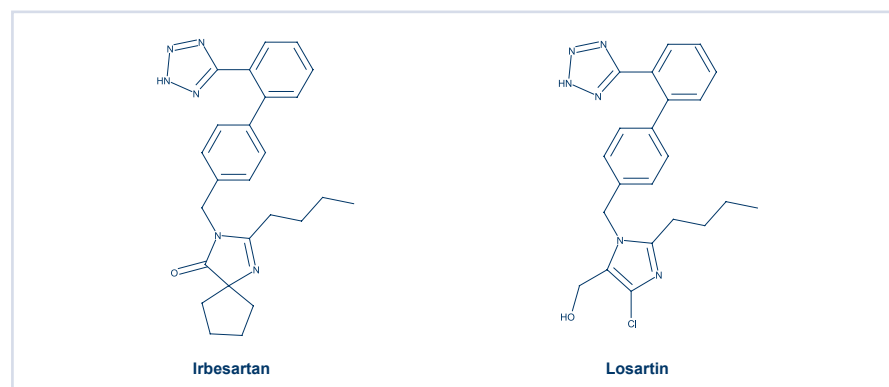
A Method for the Determination of Irbesartan in Human Plasma by LC-MS-MS

Authors:

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Introduction:

Irbesartan is used in the control of high blood pressure. Increases in blood pressure, even at modest levels, are associated with an increased risk of cardiovascular complications. Large trials have shown that treatment with blood pressure-lowering agents significantly lowers the risk of cardiovascular and microvascular complications. Irbesartan is an Angiotensin II receptor blocker, a class of effective and well-tolerated orally active antihypertensive drugs. Activation of angiotensin type 1 (AT) receptors leads to vasoconstriction, stimulation of the release of catecholamines and antidiuretic hormone, and promotion of the growth of vascular and cardiac muscle. Irbesartan thereby relaxes vascular smooth muscle, increases salt excretion, decreases cellular hypertrophy and induces antihypertensive effects without modifying heart rate or cardiac output. A rugged and sensitive method for the analysis of irbesartan was developed and validated. This presentation provides details of a LC-MS-MS method for the quantitation and the validation of irbesartan in human plasma.



Objective:

- Develop and validate a method for the quantitation of Irbesartan.
- Develop a rugged method that is simple to extract.
- Develop method suitable to analyze pharmacokinetic samples.

Methodology:

Chemicals

Irbesartan and Losartan were purchased from Toronto Research Chemicals. All other chemicals were AR grade and solvents were HPLC grade or better.

Sample Preparation

Stock solutions of irbesartan were prepared in acetonitrile/water 1:1, as were combined intermediate, calibration spiking and internal standard solutions. Quality Control (QC) solutions were prepared in human K₃-EDTA plasma and stored in 0.2-mL aliquots at -20° C. QC samples were prepared from different weighings of drugs than the calibration spiking standards.

Extraction

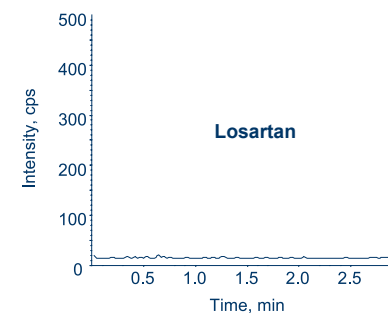
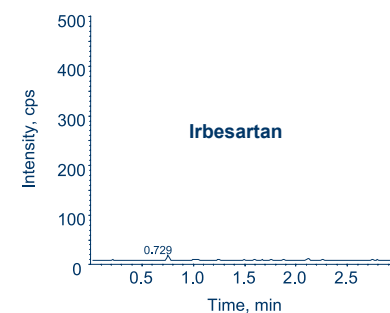
To all samples (K₃-EDTA plasma, 0.2 mL) except blank-blank samples, losartan internal standard solution is added. After adding 0.100 mL of 1.0 M potassium phosphate, the samples were extracted with 2.0 mL of ethyl acetate\ cyclohexane, 9:1. After centrifuging the samples, the organic layer was removed. The organic layer was dried with a gentle stream of nitrogen at 40 °C. The samples were reconstituted in 0.150 mL of mobile phase.

Sample Analysis by LC-MS-MS

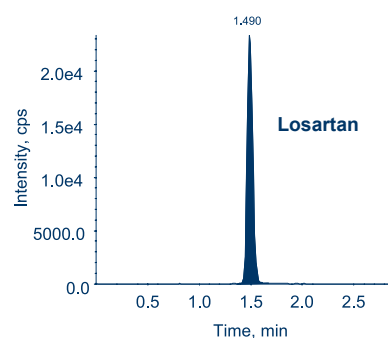
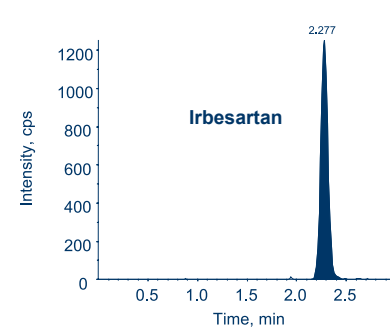
A SCIEX API-3000 LC-MS-MS in positive IonSpray MRM mode analyzed the extract. The analytes were resolved under isocratic conditions with a Thermo Scientific Aquasil C18 (3 μ, 4.6 × 50 mm) column and a mobile phase of 0.1% formic acid in acetonitrile:water, 1:1. The flow rate was 1.0 mL/min which was split approximately 3:1, waste:mass spec. Irbesartan (m/z 429 → 207) eluted at approximately 2 minutes, while the internal standard losartan (m/z 423 → 207) eluted at approximately 1.5 minutes.

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	50.0	100	250	500	1000	2000	3600	4000
Mean Found, ng/mL	48.7	104	250	521	1020	1970	3500	3850
CV (%)	2.0	4.0	1.1	2.1	5.7	1.8	2.8	2.3
% Bias	-2.6	4.0	0.0	4.2	2.0	-1.5	-2.8	-3.8

LLOQ and QC Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	50.0	Low	Med	High	VH (dil 1:10)
Mean Found, ng/mL	49.2	157	775	3030	19,100
CV (%)	3.1	5.9	5.5	3.8	5.7
% Bias	-1.6	4.7	3.3	1.0	-4.5
n	18	18	18	18	6

Stability

Concentration, ng/mL	3000	150
Mean % Change		
BTS, 24 hrs @ 22°C	0.6	10
FTS, 5 cycles	2.1	11
XTS, 118 hrs @ 22°C	1.2	12
LTS, 445 days @ -20°C	0.0	2.0

The isocratic chromatography conditions provided a quick and reliable way to analyze irbesartan, resulting in a run time of 3.0 minutes. The recovery of irbesartan and losartan was 91% and 92% respectively. Three validation runs were performed on separate days. The average precision (CV) and accuracy (%bias) across all levels of the QC range for irbesartan were within ±6.0%. The precision and accuracy at the LLOQ were within 4.0%. No chromatographic interferences or matrix effects from six different lots of plasma were observed indicating the specificity of the method. Stability of irbesartan in plasma was established for 24 hours at room temperature (BTS), 5 cycles of freezing and thawing (FTS), 445 days of storage at -20 °C (LTS), and 118 hours in the final extract (XTS).

Conclusion:

This method was successfully validated. The method proved rugged and quick in the determination of the concentrations of irbesartan in human plasma samples generated from clinical trials.