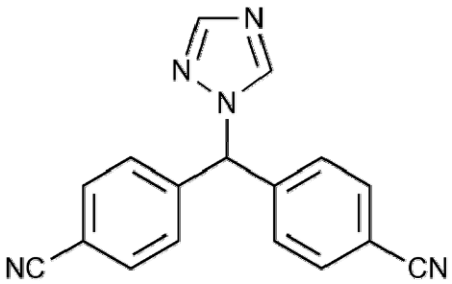
Letrozole

USP Method Letrozole RS
USP Method Letrozole Assay



Original Manufacturer: Novartis (patent expire 2011)

Brand Name: Femara

Letrozole is an oral non-steroidal aromatase inhibitor for the treatment of hormonally-responsive breast cancer after surgery.

Estrogens are produced by the conversion of androgens through the activity of the aromatase enzyme. Estrogens then bind to an estrogen receptor, which causes cells to divide.

Letrozole prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit. The action is specific, and Letrozole does not reduce production of mineralo- or corticosteroids.

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Letrozole

USP34 – NF29 S1

USP Columns:

Nucleosil C18 Assay and Related Compounds 4.6 mm x 12.5 cm, 5 µm

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	(1.51455.0001)

Recommended Solvents and Reagents:

Acetonitrile	gradient grade for liquid chromatography LiChrosolv®	(1.00030)
Water	Water for chromatography LiChrosolv®	(1.15333)
	or freshly purified water from Milli-Q water purification system	

USP Standards

Letrozole (200 mg)	USP Product Number:	1356971
Letrozole Related Compound A (25 mg)	USP Product Number:	1356982

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USP Method for Letrozole Assay

Solution A: Water
Solution B: Acetonitrile

Mobile phase
See the gradient table:

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Diluent: Acetonitrile and water (3:7)

Standard solution
10 µg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.] USP34

Sample solution
10 µg/mL of Letrozole in Diluent.
[Note—Dissolve Letrozole in acetonitrile, then dilute with water.] USP34

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 230 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Injection size: 20 µL
Flow rate: 1 mL/min

System suitability *(Sample: Standard solution)*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%

Analysis: *(Samples: Standard solution and Sample solution)*

Calculate the percentage of $C_{17}H_{11}N_5$ in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution
r_S = peak response from the Standard solution
C_S = concentration of USP Letrozole RS in the Standard solution (mg/mL)
C_U = nominal concentration of Letrozole in the Sample solution (mg/mL)

Acceptance criteria
98.0%–102.0% on the anhydrous basis

USP Method for Letrozole Assay

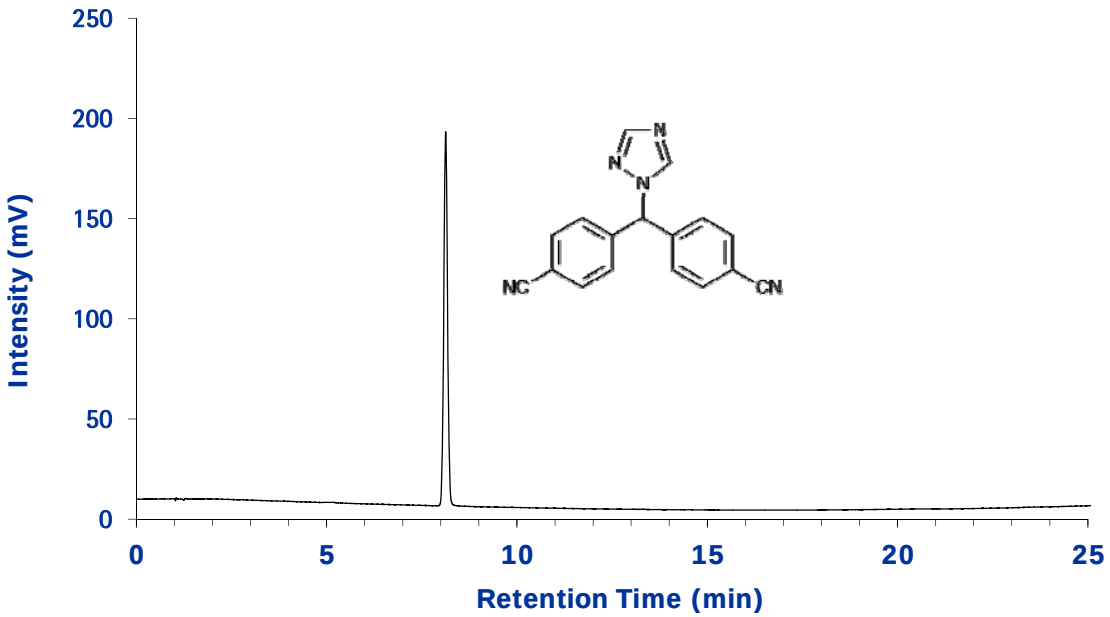
Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@230 nm	
Cell:	Semi-micro cell 2.5µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Water and Solution B: Acetonitrile	
Gradient:	See gradient table	

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Temperature:	Ambient
Diluent:	Acetonitrile and water (3:7)
Sample:	10 µg/mL of USP Letrozole RS (standard solution)
Pressure Drop:	140-96 Bar (2016-1382 psi)



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Letrozole	8.1	1.0	-	1.04

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USP Method for Letrozole RS

Solution A, Solution B, Mobile phase, Chromatographic system,USP34 and Diluent:
Proceed as directed in the Assay.

System suitability solution
2 µg/mL of USP Letrozole Related Compound A RS and 10 µg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve Letrozole and USP Letrozole Related Compound A RS in acetonitrile, then dilute with water.] USP34

Standard solution
1 µg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.] USP34

Sample solution
Transfer 25 mg of Letrozole to a 250-mL volumetric flask. Dissolve in 75 mL of acetonitrile, and dilute with water to volume. USP34

System suitability *(Samples: System suitability solution and Standard solution (USP34))*
Suitability requirements
Resolution: NLT 2.0
USP34 between Letrozole related compound A and Letrozole (System suitability solution)
Relative standard deviation: NMT 10.0%, Standard solution

Analysis
(Samples: Standard solution and Sample solution)

Calculate the percentage of each impurity in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the Sample solution
r_S = peak response of letrozole from the Standard solution
C_S = concentration of USP Letrozole RS in the Standard solution (mg/mL)
C_U = concentration of Letrozole in the Sample solution (mg/mL)

USP Method for Letrozole RS

Acceptance criteria

Individual impurities: See Impurity Table 1.
Total unspecified impurities: NMT 0.3%
[Note—Disregard any impurity peaks less than 0.05%.] USP34

Table 1.

Compound	Relative Retention Time (RRT)	Limit (%)
Letrozole related compound A[1]	0.67	0.3
Letrozole	1.0	-
4,4',4''-Methanetriyltribenzonitrile	2.4	0.1
Any other individual impurity	-	0.1

1 = 4,4¢-(1H-1,3,4-triazol-1-ylmethylene)dibenzonitrile.

USP Method for Letrozole RS

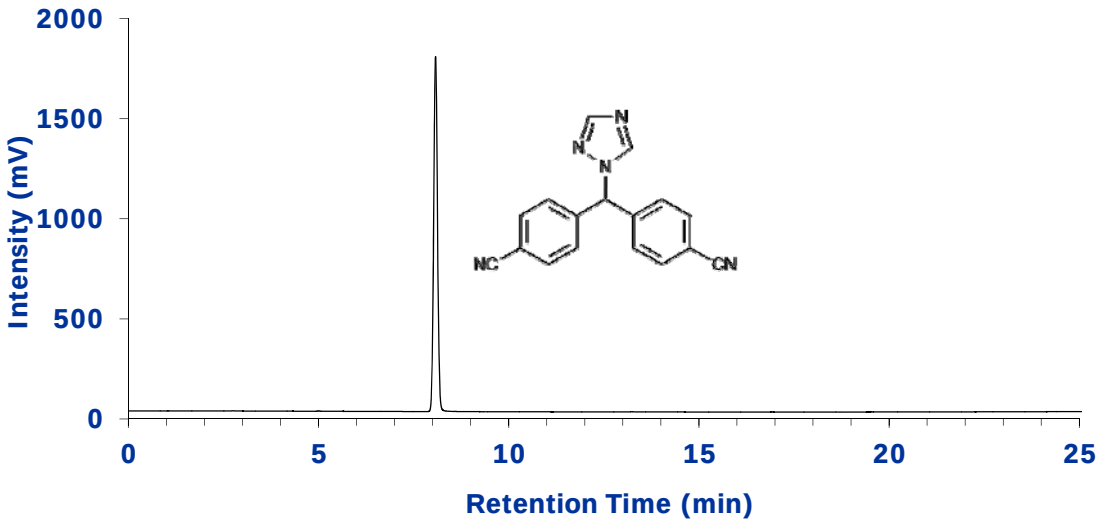
Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@230 nm	
Cell:	Semi-micro cell 2.5µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Water and Solution B: Acetonitrile	
Gradient:	See gradient table	

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Temperature:	Ambient
Diluent:	Acetonitrile and water (3:7)
Sample:	100 ppm (0.1 mg/mL) of USP Letrozole RS (sample solution)
Pressure Drop:	140-96 Bar (2016-1382 psi)



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Letrozole	8.1	1.0	-	1.05

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USP Method for Letrozole RS – SST solution

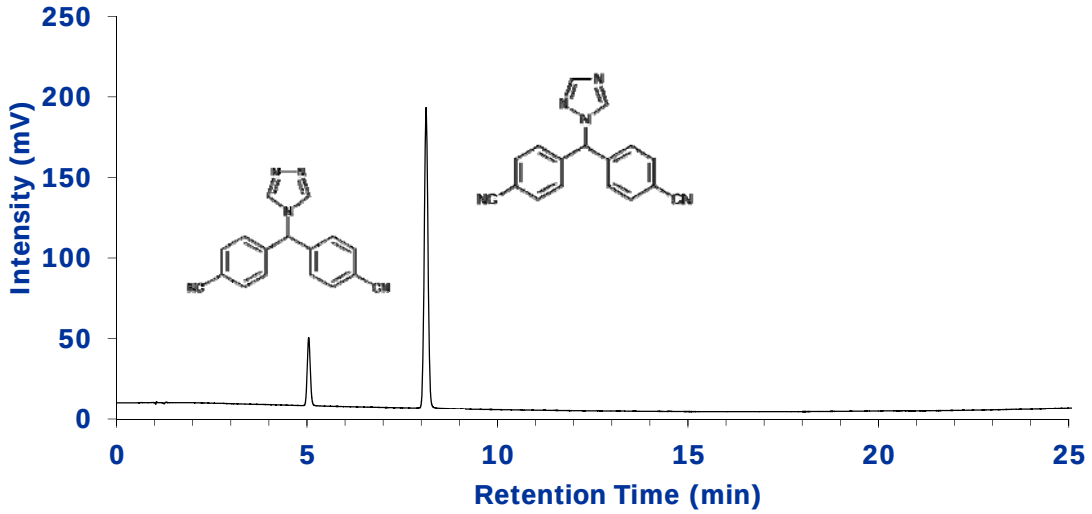
Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@230 nm	
Cell:	Semi-micro cell 2.5µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Water and Solution B: Acetonitrile	
Gradient:	See gradient table	

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Temperature:	Ambient
Diluent:	Acetonitrile and water (3:7)
Sample:	2 µg/mL of USP Letrozole Related Compound A RS and 10 µg/mL of USP Letrozole RS
Pressure Drop:	140-96 Bar (2016-1382 psi)



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Letrozole RS A	5.0	0.6	-	1.07
2	Letrozole	8.1	1.0	18.7	1.04

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