HPLC Analysis of Letrozole and its Impurities using a Purospher® STAR RP18e Column

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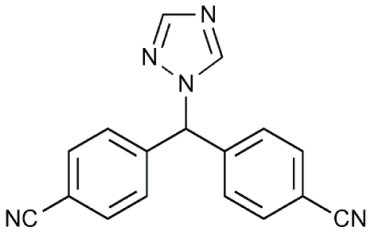
ABSTRACT

This application note follows the USP method for analysis of Letrozole and its related impurity-A by HPLC-UV. The analytes were all separated on a Purospher® STAR RP-18 endcapped column with an excellent peak shape and good detection limits. The method validation results have met the acceptance criteria cited in USP43-NF38 monograph suggesting robust method performance and increased confidence in results.

INTRODUCTION

Letrozole is an oral non-steroidal aromatase inhibitor used in treatment of hormonally-responsive breast cancer after surgery. It prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit [1]. In this work, we present an HPLC method with UV detection for the simultaneous quantification of letrozole and its impurities by following USP43-NF38 Guidelines [2].

Figure 1. Chemical Structure of Letrozole



INSTRUMENTATION

- Shimadzu Prominence UFLC XR System
- Milli-Q® Integral 3 Water purification system
- Ultrasonic bath from PCI analytics
- Millex® HV Durapore membrane filter (PVDF) 0.45 µm

Parameter	Value
Mobile phase	**Solution-A**: Milli-Q® or Ultrapure water (HPLC grade) **Solution-B**: Acetonitrile (HPLC grade). **Gradient Program:** **Time (min) A (%) B (%)** 0.01 70 30 25.0 30 70 26.0 70 30 30.0 70 30
Analytical Column	Purospher® STAR RP-18e, 125x4.6 mm, 5 µm
Diluent	Solution-A and Solution-B (7:3) v/v
Back pressure	72 - 28 Bar (1044 - 410 psi)
Injection volume	20 µL
Column temperature	25 °C

Flow rate	1 mL/min
UV detection	230 nm
Standard	Dissolve 10 mg of Letrozole USP in 30 mL of diluent, mix well and dilute to 100 mL with the diluent.
System suitability (SST) solution	Dissolve 10 mg of Letrozole USP and 20 mg of Letrozole related impurity-A containing 50 mL of diluent, mix well and dilute to 100 mL with the diluent.
Sample	Weigh accurately about 25 mg of Letrozole to a 250 mL volumetric flask containing 75 mL of acetonitrile. Dilute with water to volume, mix well and filter the resulting solution using a 0.22 µm syringe filter for HPLC analysis.

Table 1. Experimental conditions for the analysis

CHROMATOGRAPHIC DATA OF LETROZOLE AND ITS RELATED IMPURITY ANALYSIS

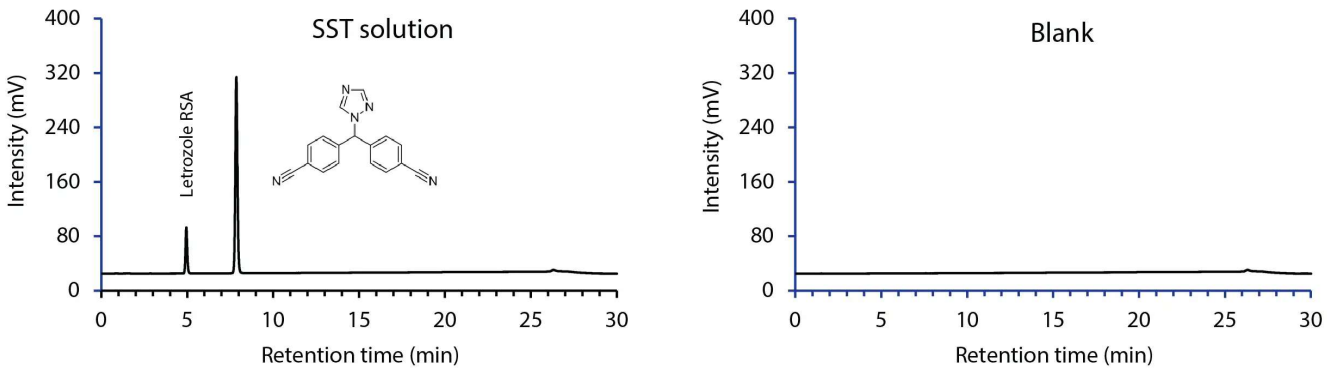


Figure 2. Chromatograms of (Left) Letrozole related impurity-A and Letrozole USP; (Right) Blank solution (diluent)

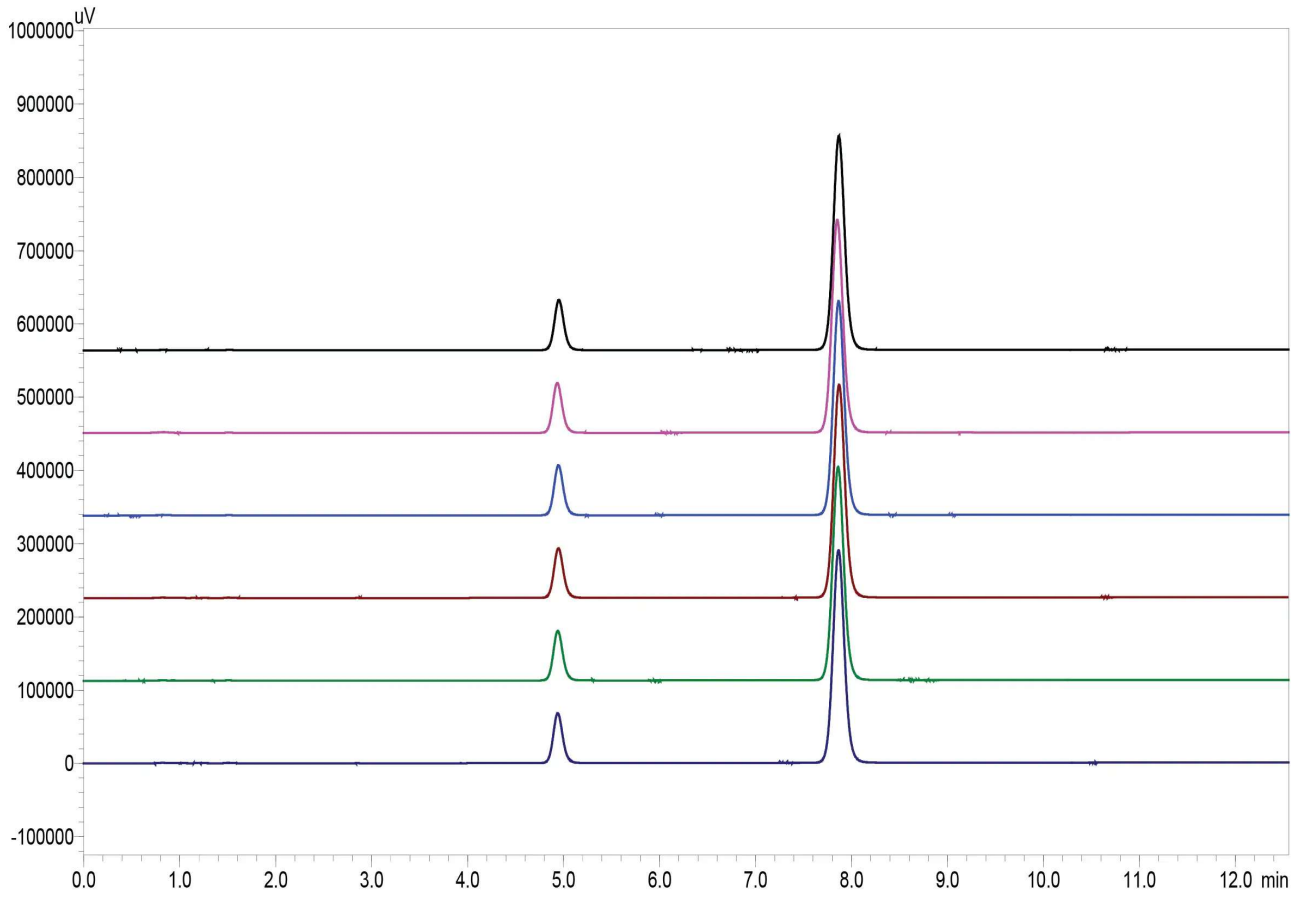


Figure 3. Overlaid Chromatogram of Letrozole and its related impurity-A using SST solution; 1). Letrozole (4.9 min); 2). Letrozole related impurity-A (7.9 min)

#	Compound	Retention Time (min)	RRT	RRT per USP43	Resolution	Resolution per USP43	Theoretical plates
1	Letrozole related compound A (RCA)	4.9	0.62	0.67	-	-	1.1
2	Letrozole	7.9	1.00	1.00	14.3	2.0	1.1

Table 2. System Suitability criteria of Letrozole and its Impurity-A

Identity	Letrozole RS-A	Letrozole
1	474836	2457649
2	474364	2458516
3	474541	2450935
4	474747	2449480
5	474431	2450486
6	473816	2451989
Average	474456	2453176
Standard Deviation	362	3895
RSD (%)	<0.1	0.2

Table 3. Repeatability of Letrozole and its related impurity-A (SST Solution)

Concentration (µg/mL)	Letrozole RS-A (Mean Area)	Concentration (µg/mL)	Letrozole (Mean 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
Line equation	y = 240765x - 1154.2	y = 250992x - 10622	
R^2	0.9998	0.9998	

Table 4. Linearity of Letrozole and its related impurity - A

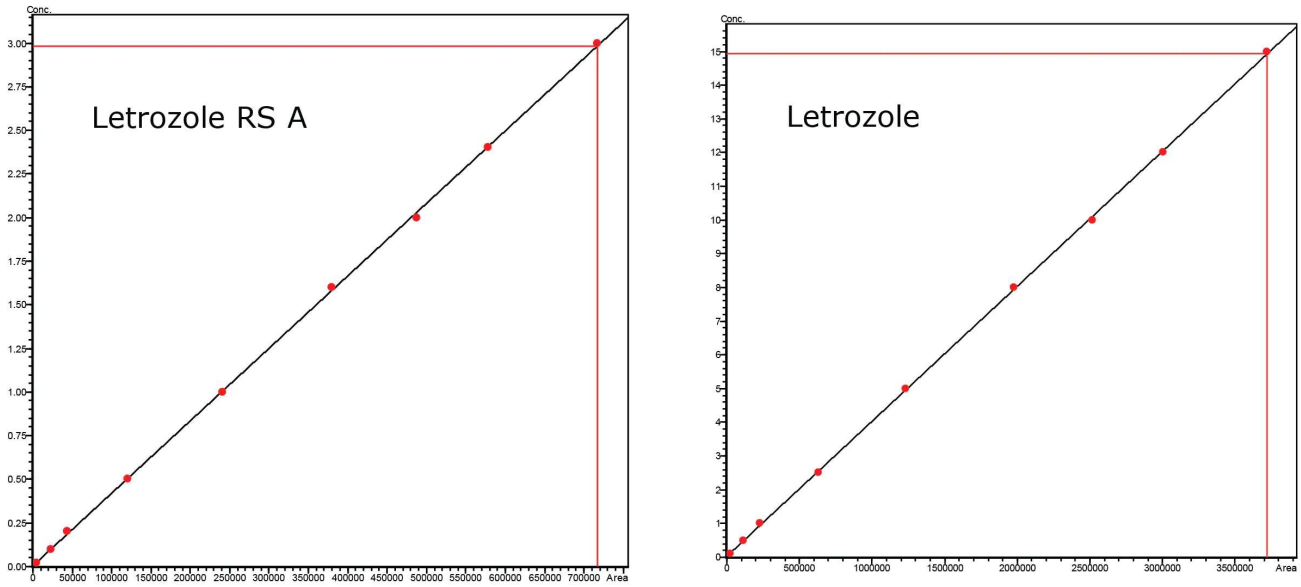


Figure 4. Linearity plot of (Left) Letrozole Related impurity-A; (Right) Letrozole

RESULTS AND DISCUSSION

An isocratic RP-HPLC method for the determination of Letrozole and its related impurity-A was tested using Purospher® STAR RP-18e column, referring to USP43-NF38. The experimental data indicated an excellent linearity with an r^2 value of ≥ 0.998 (**Figure 4**) for the selected concentration range (**Table 4**). The LOD & LOQ values for Letrozole related impurity-A were 0.03 & 0.09 µg/mL, respectively.

CONCLUSION

A HPLC-UV method was tested for the reliable estimation of Letrozole and its related impurity-A using Purospher® STAR RP-18 endcapped HPLC column. The data from linearity, system suitability,

repeatability of the method suggests that the method provided better retentivity and specificity for the assay of Letrozole and its related impurity-A by following USP43-NF38 monograph.

RELATED PRODUCTS

Product No.	Description	SDS	Pricing
1.51914	**Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 125-4.6** suitable for HPLC		Expand
1356971	**Letrozole** United States Pharmacopeia (USP) Reference Standard		Expand
1356982	**Letrozole Related Compound A** United States Pharmacopeia (USP) Reference Standard		Expand
1.00030	**Acetonitrile** gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur		Expand
1.15333	**Water** for chromatography (LC-MS Grade) LiChrosolv®		Expand
SLHVX13NK	**Millex Syringe Filter, Durapore® (PVDF), Non-sterile** 0.45 µm pore size, 13 mm diameter, Millex-HV Durapore® (PVDF) membrane, hydrophilic		Expand

References

1. Blackwell K, Burris H, Gomez P, Lynn Henry N, Isakoff S, Campana F, Gao L, Jiang J, Macé S, Tolaney SM. 2015. Phase I/II dose-escalation study of PI3K inhibitors pilaralisib or voxtalisib in combination with letrozole in patients with hormone-receptor-positive and HER2-negative metastatic breast cancer refractory to a non-steroidal aromatase inhibitor. *Breast Cancer Res Treat.* 154(2):287-297. **https://doi.org/10.1007/s10549-015-3615-9**

2. Letrozole. **https://doi.org/10.31003/uspnf_m44500_04_01**