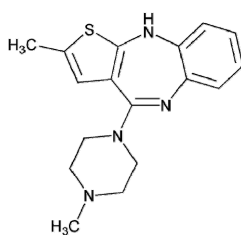


Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped



Olanzapine

Olanzapine is an atypical antipsychotic, approved for the treatment of schizophrenia and bipolar disorder. Common brand names: Zyprexa, Zalasta, Zolafren, Olzapin, Oferta, Zypadhera. Combination Drug: Symbyax (Olanzapine and Fluoxetine)

The current USP monograph method for Olanzapine and related substances specifies use of a 250x4.6 mm column with 5 µm L7 (RP-8) packing as stationary phase.

System suitability requirements for related substances are; the resolution between Olanzapine and Olanzapine related substance A should not be less than 3.0 and the tailing factor should not be more than 1.5 for the Olanzapine peak.

The following pages illustrate that the acceptance criteria are being met for the Olanzapine and related substances method by following the current USP monograph using a 250x4.6 mm Purospher® STAR RP-8 endcapped (5 µm) column.

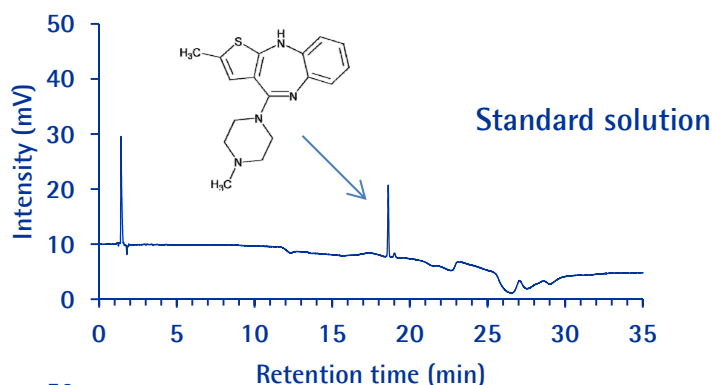
To address the current trend of monograph modernization, and improving the method in terms of selectivity, speed and sensitivity, we also scaled this method to a 100x2.1 mm UHPLC column; Purospher® STAR RP-8 endcapped (2 µm). Also in this example, all the system suitability criteria are met for the Olanzapine and related substances. This is however, a non-allowed scaling and would require a complete revalidation of the new method for approval. For gradient separations, changes in length, column inner diameter and particle size are not allowed. This is new in USP 37.

Olanzapine and Related Substances (USP)

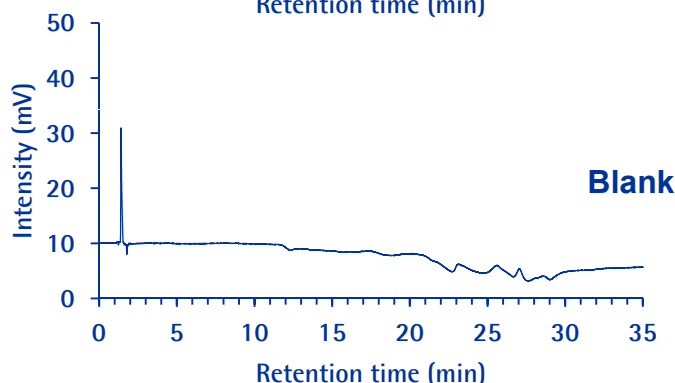
Purospher® STAR RP-8 endcapped – HPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (5µm) Hibar® 250x4.6 mm	1.51454.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water. Add 5 mL of phosphoric acid, and adjust with a sodium hydroxide solution to a pH of 2.5. A: Acetonitrile and Buffer 48:52 (v/v) B: Acetonitrile and Buffer 70:30 (v/v)	
Gradient:	See table	
Temperature:	35°C, Autosampler cooler at 5°C	
Diluent:	37 mg/L of EDTA disodium in Buffer. Acetonitrile and EDTA solution 40:60 (v/v)	
Test Solution:	20 µg/mL of USP Olanzapine, and 2 µg/mL each of Related Compound A and B in diluent	
Standard Solution:	2 µg/mL of USP Olanzapine in diluent	
Sample Solution:	0.4 mg/mL of Olanzapine in diluent	
Pressure Drop:	168 – 129 Bar (2434 – 1871 psi)	

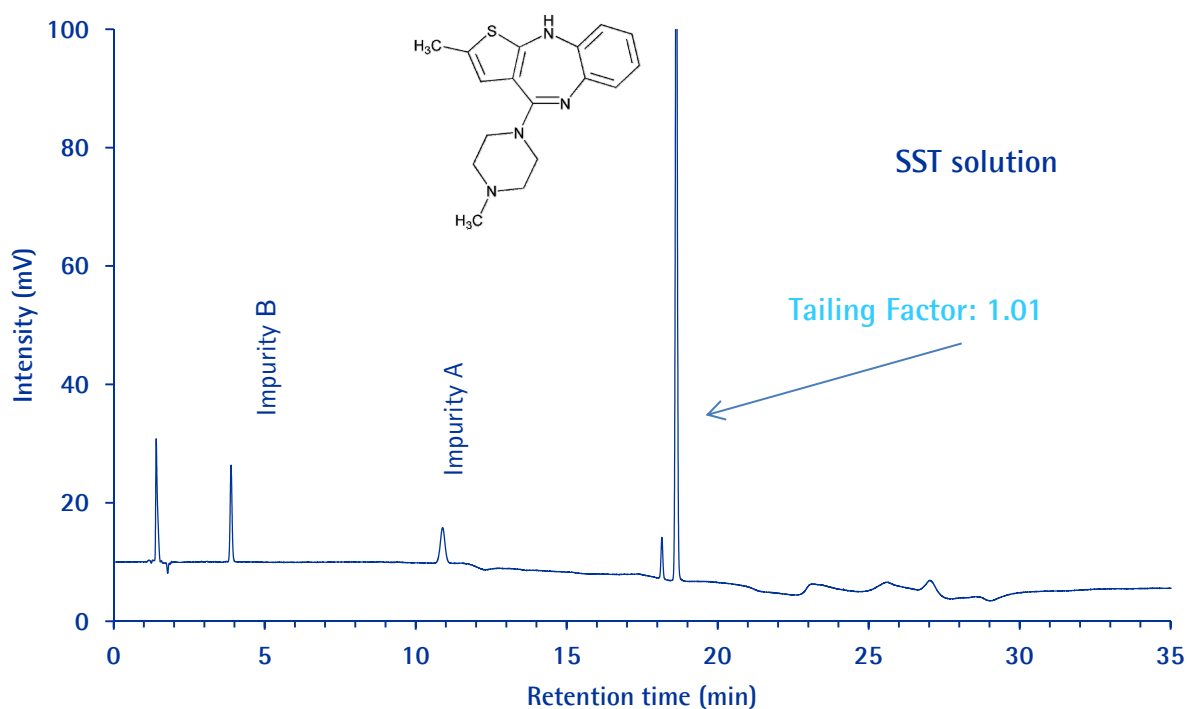


Time (min)	A (%)	B (%)
0.0	100	0
8.0	100	0
17.0	70	30
20.0	70	30
20.1	100	0
23.0	100	0



Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped - HPLC



Chromatographic Data :

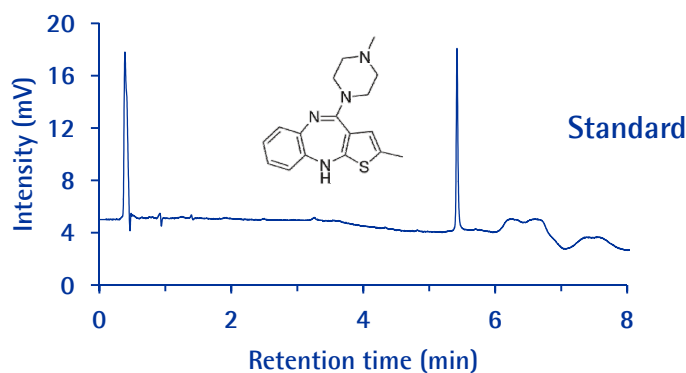
No.	Compound	Retention Time (min)	Resolution	RRT
1	Olanzapine Related compound B	3.9		0.21
2	Olanzapine Related compound A	10.9	34.3	0.59
3	Olanzapine	18.6	37.9	1.00

Olanzapine and Related Substances (USP)

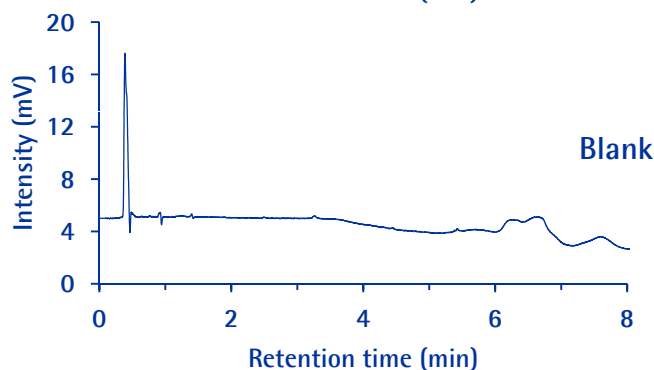
Purospher® STAR RP-8 endcapped – UHPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (2µm) Hibar® HR 100x2.1 mm	1.50629.0001
Injection:	5 µL (<i>larger injection volume than according to column tube volume reduction</i>)	
Detection:	UV 220 nm	
Cell:	2.5 µL	
Flow Rate:	0.5 mL/min (<i>faster flow rate – thus not according to same linear velocity</i>)	
Mobile Phase:	Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water. Add 5 mL of phosphoric acid, and adjust with a sodium hydroxide solution to a pH of 2.5.	
	A: Acetonitrile and Buffer 48:52 (v/v)	
	B: Acetonitrile and Buffer 70:30 (v/v)	
Gradient:	See table	
Temperature:	35°C, Sample cooler at 5°C	
Diluent:	37 mg/L of EDTA disodium in Buffer. Acetonitrile and EDTA solution 40:60 (v/v)	
Test Solution:	20 µg/mL of USP Olanzapine, and 2 µg/mL each of Related Compound A and Related Compound B in diluent	
Standard Solution:	2 µg/mL of USP Olanzapine in diluent	
Sample Solution:	0.4 mg/mL of Olanzapine in Diluent	
Pressure Drop:	408 – 311 Bar (5916 – 4510 psi)	



Time (min)	A (%)	B (%)
0.0	100	0
2.35	100	0
4.85	0	100
6.1	0	100
6.6	100	0
9.0	100	0



Scaling 250x4.6 to 100x2.1

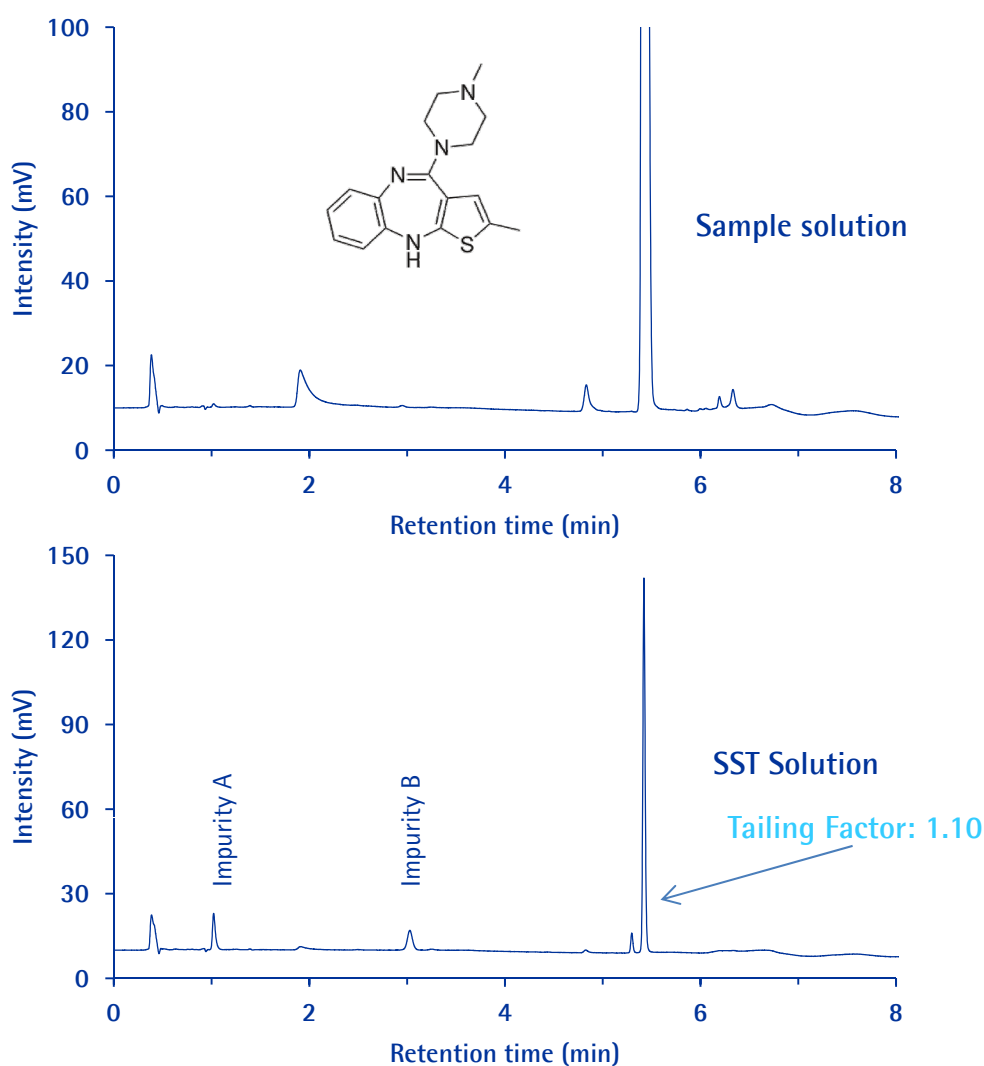
Column volume reduction: 12X
(and thereby injection volume also)

Flow rate reduction: 12X
(to maintain same retention time) or

Flow rate reduction: 4.8 X
(to maintain same linear velocity)

Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped - UHPLC



Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	RRT
1	Olanzapine Related compound A	1.0		0.19
2	Olanzapine Related compound B	3.0	26.1	0.55
3	Olanzapine	5.4	33.6	1.00