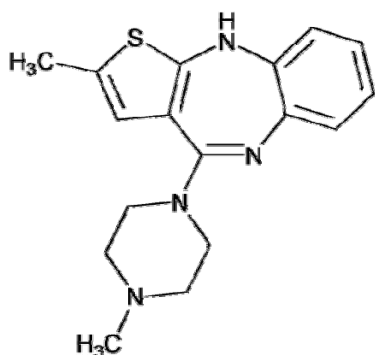


Olanzapine

USP Method Olanzapine Assay



Original Manufacturer:	Eli Lilly (patent expired October 23, 2011)
Brand Name:	Zyprexa, Zalasta, Zolafren, Olzapin, Oferta, Zypadhera
Combination Drug:	Symbyax (Olanzapine and Fluoxetine)

Olanzapine is an atypical antipsychotic, approved for the treatment of schizophrenia and bipolar disorder.

Sales of Zyprexa in 2010 was \$5.7 Billion in total.

US federal health officials approved the first generic versions of the blockbuster drug Zyprexa, October 25, 2011. The new low-cost versions of the drug will be marketed by Indian generic drug maker Dr. Reddy's Laboratories and by Teva Pharmaceutical Industries, based in Israel.



Olanzapine

USP34 – NF29 S1

USP Columns:

ZORBAX RX-C8: Assay 4.6 mm x 15 cm, Related Compounds 4.6 mm x 25 cm, both 5 µm.

Equivalent Column:

Purospher®STAR RP-8 endcapped (5 µm) 250x4.6 mm (1.51454.0001)

Recommended Solvents and Reagents:

Acetonitrile isocratic grade for liquid chromatography LiChrosolv® (1.14291)

Water Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q water purification system

Sodium dodecyl sulfate for ion pair chromatography LiChropur® (1.18309)

Phosphoric Acid Use ACS reagent grade

Sodium di-hydrogen phosphate Use ACS reagent grade

USP Standards

Olanzapine (200 mg)

USP Product Number:1478301

Olanzapine Related Compound A (25 mg)

USP Product Number:1478312

Olanzapine Related Compound B (25 mg)

USP Product Number: 1478323

USP Method Olanzapine Assay

Mobile phase

Dissolve 6.9 g of monobasic sodium phosphate in 1 L water, adjust the pH to 2.5 with phosphoric acid, then add 12 g of sodium dodecyl sulfate per L, and mix. Prepare a filtered and degassed mixture of this buffer and acetonitrile (53:47). Make adjustments if necessary (*see System Suitability under Chromatography 621*).

System suitability solution

Dissolve suitable quantities of USP Olanzapine RS and USP Olanzapine Related Compound A RS in Mobile phase to obtain a final solution having a concentration of about 0.1 mg of Olanzapine and 0.01 mg per mL of Olanzapine related compound A, respectively.

Standard preparation

Dissolve an accurately weighed quantity of USP Olanzapine RS in Mobile phase, and dilute quantitatively with Mobile phase to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation

Dissolve an accurately weighed quantity of Olanzapine in Mobile phase, to obtain a solution having a nominal concentration of about 0.1 mg per mL.

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm × 15-cm column that contains 5-μm L7 packing. The flow rate is about 1.5 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure:

- the resolution, R , between Olanzapine related compound A and Olanzapine is not less than 2.0
- the tailing factor for Olanzapine peak is between 0.8 and 1.5;
- the relative standard deviation for replicate injections for the Olanzapine peak is not more than 1.0%.

Procedure

Separately inject equal volumes (about 20 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{17}H_{20}N_4S$ in the portion of Olanzapine taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of Olanzapine in the Standard preparation and the Assay preparation, respectively; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method Olanzapine RS

Buffer solution

Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water, add 5 mL of phosphoric acid, and adjust the pH to 2.5 with a sodium hydroxide solution.

Solution A: Mix the Buffer solution and acetonitrile (52:48). Filter and degas.

Solution B: Mix acetonitrile and the Buffer solution (70:30). Filter and degas.

Mobile phase

Use variable mixtures of Solution A and Solution B. Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Diluent

Dissolve about 55 mg of edetate disodium in 1500 mL of Buffer solution. Prepare a mixture of the resulting solution and acetonitrile (3:2).

System suitability solution

Dissolve suitable quantities of USP Olanzapine RS, USP Olanzapine Related Compound A RS, and USP Olanzapine Related Compound B RS in Diluent to obtain a solution having a concentration of about 20 µg per mL of Olanzapine and 2 µg per mL each of Olanzapine related compound A and Olanzapine related compound B, respectively.

Standard solution

Dissolve an accurately weighed quantity of USP Olanzapine RS in Diluent to obtain a solution having a known concentration of about 0.002 mg per mL.

Test solution

Transfer about 10 mg of Olanzapine, accurately weighed, to a 25-mL volumetric flask. Dissolve in and dilute with Diluent to volume, and mix.

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 220-nm detector, and a 4.6-mm × 25-cm column that contains 5-µm L7 packing, is maintained at a constant temperature of about 35, and is programmed to provide a Mobile phase consisting of variable mixtures of Solution A and Solution B. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (min)	Solution A (%)	Solution B (%)	Elution
0-10	100	0	Isocratic
10-20	100→0	0→100	Linear gradient
20-25	0	100	Isocratic
25-27	0→100	100→0	Linear gradient
27-35	100	0	Isocratic

USP Method Olanzapine RS

Chromatograph the System suitability solution, and record the peak responses as directed for Procedure. Identify the peaks using the approximate RRT values given in Table 1;

- the resolution, R , between Olanzapine related compound A and Olanzapine is not less than 3.0;
- the tailing factor for the Olanzapine peak is not more than 1.5;
- the relative standard deviation for four replicate injections for the Olanzapine peak is NMT 2.0%.

Peak Identification	Rel. Ret. Time (RRT)	Rel. Resp. Factor (RRF)	Limit (%)
Olanzapine RS B	0.3	2.3	0.1
Olanzapine RS A	0.8	1.0	0.1
Olanzapine	1.0	-	-
Any individual unspecific impurity	-	-	0.1
Total	-	-	0.4
Olanzapine RS A = 5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile			
Olanzapine RS B = 2-Methyl-10H-thieno-[2,3-b][1,5] benzodiazepin-4[5H]-one			

Procedure

Separately inject equal volumes (about 20 μ L) of the Standard solution and the Test solution, maintained at a temperature of about 5 degrees Celsius, into the chromatograph, record the peaks, and measure the responses for the peaks. Calculate the percentage of each impurity in the portion of Olanzapine taken by the formula:

$$100(1/F)(C_S / C_T)(r_i / r_S)$$

in which F is the relative response factor for each impurity from Table 1; C_S is the concentration, in mg per mL, of USP Olanzapine RS in the Standard solution; C_T is the concentration, in mg per mL, of Olanzapine in the Test solution; r_i is the response for each impurity in the Test solution; and r_S is the response of the Olanzapine peak in the Standard solution. The impurity limits are given in Table 1.

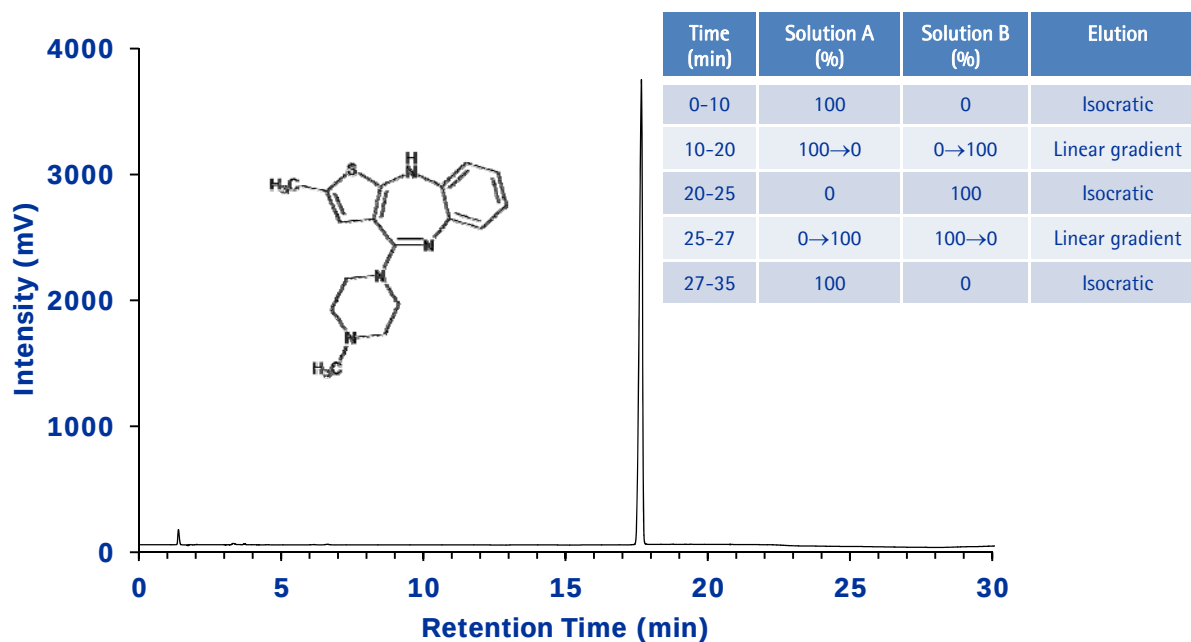
USP Method for Olanzapine RS

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column: Purospher®STAR RP-8 endcapped (5 µm) 250x4.6 mm 1.51454.0001
Injection: 20 µL
Detection: Shimadzu Prominence 2010, UV@220 nm
Cell: 10 µL
Flow Rate: 1.5 mL/min

Mobile Phase (v/v): Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water, add 5 mL of phosphoric acid, and adjust the pH to 2.5 with a sodium hydroxide solution.
Solution A: Buffer solution and acetonitrile (52:48)
Solution B: acetonitrile and the Buffer solution (70:30)
Gradient: See Table:
Temperature: 30° Celsius
Diluent: 55 mg of disodium EDTA in 1500 ml buffer. Mix resulting solution & acetonitrile (3:2)
Sample: Test solution - 400 ppm (0.4 mg/mL) of Olanzapine
Pressure Drop: 146 Bar (2117 psi)



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Olanzapine	17.7	0.74	1.0	