

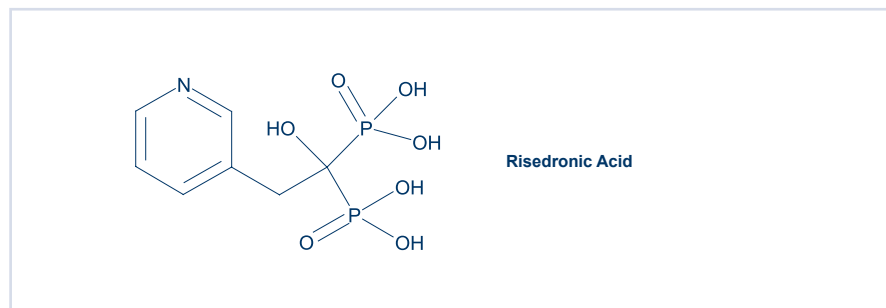
Determination of Risedronic Acid in Human Urine by Solid-Phase Extraction, On-column Methylation and LC/MS/MS

Authors:

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Introduction:

Risedronic acid is a bone resorption inhibitor used to treat osteoporosis in postmenopausal women. A rugged and sensitive method for the analysis of risedronic acid in human urine was developed and validated. This presentation provides details of this method.



Objective:

- To develop an efficient and rugged procedure for the accurate determination of risedronic acid in human urine by LC/MS/MS.
- To validate analysis of risedronic acid over the range 1.00 to 1000 ng/mL.

Methodology:

Chemicals

Risedronic acid and the internal standard risedronic acid-D₄ were obtained from SynFine Research, Inc. All solvents were HPLC grade and additional reagents were ACS Reagent grade or better.

Sample Preparation

Risedronic acid stock solution was prepared in acetonitrile/water (1:1,v/v) containing a small amount of ammonium hydroxide to aid dissolution. This stock was used to prepare an intermediate solution in acetonitrile/water at a known concentration. Calibration standards covering the required range were then prepared in the same solvent. Risedronic acid-D₄ stock solution was likewise prepared in acetonitrile/water (1:1,v/v) containing a small amount of ammonium hydroxide, and an internal standard working solution in acetonitrile/water was prepared from this stock.

For validation, Quality Control samples were prepared in human urine at three different concentration levels (QC Low = 3.00 ng/mL; QC Medium = 200 ng/mL; QC High = 800 ng/mL). These QC samples were stored in 0.200 mL aliquots at -20 °C.

Extraction

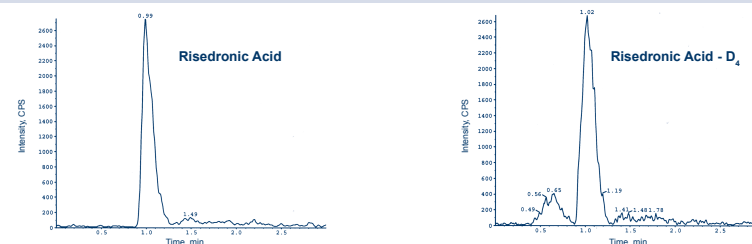
- Aliquot 0.200 mL human urine.
- Spike the standards with 20.0 µL of spiking solution.
- Add 20.0 µL of working internal standard solution.
- Add 700 µL of ammonium acetate (20 mM).
- Condition Strata SAX SPE cartridges (Phenomenex, 100 mg/1 mL) with 1 mL of methanol followed by 1 mL of ammonium acetate.
- Load samples onto SPE cartridges.
- Wash cartridges with 1 mL of water followed by 1 mL of methanol.
- Dry the cartridges for 5 min with full vacuum.
- Derivatize with 0.400 mL of diazomethane in ether.
- Elute with 0.8 mL of methanol.
- Evaporate with nitrogen at 40°C and reconstitute in 150 µL of mobile phase.
- Analyze by LC-MS-MS.

Sample Analysis by LC-MS-MS

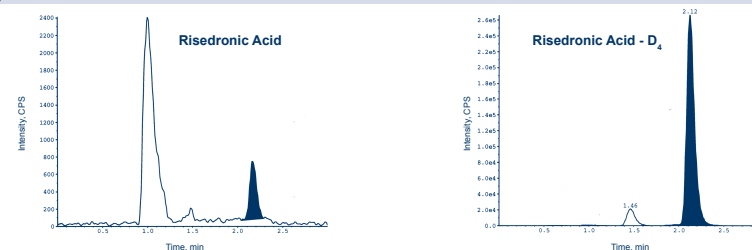
HPLC was performed on a Phenomenex Gemini C18 column (4.6x50mm) with a mobile phase of water/methanol/acetonitrile/formic acid/ammonium hydroxide 650/250/100/1/0.6 at 0.4mL/min. Mass spectrometry detection of the tetramethyl derivatives was carried out on a Sciex API 4000 instrument equipped with a TurboIonSpray source. Mass spectra were acquired in positive ion mode with MRM monitoring of mass transitions of 340→214 for risedronic acid (tetramethylated) and 344→218 for risedronic acid-D₄ (tetramethylated).

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	1.00	2.00	5.00	20.0	100	400	900	1000
Mean Found, ng/mL	0.968	2.08	5.21	21.6	101	392	853	931
CV (%)	0.6	1.2	2.8	3.7	0.6	1.8	1.9	1.8
% Bias	-3.2	4.0	4.2	8.0	1.0	-2.0	-5.2	-6.9

LLOQ and QC Precision and Accuracy

Mean of Three Validation Runs (n=18)

Amount Added, ng/mL	1.00	Low 3.00	Med 200	High 800
Mean Found, ng/mL	0.944	3.15	195	763
CV (%)	7.7	6.3	2.8	4.3
% Bias	-5.6	5.0	-2.5	-4.6

Stability

Concentration, ng/mL	3.00	8.00
Mean % Change		
BTS, 25 hrs @ 22°C	1.1	-7.6
FTS, 5 cycles	2.5	-7.5
XTS, 189 hrs @ 22°C	-2.5	-6.0

No matrix interferences were observed for risedronic acid or the internal standard in six different lots of urine. Acceptable intraday and interday precision (<11.4% CV) and accuracy (< 5.6% bias) were observed over the linear range of 1.00 to 1000 ng/mL. The mean coefficient of variation (1/x² weighting) was 0.9962. Stability was confirmed at 25 hours at room temperature in plasma, 5 cycles of freezing and thawing, and 189 hours in extract form. The extraction recovery at three different levels was from 92.7 to 107.5%.

Conclusion:

An LC-MS-MS method for quantitation of risedronic acid in human urine has been developed. This method was shown to be specific, accurate, sensitive and rugged.