Raloxifene Hydrochloride (USP)

![Image of Raloxifene molecule](image)

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.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/perfect-solution
Definition:
Raloxifene Hydrochloride contains NLT 97.5% and NMT 102.0% of raloxifene hydrochloride (C_{28}H_{27}NO_{4}S·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 (C28H27NO4S·HCl) in the portion of Raloxifene Hydrochloride taken:

\[ \text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{CS}{CU} \right) \times 100 \]

\( r_U \) = peak response from the Sample solution
\( r_S \) = 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

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.]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>9.00</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>40.25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>42.25</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>49.00</td>
<td>75</td>
<td>25</td>
</tr>
</tbody>
</table>
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:

Result = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100

r_U = peak response of each impurity in the Sample solution
r_S = peak response of raloxifene in the Standard solution
C_S = concentration of USP Raloxifene Hydrochloride RS in the Standard solution (mg/mL)
C_U = concentration of the Sample solution (mg/mL)

Acceptance criteria: See Table 2. The reporting level for impurities is 0.05%.
Raloxifene Hydrochloride (USP)

<table>
<thead>
<tr>
<th>Name</th>
<th>RRT</th>
<th>Acceptance criteria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raloxifene 3,7-diketone⁴</td>
<td>0.74</td>
<td>0.20</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>Other impurities</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Total impurities</td>
<td>-</td>
<td>0.5</td>
</tr>
</tbody>
</table>


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)benzo[b]thiophene-3-carbonyl]phenoxy)ethyl)piperidine 1-oxide.  \(\text{C}_{28}\text{H}_{27}\text{NO}_{5}\text{S}\)

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) ESMURE® ACS,ISO,Reag. Ph Eur  104877
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)
ortho-Phosphoric acid 85% for analysis ESMURE® ACS,ISO,Reag. Ph Eur  100573
Potassium hydroxide solution 47% for analysis ESMURE®  105545
Acetonitrile (isocratic grade for liquid chromatography LiChrosolv®) 1.14291
Raloxifene Hydrochloride (USP)

Identification

A. INFRARED ABSORPTION <197K>  

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

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.

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)

Sample solution:

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)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Resolution</th>
<th>Plates</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0 void volume</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raloxifene RS</td>
<td>3.3</td>
<td>9337</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>Raloxifene RS C</td>
<td>3.8</td>
<td>11166</td>
<td>1.05</td>
<td></td>
</tr>
</tbody>
</table>

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/perfect-solution
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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>RRT</th>
<th>Tailing factor</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raloxifene HCl</td>
<td>3.34</td>
<td>0.84</td>
<td>1.16</td>
<td>–</td>
</tr>
<tr>
<td>Raloxifene RS C</td>
<td>3.95</td>
<td>1.00</td>
<td>1.05</td>
<td>3.4</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>[Raloxifene] (ppm)</th>
<th>Area (mAU*min)</th>
<th>[Raloxifene RS C] (ppm)</th>
<th>Area (mAU*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.378</td>
<td>1.5</td>
<td>0.002</td>
</tr>
<tr>
<td>200</td>
<td>4.305</td>
<td>3.0</td>
<td>0.004</td>
</tr>
<tr>
<td>500</td>
<td>11.498</td>
<td>7.5</td>
<td>0.012</td>
</tr>
<tr>
<td>1000</td>
<td>22.221</td>
<td>15</td>
<td>0.026</td>
</tr>
<tr>
<td>5000</td>
<td>117.685</td>
<td>75</td>
<td>0.124</td>
</tr>
<tr>
<td>10000</td>
<td>229.634</td>
<td>150</td>
<td>0.255</td>
</tr>
</tbody>
</table>

| STEYX              | 0.611          | 0.00066                 |
| SLOPE              | 0.024          | 0.00166                 |
| LOD                | 85             | 1.3                     |
| LOQ                | 259            | 4.0                     |

Raloxifene

\[
y = 0.0231x + 0.0355
\]
\[R^2 = 0.9998\]

Raloxifene RS C

\[
y = 0.0017x - 0.0007
\]
\[R^2 = 0.9998\]
Raloxifene Hydrochloride (USP) – Assay

Purospher® STAR RP-8 endcapped – UHPLC

Chromatographic Conditions

Column: Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm

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.

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)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Resolution</th>
<th>Plates</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0 void volume</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raloxifene RS</td>
<td>2.3</td>
<td>7051</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>Raloxifene RS C</td>
<td>2.6</td>
<td>8119</td>
<td>1.11</td>
<td></td>
</tr>
</tbody>
</table>

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/perfect-solution
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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>RRT</th>
<th>Tailing factor</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raloxifene HCl</td>
<td>2.32</td>
<td>0.88</td>
<td>1.13</td>
<td>-</td>
</tr>
<tr>
<td>Raloxifene RS C</td>
<td>2.64</td>
<td>1.00</td>
<td>1.1</td>
<td>2.8</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>[Raloxifene] (ppm)</th>
<th>Area (counts)</th>
<th>[Raloxifene RS C] (ppm)</th>
<th>Area (counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1548778</td>
<td>7.5</td>
<td>21459</td>
</tr>
<tr>
<td>200</td>
<td>3093681</td>
<td>15</td>
<td>43233</td>
</tr>
<tr>
<td>500</td>
<td>8091485</td>
<td>75</td>
<td>220077</td>
</tr>
<tr>
<td>1000</td>
<td>16499725</td>
<td>150</td>
<td>455731</td>
</tr>
<tr>
<td>5000</td>
<td>82905592</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td>162787912</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

STEYX: 62932
SLOPE: 16616
LOD: 12.5
LOQ: 38

Raloxifene

\[ y = 16616x + 62932 \]

\[ R^2 = 0.9999 \]

Raloxifene RS C

\[ y = 2944x - 219 \]

\[ R^2 = 0.9997 \]
Raloxifene HCl (USP) – Related Substances

Purospher® STAR RP-8 endcapped

HPLC

Column: Purospher® STAR RP-8 endcapped (5µm) 250x4.6 mm

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

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)

Chromatographic Data: (SST)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>RRT</th>
<th>Resolution</th>
<th>Plates</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0 void volume</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raloxifene RS</td>
<td>17.3</td>
<td>0.86</td>
<td>66831</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Raloxifene RS C</td>
<td>19.9</td>
<td>1.00</td>
<td>72260</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>RRT</th>
<th>Tailing factor</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raloxifene HCl</td>
<td>17.3</td>
<td>0.89</td>
<td>1.09</td>
<td>–</td>
</tr>
<tr>
<td>Raloxifene RS C</td>
<td>19.9</td>
<td>1.0</td>
<td>1.12</td>
<td>6.09</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>[Raloxifene] (ppm)</th>
<th>Area (mAU*min)</th>
<th>[Raloxifene RS C] (ppm)</th>
<th>Area (mAU*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>5.599</td>
<td>1.5</td>
<td>0.010</td>
</tr>
<tr>
<td>200</td>
<td>10.812</td>
<td>3.0</td>
<td>0.022</td>
</tr>
<tr>
<td>500</td>
<td>27.328</td>
<td>7.5</td>
<td>0.114</td>
</tr>
<tr>
<td>1000</td>
<td>55.919</td>
<td>15</td>
<td>0.216</td>
</tr>
<tr>
<td>5000</td>
<td>273.231</td>
<td>75</td>
<td>0.010</td>
</tr>
<tr>
<td>10000</td>
<td>556.720</td>
<td>150</td>
<td>0.022</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>STEYX</th>
<th>SLOPE</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.375</td>
<td>0.056</td>
<td>22</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>8.3E-05</td>
<td>0.00154</td>
<td>0.18</td>
<td>0.54</td>
</tr>
</tbody>
</table>

![Graphs showing linearity](image_url)