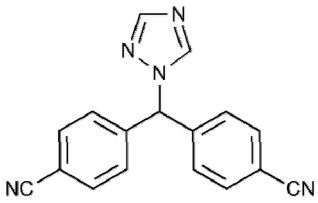
Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped



Letrozole

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. The commercial trade name of Letrozole is Femara.

The current USP monograph method for Letrozole and related substances specifies use of a 125x4.6 mm column with L1 (RP-18/ODS) (5 µm) packing as stationary phase, and with identical experimental conditions as described in the assay method. System suitability requirements for related substances are provided by means of resolution between Letrozole and Letrozole related substance A and should not be less than 2.0, as well as a relative retention time RRT of 0.67 Letrozole related substance A, 1.0 for Letrozole and 2.4 for 4,4',4''-Methanetriyltribenzonitrile. The tailing factor should be 0.8-1.5 for Letrozole.

The following pages illustrate a complete procedure for the method validation within limits of allowed changes. All acceptance critera are met for the Letrozole and related substances method by using the identical matched column; 125x4.6 mm Purospher® STAR RP-18 endcapped (5 µm) column for Letrozole analysis.

Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 125x4.6 — 1.51914.0001

Injection: 20 µL

Detection: UV 230 nm

Cell: 10 µL

Flow Rate: 1.0 mL/min

Mobile Phase: A: Water
B: Acetonitrile

Gradient: See table

Time (min)	A (%)	B (%)
0.01	70	30
25.0	30	70
26.0	70	20
30.0	70	30

Temperature: 25°C

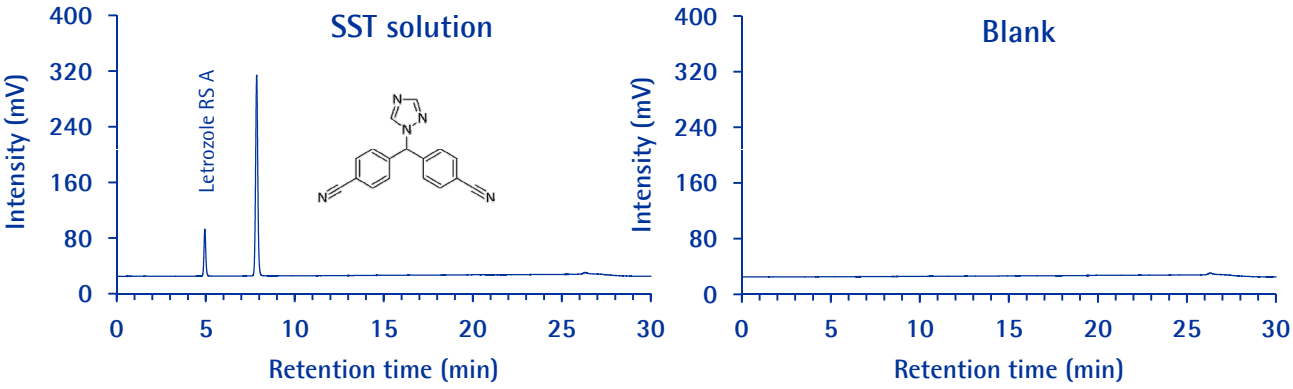
Diluent: Acetonitrile and water; 3:7 (v/v)

SST solution: 2 µg/mL of Letrozole Related Compound A and 10 µg/mL of Letrozole in Diluent

Standard solution: 1 µg/mL of USP Letrozole in Diluent.

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.

Pressure Drop: 72 - 28 Bar (1044 - 410 psi)



Chromatographic Data :

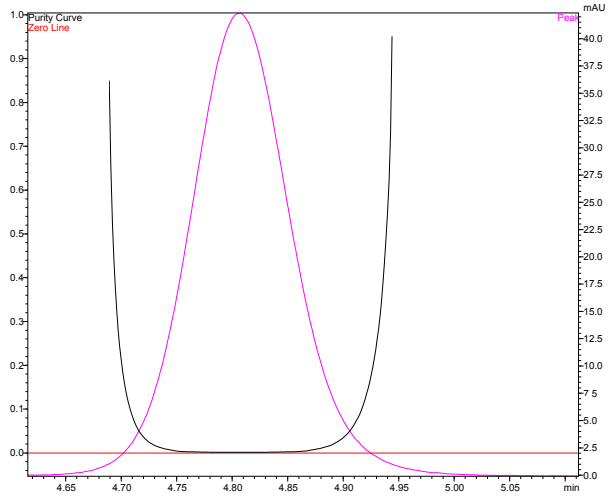
No.	Compound	Retention Time (min)	RRT	Resolution	Theoretical Plate	Assymetry
1	Letrozole related compound A	4.9	0.62		11641	1.10
2	Letrozole	7.9	1.0	14.3	20133	1.07

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/chromatography
chromatography@merckgroup.com

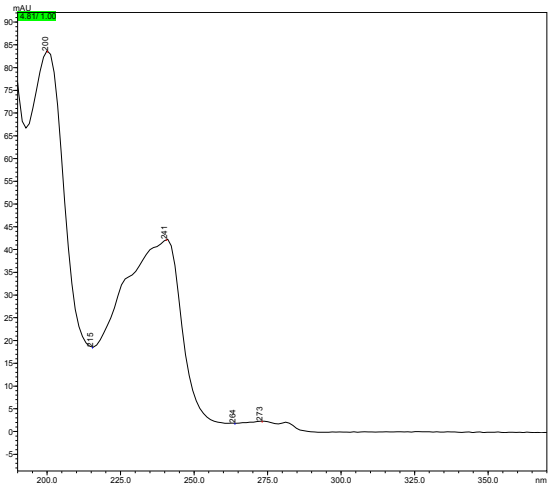
Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

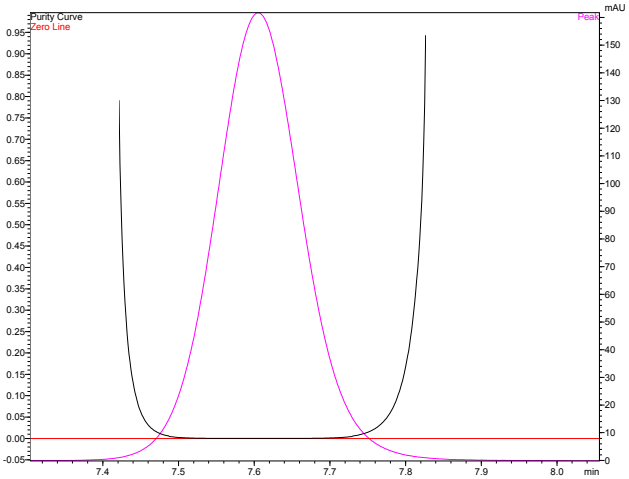
Peak Purity curve: Letrozole RS A



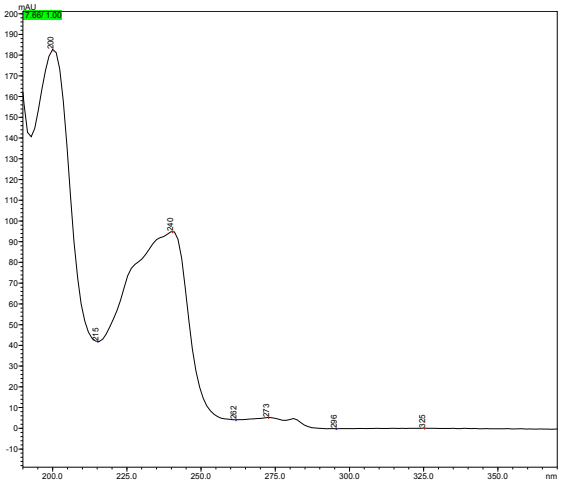
UV spectra: Letrozole RS A



Peak Purity curve: Letrozole



UV spectra: Letrozole



Letrozole and Related Substances (USP)

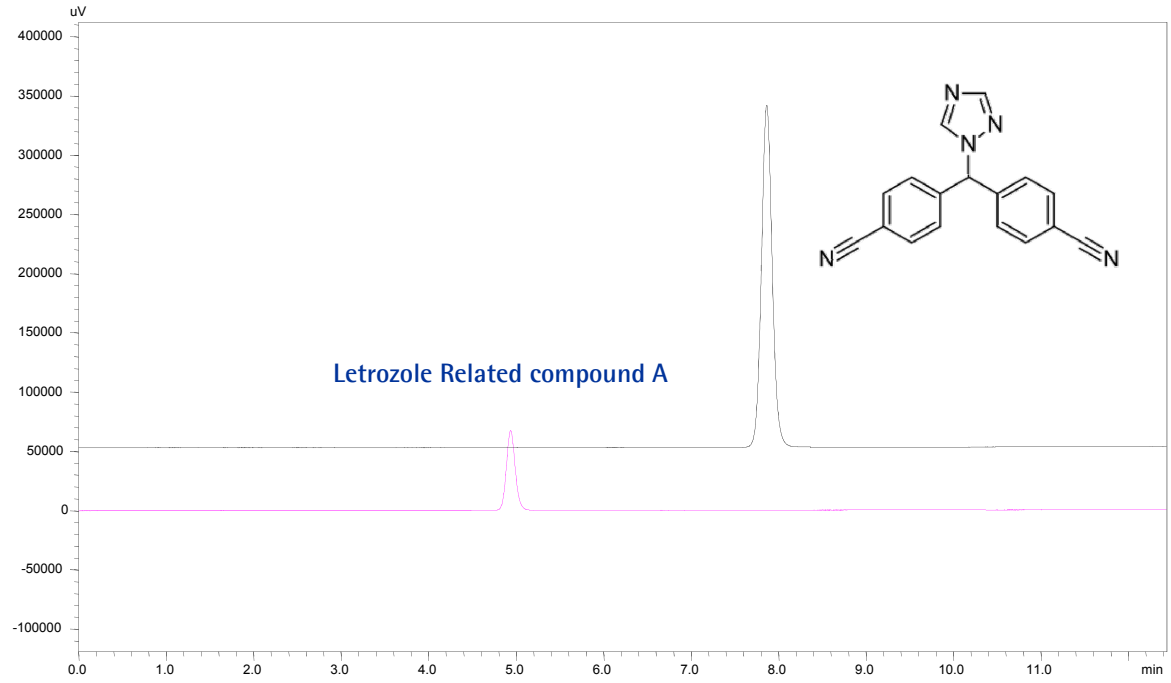
Purospher® STAR RP-18 endcapped

Method Validation

Following parameters have been checked during the validation of Letrozole

- Specificity
- Precision and Robustness
- Linearity
- Limit of Detection (LOD) and Limit of Quantitation (LOQ)
- LOQ repeatability

Specificity :
Each individual component of the sample has been analysed using the same conditions.
Not a single component or blank has any interference on each other.
The method stands specific using the Purospher® STAR RP-18 endcapped column.



Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Precision:

The retention time precision achieved for both cases is < 0.5 % RSD.
The area precision achieved for both the cases is < 1 % RSD.

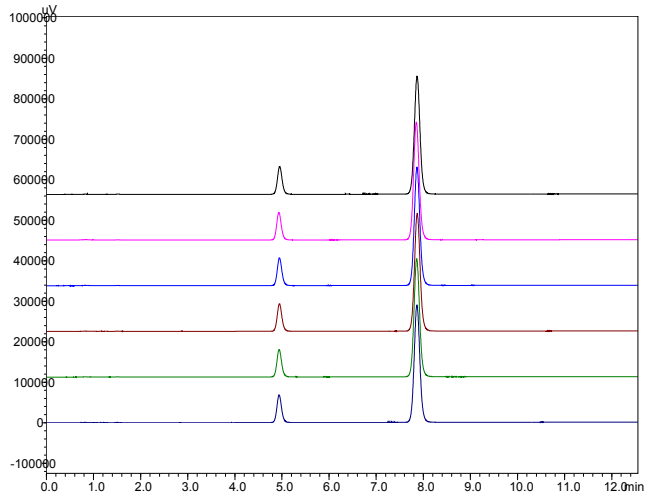


Table I: System Precision

	Retention Time (min)		Area	
Injection	RS A	Letrozole	RS A	Letrozole
1	4.98	7.90	474836	2457649
2	4.97	7.89	474364	2458516
3	4.97	7.89	474541	2450935
4	4.96	7.87	474747	2449480
5	4.95	7.86	474431	2450486
6	4.93	7.84	473816	2451989
Average	4.96	7.874	474456	2453176
Std. Dev.	0.02	0.023	362	3895
% RSD	0.40	0.29	0.08	0.16

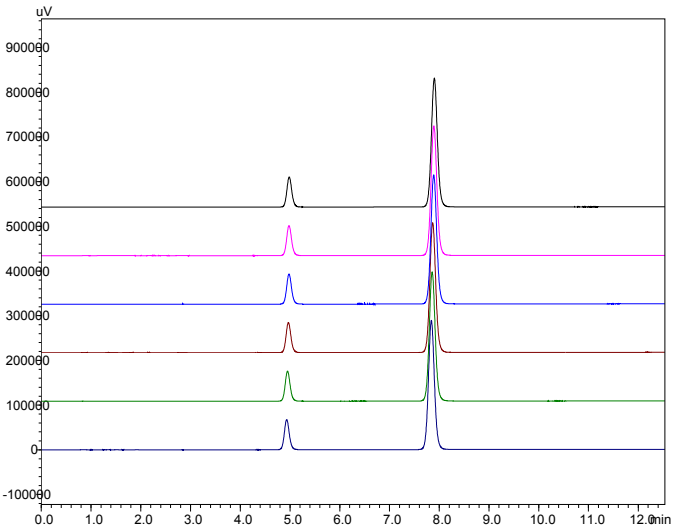


Table II: Method Precision

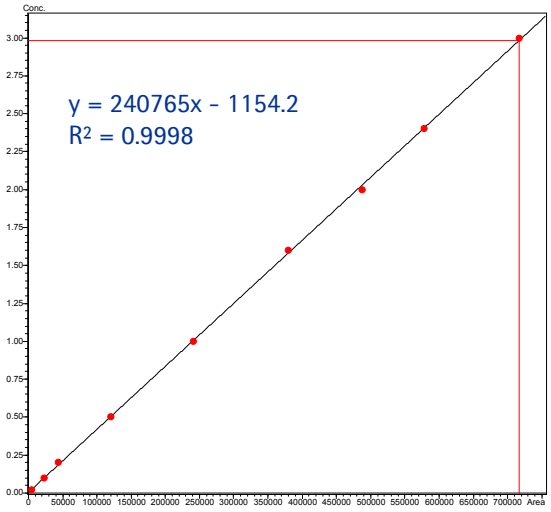
	Retention Time (min)		Area	
Injection	RS A	Letrozole	RS A	Letrozole
1	4.93	7.85	478983	2473247
2	4.95	7.87	479259	2478348
3	4.95	7.87	480187	2479408
4	4.94	7.86	479478	2479989
5	4.94	7.86	480206	2477724
6	4.95	7.87	478293	2486492
Average	4.94	7.863	480206	2479201
Std. Dev.	<0.01	<0.01	3294	4294
% RSD	0.12	0.09	0.69	0.17

Letrozole and Related Substances (USP)

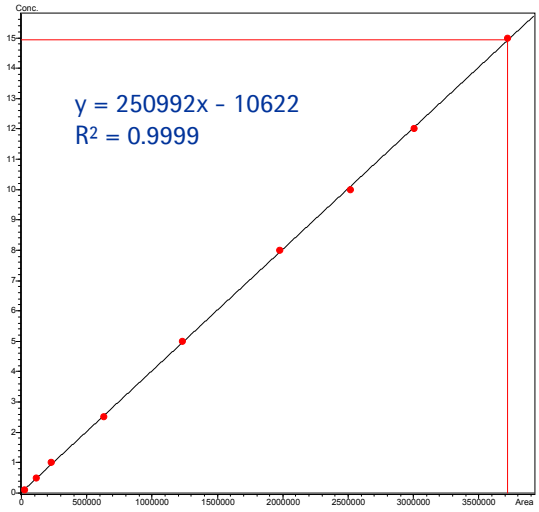
Purospher® STAR RP-18 endcapped

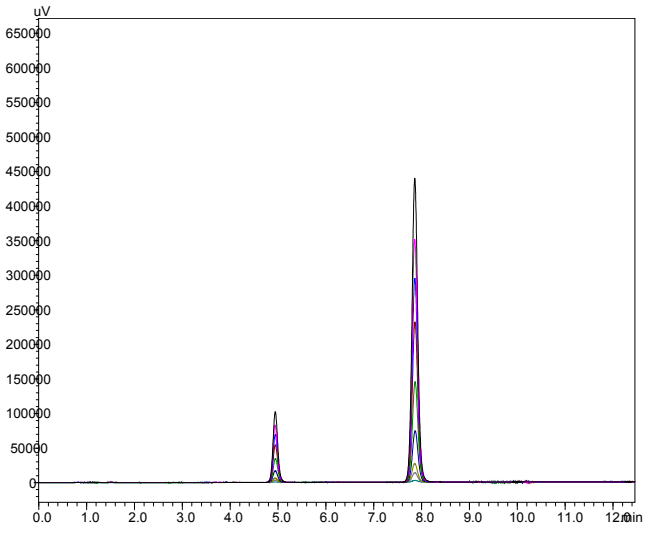
Linearity:

Letrozole Related Substance A



Letrozole





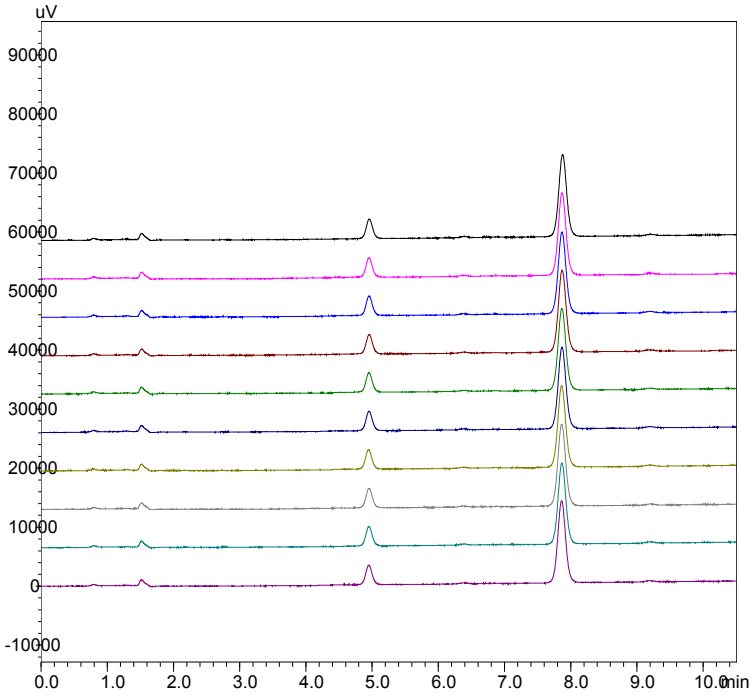
Letrozole RS A		Letrozole	
Conc (ppm)	Area	Conc (ppm)	Area
0.02	4394	0.1	22557
0.1	22630	0.5	116715
0.2	43882	1.0	229577
0.5	120464	2.5	631260
1.0	240666	5.0	1231279
1.6	380178	8.0	1976461
2.0	487146	10.0	2519018
2.4	578138	12.0	3001958
3.0	717196	15.0	3722293

LOD for Related Compound A 0.03 ppm
LOQ for Related Compound A 0.1 ppm

Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

LOQ repeatability Related Substance A:



Injection	Area
1	22718
2	22826
3	22825
4	22730
5	22799
6	22498
7	22622
8	22346
9	22291
10	22555
Average	22621
Std. Dev	194.54
% RSD	0.86 %

Relative standard deviation (RSD in %) for Letrozole Related Substance A at LOQ level 0.86%

Conclusion:

The analytical HPLC method for Letrozole related substance has been validated and it passed all method validation criteria with the Purospher® STAR RP-18 endcapped column.