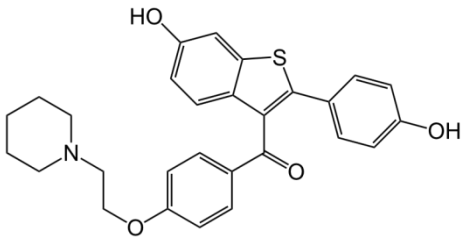
Raloxifene Hydrochloride (USP)



Raloxifene is an oral selective estrogen receptor modulator (SERM) that has estrogenic actions on bone and anti-estrogenic actions on the uterus and breast. Evista is the common commercial brand name and it was developed by Eli Lilly and Company.

We have followed the experimental conditions in the current Raloxifene hydrochloride USP monograph (USP38-NF33):

Identification – FTIR (197K)
Assay – HPLC and UHPLC (isocratic methods)
Related Substances (RS) – HPLC (gradient method)

The Assay and Related Substances (RS) have been carried out with HPLC using RP-8 and RP-18 endcapped columns. The assay method has also been scaled to a shorter column dimension with smaller particle size, to shorten the analysis time and to improve the sensitivity of the method. This is an allowed change within partial revalidation, as it is an isocratic method.

Raloxifene Hydrochloride (USP)

Definition:

Raloxifene Hydrochloride contains NLT 97.5% and NMT 102.0% of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$), calculated on the dried basis.

Identification

A. Infrared Absorption <197K>
B. Identification Tests—General, Chloride191: It meets the requirements, the sample being dissolved in methanol.

Assay: HPLC

Buffer: Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.5 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1.

Mobile phase: Acetonitrile and Buffer (33:67)

System suitability solution: Prepare as directed in the test for Organic Impurities.

Standard solution: 0.05 mg/mL of USP Raloxifene Hydrochloride RS in Mobile phase

Sample solution: 0.05 mg/mL of Raloxifene Hydrochloride in Mobile phase

Chromatographic system (See Chromatography 621, System Suitability.)

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 3.5 µm base-deactivated packing L7

Column temperature: 35°C

Flow rate: 1.5 mL/min *(we used 1.0 mL/min for HPLC method and 0.21 mL/min for UHPLC method)*

Injection volume: 10 µL

Raloxifene Hydrochloride (USP)

System suitability
Sample: System suitability solution

Suitability requirements
Resolution: NLT 2.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for raloxifene
Relative standard deviation: NMT 0.7% for raloxifene

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) in the portion of Raloxifene Hydrochloride taken:

$$\text{Result} = (rU/rS) \times (CS/CU) \times 100$$

rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of USP Raloxifene Hydrochloride RS in the Standard solution (mg/mL)
CU = concentration of the Sample solution (mg/mL)

Acceptance criteria: 97.5%–102.0% on the dried basis

IMPURITIES - Organic Impurities — HPLC

Solution A: Dissolve 9.0 g of monobasic potassium phosphate in 1000 mL of water. Add 0.6 mL of phosphoric acid, and adjust with phosphoric acid or potassium hydroxide solution to a pH of 3.0 ± 0.1.
Solution B: Acetonitrile
Mobile phase: See Table 1.
[Note—Adjust the start time of the gradient step on the basis of the instrument's dwell volume.]

Time (min)	Solution A (%)	Solution B (%)
0.00	75	25
9.00	75	25
40.25	50	50
42.25	75	25
49.00	75	25

Raloxifene Hydrochloride (USP)

Diluent A: Solution A and acetonitrile (70:30)
Diluent B: Tetrahydrofuran and methanol (70:30)

Raloxifene related compound C solution: 0.15 mg/mL of USP Raloxifene RS C in Diluent B
System suitability solution: Transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of Raloxifene related compound C solution, and dilute with Diluent A to volume.
Standard solution: 0.003 mg/mL of USP Raloxifene Hydrochloride RS in Diluent A
Sample solution: 3 mg/mL of Raloxifene Hydrochloride in Diluent A

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5 µm base-deactivated packing L7
Column temperature: 35°C
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: System suitability solution

Suitability requirements
Resolution: NLT 3.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for raloxifene

Analysis
Samples: Standard solution and Sample solution

Record the chromatograms for NLT two times the retention time of the raloxifene peak, and measure all of the peak responses.

Calculate the percentage of each impurity in the portion of Raloxifene Hydrochloride taken:

$$\text{Result} = (rU/rS) \times (CS/CU) \times 100$$

rU = peak response of each impurity in the Sample solution
rS = peak response of raloxifene in the Standard solution
CS = concentration of USP Raloxifene Hydrochloride RS in the Standard solution (mg/mL)
CU = concentration of the Sample solution (mg/mL)

Acceptance criteria: See Table 2. The reporting level for impurities is 0.05%.

Raloxifene Hydrochloride (USP)

Name	RRT	Acceptance criteria (%)
Raloxifene 3,7-diketone[a]	0.74	0.20
Raloxifene	1.00	-
Other impurities	-	0.10
Total impurities	-	0.5

a) Methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3,7-diyl]bis[4-[2-(1-piperidinyl)ethoxy]phenyl].

ADDITIONAL REQUIREMENTS

Packaging and Storage: Preserve in tight containers, and store at controlled room temperature.

USP Reference Standards

USP Raloxifene Hydrochloride RS
USP Raloxifene Related Compound C RS
1-(2-{4-[6-Hydroxy-2-(4-hydroxyphenyl)benzothiophene-3-carbonyl]phenoxy}ethyl)piperidine 1-oxide. ($C_{28}H_{27}NO_5S$)

Recommended products:

FTIR – Identification (197K)

Potassium bromide for IR spectroscopy Uvasol® (1.04907)

HPLC Assay and Related Substances

Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm (1.50009.7220) for assay scaled to
Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm (1.50629.0001)
Purospher® STAR RP-8 endcapped (5µm) 250x4.6 mm (1.51454.0001) for RS analysis
Potassium dihydrogen phosphate for analysis (<= 0.005% Na) EMSURE® ACS,ISO,Reag. Ph Eur 104877
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur 100573
Potassium hydroxide solution 47% for analysis EMSURE® 105545
Acetonitrile (isocratic grade for liquid chromatography LiChrosolv®) 1.14291

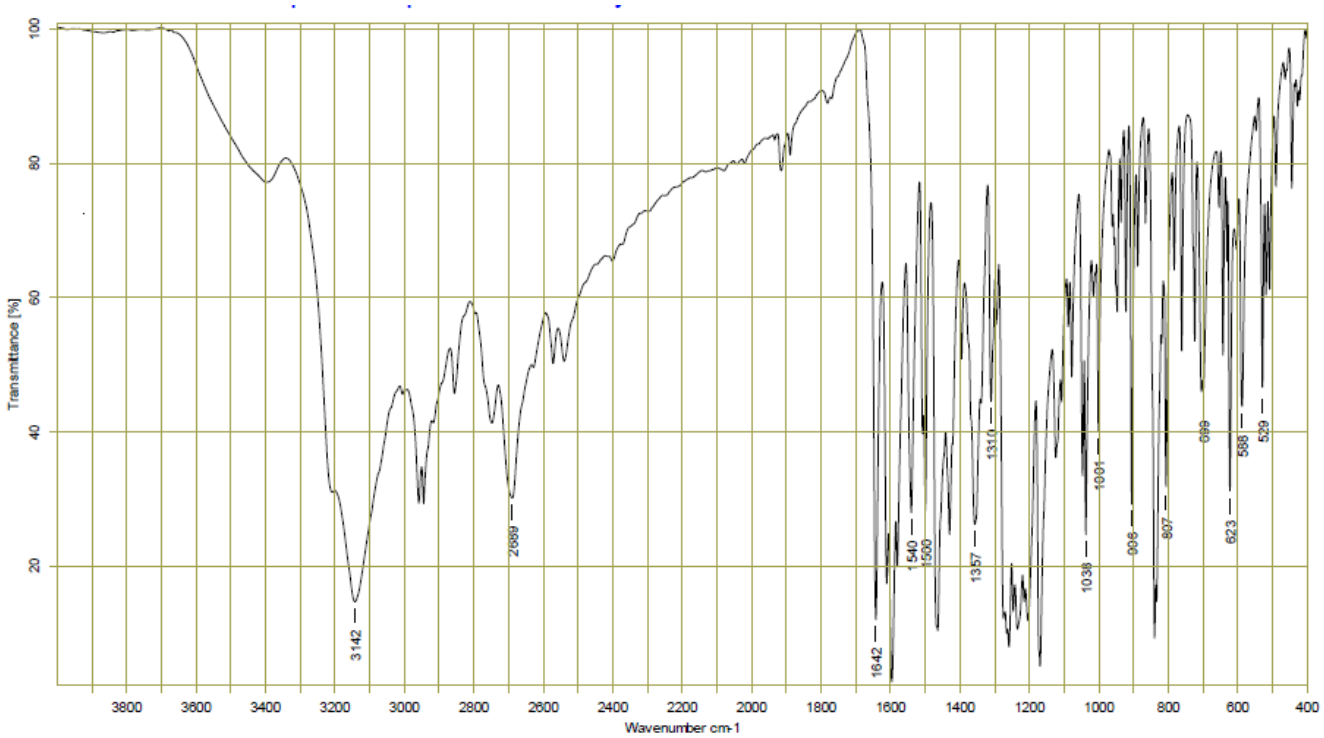
Raloxifene Hydrochloride (USP)

Identification

A. INFRARED ABSORPTION <197K> FTIR

The reference 197K in a monograph signifies that the substance under examination is mixed intimately with potassium bromide.

We recommend Potassium bromide for IR spectroscopy Uvasol® (1.04907) .

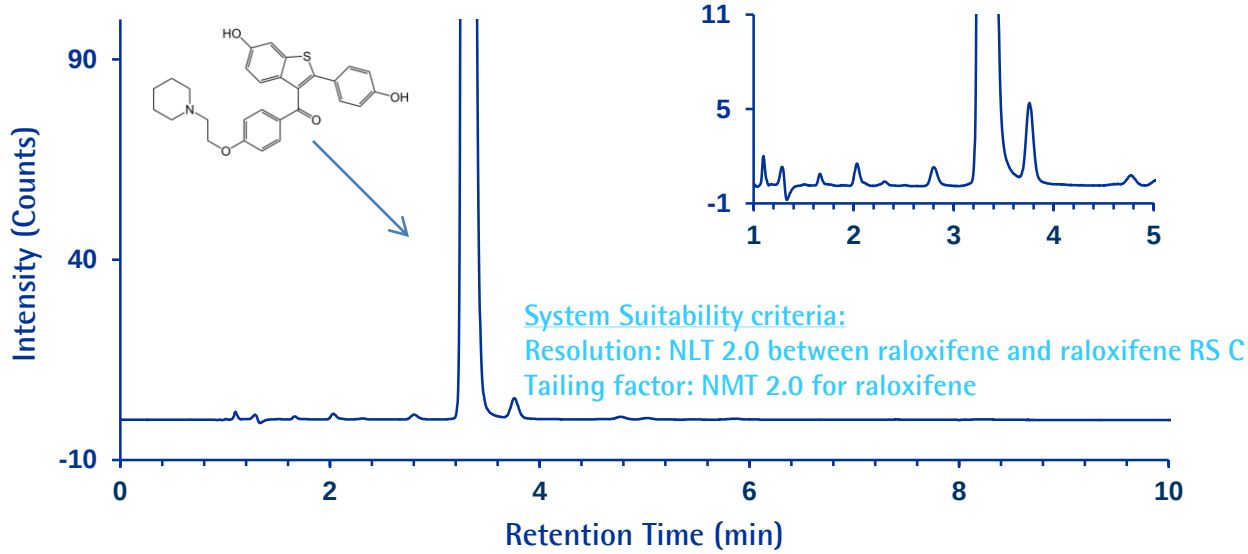


Raloxifene Hydrochloride (USP) – Assay

Purospher® STAR RP-8 endcapped HPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm	1.50009.7220
Injection:	10 µL	
Detection:	UV 280nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.3 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1. Mix acetonitrile and buffer (33:67 v/v)	
Temperature:	35°C	
Diluent:	Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.	
Samples:	**Standard solution:** 0.05 mg/mL of USP Raloxifene Hydrochloride RS in Mobile phase **Sample solution:** 0.05 mg/mL of Raloxifene Hydrochloride in Mobile phase **System suitability solution:** transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of Raloxifene related compound C solution, and dilute with Diluent A to volume.	
Pressure Drop:	172 Bar (2494psi)	



Chromatographic Data: (SST)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	1.3			
Raloxifene RS	3.3		9337	1.14
Raloxifene RS C	3.8	3.4	11166	1.05

Raloxifene Hydrochloride (USP) - Assay

Validation and Verification HPLC - Assay

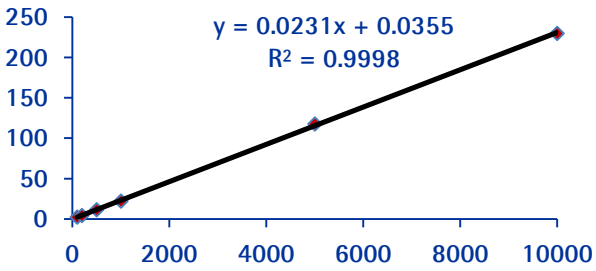
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Raloxifene HCl and Raloxifene RS C using a Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Raloxifene HCl	3.34	0.84	1.16	-
Raloxifene RS C	3.95	1.00	1.05	3.4

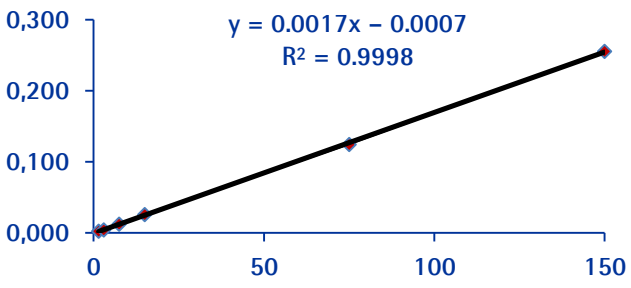
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 100-10000 ppm of Raloxifene HCl, and six (6) concentration levels ranging from 1.5-150 ppm of Raloxifene RS C.

	[Raloxifene] (ppm)	Area (mAU*min)	[Raloxifene RS C] (ppm)	Area (mAU*min)
	100	2.378	1.5	0.002
	200	4.305	3.0	0.004
	500	11.498	7.5	0.012
	1000	22.221	15	0.026
	5000	117.685	75	0.124
	10000	229.634	150	0.255
STEYX		0.611		0.00066
SLOPE		0.024		0.00166
LOD		85		1.3
LOQ		259		4.0

Raloxifene



Raloxifene RS C

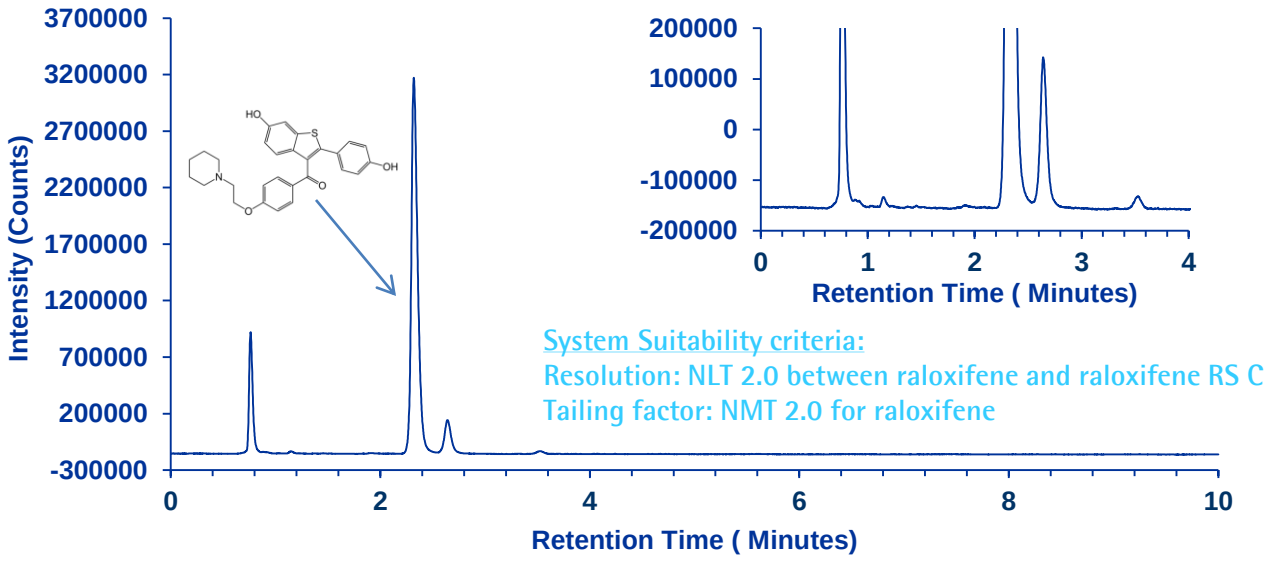


Raloxifene Hydrochloride (USP) – Assay

Purospher® STAR RP-8 endcapped UHPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm	1.50629.0001
Injection:	2 µL	
Detection:	UV 280nm	
Cell:	1.4 µL	
Flow Rate:	0.21 mL/min	
Mobile Phase:	Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.3 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1. Mix acetonitrile and buffer (33:67 v/v)	
Temperature:	35°C	
Diluent:	Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.	
Samples:	**Standard solution:** 0.05 mg/mL of USP Raloxifene Hydrochloride RS in Mobile phase **Sample solution:** 0.05 mg/mL of Raloxifene Hydrochloride in Mobile phase **System suitability solution:** Transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of Raloxifene related compound C solution, and dilute with Diluent A to volume.	
Pressure Drop:	261 Bar (3785psi)	



System Suitability Solution

System Suitability criteria:
Resolution: NLT 2.0 between raloxifene and raloxifene RS C
Tailing factor: NMT 2.0 for raloxifene

Chromatographic Data: (SST)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	0.9			
Raloxifene RS	2.3		7051	1.13
Raloxifene RS C	2.6	2.8	8119	1.11

Raloxifene Hydrochloride (USP) - Assay

Validation and Verification UHPLC - Assay

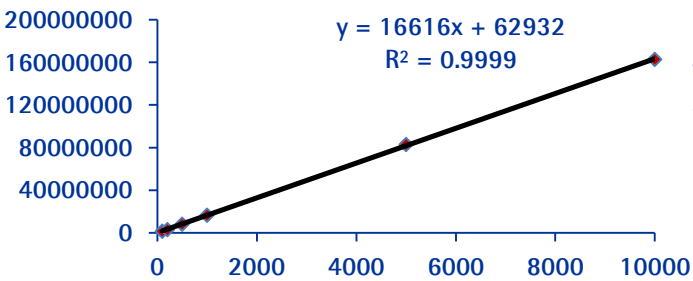
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Raloxifene HCl and Raloxifene RS C using a Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Raloxifene HCl	2.32	0.88	1.13	-
Raloxifene RS C	2.64	1.00	1.1	2.8

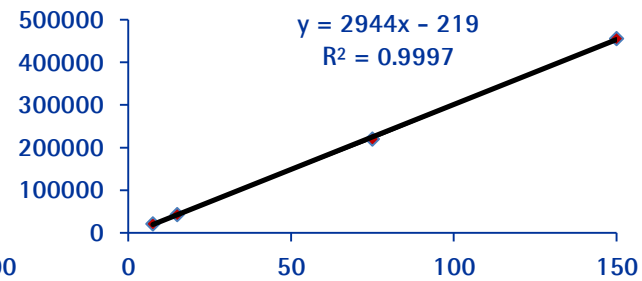
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 100-10000 ppm of Raloxifene HCl, and four (4) concentration levels ranging from 7.5-150 ppm of Raloxifene RS C.

	[Raloxifene] (ppm)	Area (counts)	[Raloxifene RS C] (ppm)	Area (counts)
	100	1548778	7.5	21459
	200	3093681	15	43233
	500	8091485	75	220077
	1000	16499725	150	455731
	5000	82905592		
	10000	162787912		
STEYX		62932		219
SLOPE		16616		2944
LOD		12.5		0.25
LOQ		38		0.75

Raloxifene



Raloxifene RS C



Raloxifene HCl (USP) – Related Substances

Purospher® STAR RP-8 endcapped HPLC

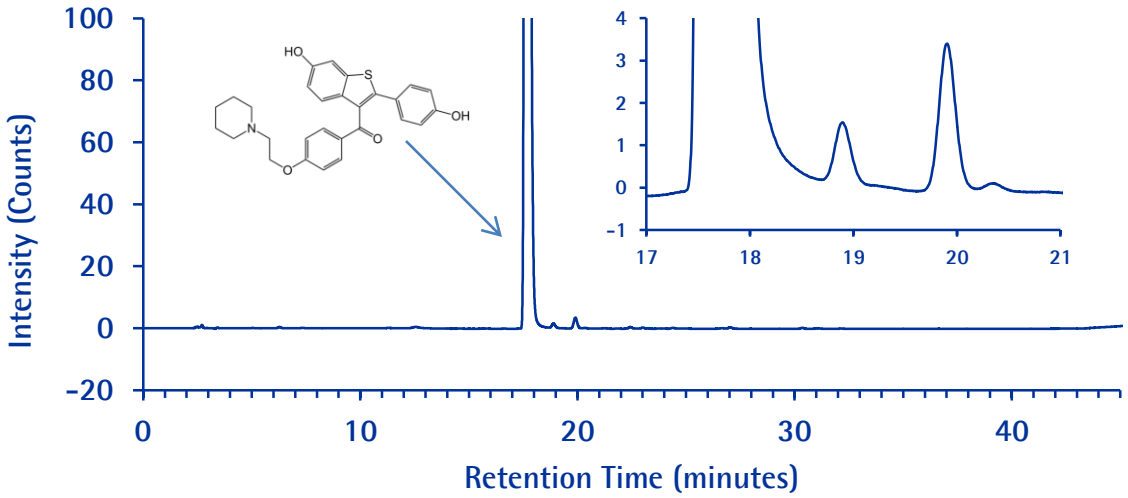
Column:	Purospher® STAR RP-8 endcapped (5µm) 250x4.6 mm	1.51454.0001
Injection:	10 µL	
Detection:	UV 280nm	
Cell:	11 µL	
Flow Rate:	1.5mL/min	
Solution A:	Assay solution A : Acetonitrile 75:25	
Solution B:	Assay solution A : Acetonitrile 50:50	
Gradient:	See table	
Temperature:	35°C	
Diluent	Acetonitrile:Buffer 60:40	

Time (min)	Solution A (%)	Solution B (%)
0.00	75	25
9.00	75	25
40.25	50	50
42.25	75	25
49.00	75	25

SST stock solution: Transfer 15mg of USP Raloxifene Hydrochloride RS to a 50 mL volumetric flask and add 15mL Acetonitrile, 3mL water and 5mL of 30% hydrogen peroxide. Shake the solution for 30min, followed by 30min sonication. Let it stand at least for 6h at 30°C. Dilute with diluent 1 to 50mL

SST solution: 15mg Raloxifene HCl to a 50mL volumetric flask, add 5mL of system suitability stock solution and 20mL of Diluent 2. Dilute with impurity solution A.

Pressure Drop: 81-145Bar (1175-2103psi)



System Suitability criteria:
Resolution: NLT 3.0 between raloxifene and raloxifene RS C
Tailing factor: NMT 2.0 for raloxifene

Chromatographic Data: (SST)

Compound	Retention Time (min)	RRT	Resolution	Plates	Tailing Factor
t0 void volume	3.4	-			
Raloxifene RS	17.3	0.86		66831	1.10
Raloxifene RS C	19.9	1.00	6.1	72260	1.06

Raloxifene HCl (USP) – Related Substances

Validation and Verification HPLC

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Raloxifene HCl and Raloxifene RS C using a Purospher® STAR RP-8 endcapped (5 µm) 250x4.6 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Raloxifene HCl	17.3	0.89	1.09	-
Raloxifene RS C	19.9	1.0	1.12	6.09

2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 100-10000 ppm of Raloxifene HCl, and six (6) concentration levels ranging from 1.5-150 ppm of Raloxifene RS C.

	[Raloxifene] (ppm)	Area (mAU*min)	[Raloxifene RS C] (ppm)	Area (mAU*min)
	100	5.599	1.5	0.010
	200	10.812	3.0	0.022
	500	27.328	7.5	0.114
	1000	55.919	15	0.216
	5000	273.231	75	0.010
	10000	556.720	150	0.022
STEYX		0.375		8.3E-05
SLOPE		0.056		0.00154
LOD		22		0.18
LOQ		67		0.54