

Sensitive Determination of Anastrozole in K₂-EDTA Human Plasma by LC-MS-MS

Authors:

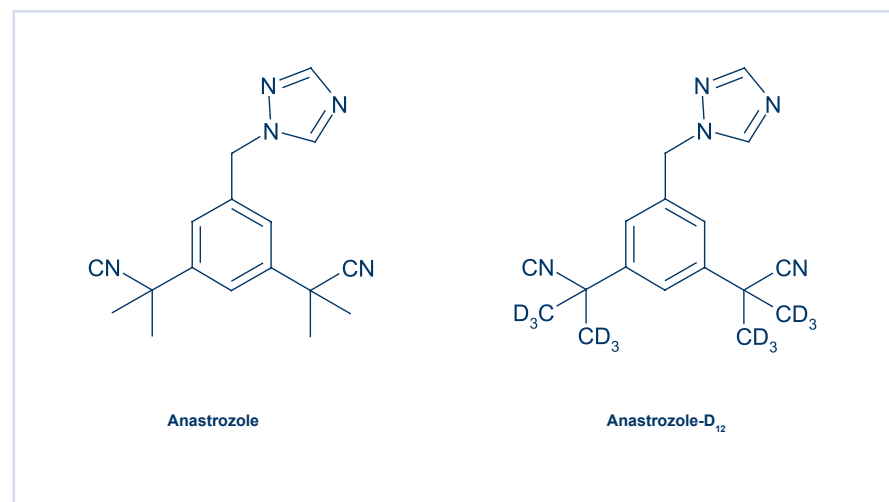
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Introduction:

Anastrozole is a potent and selective non-steroidal aromatase inhibitor. The drug is used for the treatment of postmenopausal women with hormone receptor positive breast cancer. This presentation provides details for the determination of anastrozole in K₂-EDTA human plasma by LC-MS-MS.

Objective:

Develop a rapid LC-MS-MS assay to measure anastrozole in human plasma with a range of 0.100 to 30.0 ng/mL.



Methodology:

Chemicals

Anastrozole and anastrozole-D₁₂ were purchased from Toronto Research Chemicals. All other chemicals were AR grade and solvents were HPLC grade or better.

Sample Preparation

Stock solutions of anastrozole were prepared in dimethylformamide, as were intermediate, calibration spiking and internal standard solutions. Quality Control (QC) samples were prepared in human K₂-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

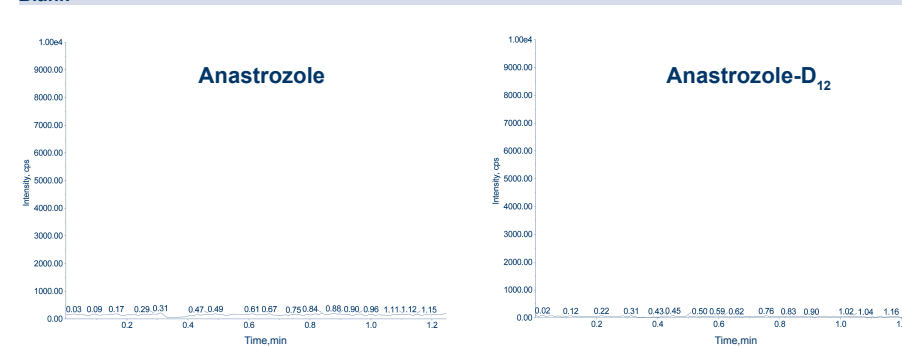
To all samples (K₂-EDTA plasma, 0.200 mL) except blank-blank samples, anastrozole-D₁₂ internal standard solution was added. After addition of 0.200 mL of sodium phosphate buffer (1.0 M, pH = 9.0) samples were extracted with 2.0 mL of ethyl acetate/MTBE, 1:1. After centrifuging the samples, the organic layer was removed and dried with a gentle stream of nitrogen at 40 °C. The samples were reconstituted in 100 µL of mobile phase.

Sample Analysis by LC-MS-MS

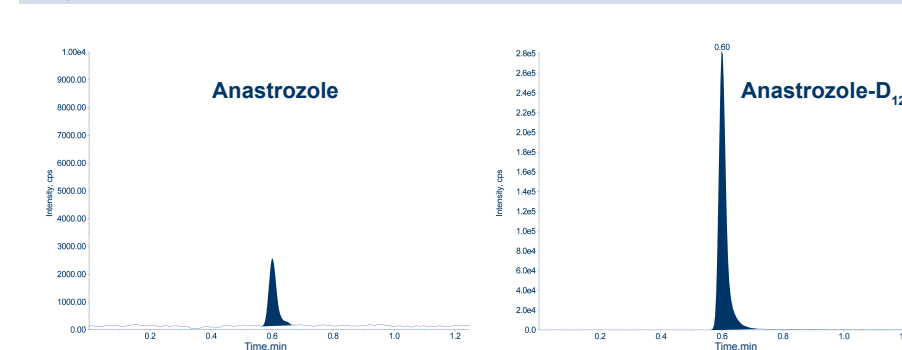
Extracts were injected onto a Luna Silica, 50 x 4.6 mm HPLC column and separated with ethyl acetate/methanol/water/formic acid (1000:80:40:1) at 1.5 ml/min. The eluant was analyzed by a Sciex API 4000 LC-MS-MS in positive ion APCI mode with a run time of 1.3 min. Mass spectra were acquired in multiple reaction monitoring mode using the mass transition of m/z 294→225.

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	0.100	0.200	0.600	3.00	12.0	18.0	27.0	30.0
Mean Found, ng/mL	0.0998	0.202	0.610	2.94	11.8	18.1	27.0	30.1
CV (%)	1.0	3.4	1.8	2.3	2.1	1.3	1.5	1.0
% Bias	-0.2	1.0	1.7	-2.0	-1.7	0.6	0.0	0.3

LLOQ and QC Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	0.100	Low	Med	High	VH (dil 1:10)
Mean Found, ng/mL	0.0992	0.315	6.13	24.7	162
CV (%)	12.3	3.5	1.5	2.0	1.9
% Bias	-0.8	5.0	2.2	2.9	8.0
n	18	18	18	18	6

Stability

Concentration, ng/mL	24.0	0.300
Mean % Change		
BTS, 24 hrs @ 22°C	1.4	7.0
FTS, 5 cycles	1.5	10.1
XTS, 74 hrs @ 22°C	0.3	6.1
LTS, 89 days @ -20°C	-4.6	-5.0

The linear quantitative range was established from 0.100 to 30.0 ng/mL using freshly fortified calibration samples. Precision and accuracy of the method were determined to be acceptable after evaluating LLOQ samples and QC samples over three days of validation. The correlation coefficient of the standard curve was 0.9998 (1/x weighting). Stability of QC samples in plasma during normal handling was determined to be acceptable (less than 15% change) after stressing with five freeze-thaw cycles, 24 h room temperature exposure and 89 days in -20 °C frozen storage. The average extraction recovery of three concentration levels was 85.0 %. 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 with a considerably short analysis time.