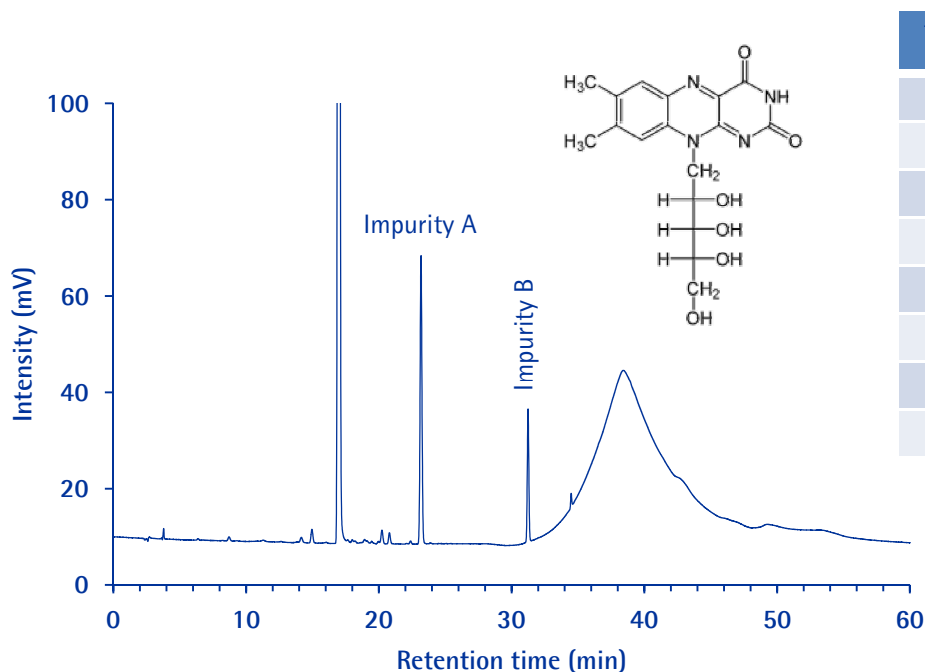


# Riboflavin and Related Substances

## Purospher® STAR RP-18 endcapped (HPLC)

### Chromatographic Conditions

**Column:** Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm 1.51456.0001  
**Injection:** 10 µL  
**Detection:** UV 267 nm  
**Cell:** 10 µL  
**Flow Rate:** 1.0 mL/min  
**Mobile Phase:** Mobile Phase A: Mix water & ortho-phosphoric acid 1000:1 (v/v)  
 Mobile Phase B: Acetonitrile  
**Gradient:** See table  
**Temperature:** 25 °C  
**Sample:** In order to see impurity A & B, dissolve 10 mg substance in 1 mL 0.5 M NaOH solution. Expose to daylight for 1.5 hour. Add 0.5 mL of acetic acid and dilute to 100 mL with Milli-Qwater.  
**Pressure Drop:** 156 to 134 Bar(2262 to 1943 psi)



Time (min)	%A	%B
0.0	90	10
5.0	90	10
20.0	80	20
25.0	80	20
35.0	50	50
45.0	50	50
50.0	90	10
60.0	90	10

### Chromatographic Data

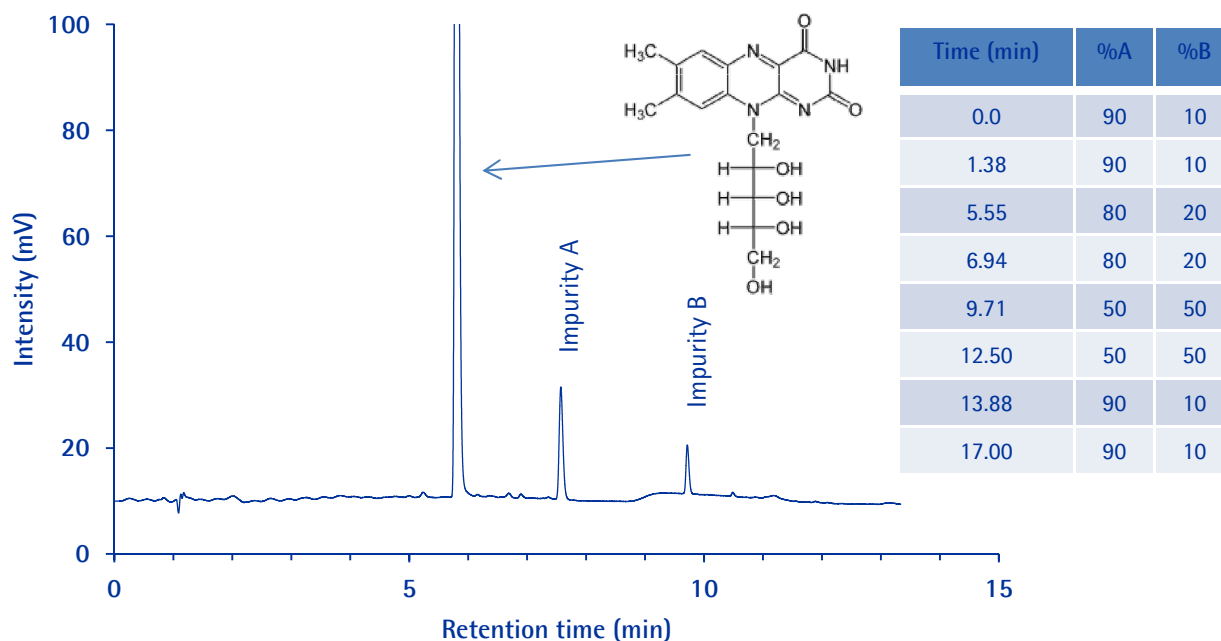
No.	Compound	Retention Time (min)	RRT	Resolution
1	Riboflavin	17.0	1.00	
2	Impurity A	23.2	1.36	
3	Impurity B	31.2	1.84	32.7

# Riboflavin and Related Substances

## Purospher® STAR RP-18 endcapped (UHPLC)

### Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100x2.1 mm	1.50648.0001
Injection:	2 µL	
Detection:	UV 267 nm	
Cell:	2.5 µL (micro flowcell)	
Flow Rate:	0.3 mL/min	
Mobile Phase:	Mobile Phase A: Mix water & ortho-phosphoric acid 1000:1 (v/v) Mobile Phase B: Acetonitrile	
Gradient:	See table	
Temperature:	25 °C	
Sample:	In order to see impurity A & B, dissolve 10 mg substance in 1 mL 0.5 M NaOH solution. Expose to daylight for 1.5 hour. Add 0.5 mL of acetic acid and dilute to 100 mL with Milli-Qwater.	
Pressure Drop:	375 to 330 Bar(5438 to 4785 psi)	



### Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Resolution
1	Riboflavin	5.8	1.00	
2	Impurity A	7.8	1.34	
3	Impurity B	9.7	1.67	20.5