

Quantification of Letrozole in Human Plasma by LC-MS/MS Employing A Core-Shell Technology Column

Yafei Xu; Jing Ke; Harry Zhao; Zhongping (John) Lin,
Frontage Laboratories, Inc., 105 Great Valley Parkway, Malvern, PA 19355

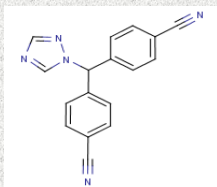
Introduction

Letrozole is an oral, anti-estrogen drug used for treating postmenopausal women with breast cancer. A number of methods have been developed for the quantification of letrozole in various matrices. However, few methods have been reported for the determination of low levels of letrozole (< 1 ng/mL) in plasma using LC-ESI/MS/MS. In this work, a simple, rapid, and sensitive method was developed and validated using a new Core-Shell Technology PFP HPLC column with ESI-MS/MS detection.

Objective

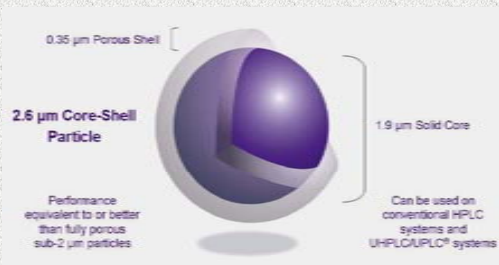
To develop and validate a rapid, simple, and sensitive LC-MS/MS method for the determination of low concentrations of letrozole in human plasma using a new Core-Shell Technology PFP column.

Figure 1. Structure of Letrozole



The PFP column was purchased from Phenomenex Inc. and was packed with Kinetex™ core-shell particles prepared using sol-gel processing techniques that incorporate nano-structuring technology to produce a durable, homogenous porous shell on a solid silica core. This uniform particle size distribution generates high plate counts with relatively low back pressure. The structure of a core-shell particle is shown in Figure 2.

Figure 2. Structure of Core-Shell Particle*



* Copy from the presentation of Phenomenex Inc.

Method and Conditions

Human plasma sample (200 µL) was extracted by protein precipitation using 1 mL of methanol as the extraction solvent. The extract was further diluted with solution (0.1% formic acid in 50:50 methanol/H₂O) to reduce matrix effect.

Chromatographic Conditions

Column: Kinetex PFP, 50 x 3.0 mm, 2.6 micron
Mobile Phase A: 0.1 % formic acid in H₂O
Mobile Phase B: 0.1 % formic acid in MeOH
Flow Rate: 0.4 mL/min
Injection Volume: 10 µL
Needle Wash Solvent: 0.1 % formic acid in 50:50 MeOH:H₂O
Elution: Gradient
Run time: 4.2 min

MS/MS Detection

Mass spectrometer: Sciex API 4000
Interface: TurbolonSpray
Detection mode: Positive Ion, SRM
(Selective Monitoring Reaction)

Table 1. SRM Transitions

Compound	Q1 Mass m/z	Q3 Mass m/z
Letrozole	286.0	190.1
Letrozole-d ₄ (IS)	290.0	194.1

Results

The method was fully validated for accuracy, precision, selectivity, bench-top and freeze/thaw stability, etc. Calibration curves were linear over the concentration range of 0.5-150 ng/mL ($r^2 > 0.995$) with a weighting factor of $1/x^2$. The overall accuracy of this method was 99.6-103.5%. The QC inter-run precision range (%CV) was 2-6.3%. The stability of QC samples at three concentration levels were evaluated regarding their freeze/thaw (3 cycles) stability, bench-top stability (6 hours), and processed stability (70 hours). Recovery of letrozole and letrozole-d₄ was 97.8% and 98.8%, respectively.

Table 2 Intra-Run and Inter-Run Precision and Accuracy

Run ID	Precision and Accuracy	Letrozole Concentration, ng/mL		
		1.5	38	115
Intra-Run1 N=6	Mean	1.54	39.9	121
	%CV	1.5	1.9	1.4
	%Nominal	102.7	105.0	105.4
Intra-Run2 N=6	Mean	1.48	37.3	117
	%CV	9.6	1.1	0.8
	%Nominal	98.7	98.2	101.3
Intra-Run3 N=6	Mean	1.46	39.8	119
	%CV	5.1	1.7	1
	%Nominal	97.5	104.7	103.7
Inter-Run N=18	Mean	1.49	39	119
	%CV	6.3	3.5	2
	%Nominal	99.6	102.6	103.5

Table 3 Stability Summary

Stability Conditions	Minimum Stability
Freeze/Thaw	3 Freeze/Thaw Cycles
Extract Stability	70 Hours at Room Temperature
Matrix Stability	6 Hours at Room Temperature

Figure 3. Standard Curve for Letrozole

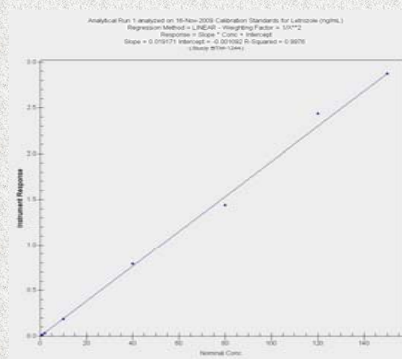
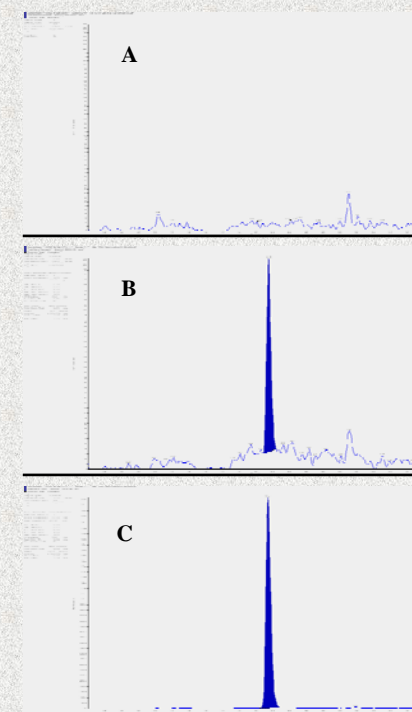


Figure 4. Chromatograms of Human Blank Plasma (A), Letrozole (0.5 ng/mL) (B) and Letrozole-d₄ (IS) (C)



Conclusions

A simple, rapid, and sensitive LC-MS/MS method was developed and validated for the determination of low levels of letrozole in human plasma. A new Kinetex Core-Shell Technology PFP column was used to improve LC chromatographic separation. The method was fast, robust, sensitive, and reliable. The validated method will be used to support a bioequivalence study.