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Analytical Method Development for the Estimation of Olanzapine in the Pharmaceutical Dosage Form

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[*1]Nagunath S, [2]Guthi Surya Teja

[1]Assistant Professor, Department of Pharmaceutical Analysis, Sarojini Ramullama College of Pharmacy, Mahabubnagar, India,

[2]Department of Pharmaceutical Analysis, Sarojini Ramullama College of Pharmacy, Mahabubnagar, India.

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ABSTRACT

A new simple, rapid, specific, economical, precise, and accurate method for the estimation of olanzapine by using reverse-phase high-performance liquid chromatography (RP-HPLC), has been developed and validated according to the ICH guidelines. The separation was achieved by the Intersil (C8-3),(250 mm x 4.6mm), 5µm particle size column, and Acetonitrile and buffer solution (52:70 v/v) used as mobile phase, at a flow rate of 1.5 ml /min and the column temperature was 35°C. Detection was carried out at 220nm. The retention time of Olanzapine was found to be 35 mins. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy, limits of detection, and quantification for the impurities following the ICH guidelines. Linearity observed for the olanzapine is within the limits, % RSD for repeatability was found to be 0.143. The mean recovery was found to be within the limits of 85-115%. The developed method was found to be simple, rapid, accurate, and specific for the estimation of olanzapine in pure and their pharmaceutical dosage form.

INTRODUCTION:

Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2, 3b][1,5]benzodiazepine), is the most commonly prescribed second-generation neuroleptic for the treatment of psychiatric patients suffering from schizophrenia. Since its introduction in the therapy of psychiatric disorders in 1997, the need for reliable, sensitive, and fast methods for its analysis in bulk samples and pharmaceutical preparations is obvious. Several methods have been already reported for the determination of olanzapine, including hyphenated techniques: spectrophotometric, HPLC-MS, HPLC, Capillary zone electrophoresis, and GC-MS.

MATERIALS AND METHODS:

Chemicals and solvents:

Ammonium dihydrogen orthophosphate and orthophosphoric acid (AR grade, Qualigens) were used for preparing the buffer. HPLC grade methanol (Qualigens) was used for mobile phase preparation. A pure sample of Olanzapine was a gift sample from the local pharmaceutical industry. Commercial samples of tablets containing the drug olanzapine were purchased from the local pharmacy.

Method validation *parameters*:

System suitability: Accurately weigh about 20 mg of Olanzapine working standard and each 2 mg of Olanzapine Related compound- A and Olanzapine Related compound-B into 100 mL volumetric flask and take 1.0 mL of the above solution into 10 mL volumetric flask. Dissolve and dilute to the volume with diluent.

Specificity: Preparation of Related compound-A stock solution: Weigh accurately about 2.0 mg of the related compound- A into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent. Preparation of Related compound-B stock solution: Weigh accurately about 2.0 mg of related compound-B into a 100mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of In-house Related compound-A stock solution:

Weigh accurately about 2.0 mg of In-house Related compound-A into a 100 mL volumetric flask, dissolve and dilute the volume with diluent. Preparation of In-house Related

compound-C stock solution: Weigh accurately about 2.0 mg of In-house Related compound-C into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of In-house Related compound-03 stock solution:

Weigh accurately about 2.0 mg of In-house Related compound-03 into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of standard solution: Dilute each 1.0 mL of Related compound-A, Related compound-B, in-house Related compound-A, In-house Related compound-C, In-house Related compound-03, and Olanzapine (Form-1) stock solution into a 50 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of sample solution: Weigh accurately about 2.0 mg of Olanzapine (Form-1) standard into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Procedure: Equilibrate the column with the mobile phase at a flow rate of 1.5 mL min for at least one hour. Inject 20 µL of diluent as blank into the system and record the chromatograms for 35 minutes. Program the data processor to inhibit the integration of peaks due to diluent. Inject 20 µL of system suitability solution into the system four times and record the chromatograms for 35 minutes.

Linearity:

Preparation of Linearity stock solution: Weigh accurately about 2.0 mg of Related compound-A, Related compound-B, Inhouse Related compound-A, In-house Related compound-C, In-house Related compound-03, and Olanzapine Form-1 standards into 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of Linearity level-1(LOQ) solution: Dilute each 1.5 mL of Linearity stock solution into 50 ml of the volumetric flask, dissolve and dilute to the volume with diluent. Preparation of Linearity level-2(25%) solution: Dilute each 0.25 mL of Linearity stock solution into 50 ml of the volumetric flask, dissolve and dilute to the volume with diluent. Preparation of Linearity level-3(50%) solution: Dilute each 0.5 mL of Linearity stock solution into a 50 ml of the volumetric flask, dissolve and dilute to the volume with diluent. Preparation of Linearity level-4(75%) solution: Dilute each 0.75mL of Linearity stock solution into a 50 mL of the volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of Linearity level-5(100%) solution: Dilute each 1.0 mL of Linearity stock solution into 50 ml of the volumetric flask, dissolve and dilute to the volume with diluent.

Procedure: Inject each level into the Chromatographic system and measure the peak area. Calculate correlation coefficient -intercept, slope, sensitivity plot, and residuals of the square from linearity levels.

Precision: Repeatability: Preparation of standard solution: Accurately weigh each 2.0 mg of all the impurities and Olanzapine Form-1 into 100 mL of volumetric flask dissolve and dilute to the volume with diluent. Dilute 1.0 ml of the above solution into a 50 ml of volumetric flask dissolve and dilute to the volume with diluent.

Preparation of Related compound-A stock solution: Weigh accurately about 2.0 mg of Related compound-A into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent. **Preparation of Related compound-B stock solution**: Weigh accurately about 2.0 mg of related compound-B into 100 mL of the volumetric flask, dissolve and dilute to the volume with diluent. **Preparation of In-house Related compound-A stock solution**: Weigh accurately about 2.0 mg of In-house Related compound-A into a 100 mL of volumetric flask dissolve and dilute to the volume with diluent.

Preparation of In-house Related compound-C stock solution: Weigh accurately about 2.0 mg of In-house Related compound-C into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of In-house Related compound-03 stock solution: Weigh accurately about 2.0 mg of In-house Related compound-03 into a 100 mL volumetric flask dissolve and dilute to the volume with diluent.

Intermediate precision: To evaluate the intermediate precision of the method, precision was performed on different days by maintaining the same conditions.

Procedure: On the different days the solution was injected six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

Preparation of standard solution: Weigh accurately each 2.0 mg of Related compound-A, Related compound-B, in-house Related compound-A, in-house Related compound-C, in-house Relatedcompound-03, and Olanzapine Form-1 stock solution into 100 ml of volumetric flask dissolve and dilute to the volume with diluent. Dilute 1.0 ml of the above solution into a 50 ml of volumetric flask dissolve and dilute to the volume with diluent.

Preparation of accuracy stock solution: Weigh accurately about 2.0 mg of Related compound-A, Related compound-B, in-house Related compound-A, in-house Related compound-C, in-house Related compound-03, and Olanzapine Form-1 into 100 mL volumetric flask dissolve and dilute to the volume with diluent.

Preparation of Test Accuracy (LOQ) level-1 solution: Weigh accurately about 10 mg of the test sample into a 25 mL volumetric flask, dissolve and dilute to the volume with LOQ solution. **Preparation of Test+ Accuracy (50%) level-2 solution**: Weigh accurately about 10 mg of the test sample into a 25 mL volumetric flask, add 0.25 mL of Accuracy stock solution, dissolve and dilute to the volume with diluent.

Preparation of Test+ Accuracy (100%) level-3 solution: Weigh accurately about 10 mg of the test sample into 25 mL of the volumetric flask, add 0.5 mL of accuracy stock solution, dissolve and dilute to the volume with diluent.

Preparation of Test + Accuracy (150%) level-4 solution: Weigh accurately about 10 mg of test sample into a 25 mL volumetric flask, add 0.75 mL of Accuracy stock solution, dissolve and dilute to the volume with diluent.

Limit of detection: The lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

Preparation of Related compound-A stock solution: Weigh accurately about 2.0 mg of Related compound-A into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent. **Preparation of Related compound-B stock solution:** Weigh accurately about 2.0 mg of related compound-B into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent. **Preparation of In-house Related compound-A stock solution:** Weigh accurately about 2.0 mg of In-house Related compound-A into a 1000 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of In-house Related compound-C stock solution: Weigh accurately about 2.0 mg of In-house Related compound-C into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent. **Preparation of In-house Related compound-03 stock solution:** Weigh accurately about 2.0 mg of In-house Related compound-03 into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of LOD solution: Prepare the concentration of limit of detection solution based on the 0.01% solution S/N ratio of the respectively related compound and Olanzapine Form-1 to get the expected S/N ratio of 3.0 to 5.0 by using the above-prepared individual solutions. Inject 20 µL of diluent as blank into the system and record the chromatograms for 35 minutes. Inject 20 µL of LOD solution in six replicates and record the chromatograms. Calculate the S/N ratio of Related compound-A, Related compound-B, in-house Related compound-A, in-house Related compound-C, Related compound-03, and Olanzapine Form-1 from Limit of detection solution through system software.

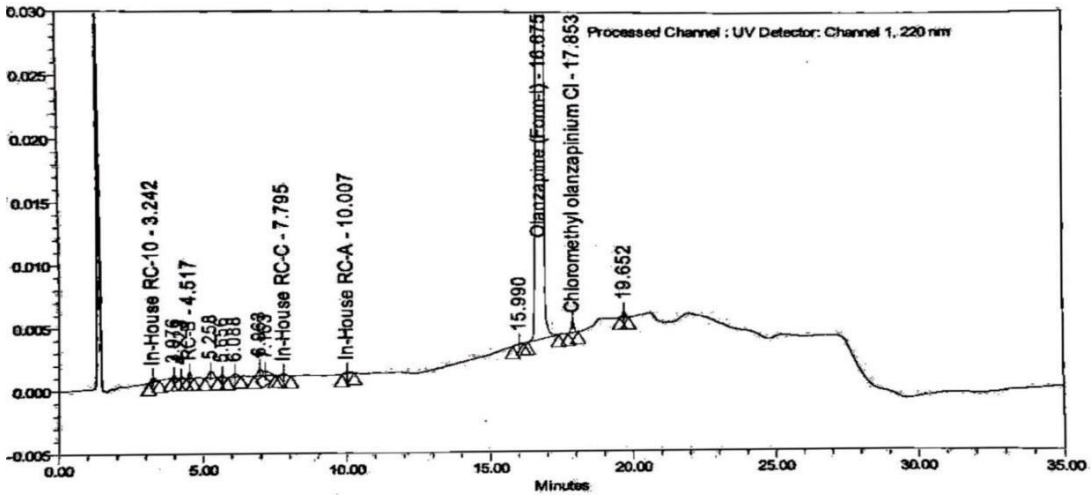
Limit of quantitation: Preparation of Olanzapine Form-1 stock solution: Weigh accurately about 2.0 mg of Olanzapine Form-1 standard into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent.

Preparation of standard solution: Dilute each 1.0 mL of Related compound-A, Related compound-B, In-house Related compound-A, In-house Related compound-C, in-house Related compound-03, and Olanzapine Form-1 stock solution into a 50 ml of volumetric flask dissolve and dilute to the volume with diluent.

Preparation of LOQ solution: If the S/N ratio of all the components is above 10 from 0.01% solution, consider 0.01% solution as a LOD solution or prepare the LOD solution based on the s/n ratio. Inject 20 µL of diluent as blank into the system and record the chromatograms for 35 minutes. Inject 20 µL of LOD solution in six replicates and record the chromatograms. Calculate the S/N ratio of Related compound-A, Related compound-B, In-house Related compound-A, in-house Related compound-C, and in-house Related compound-03 and Olanzapine Form-1 from LOD solution injection-1 through system software

Optimized Chromatogram:

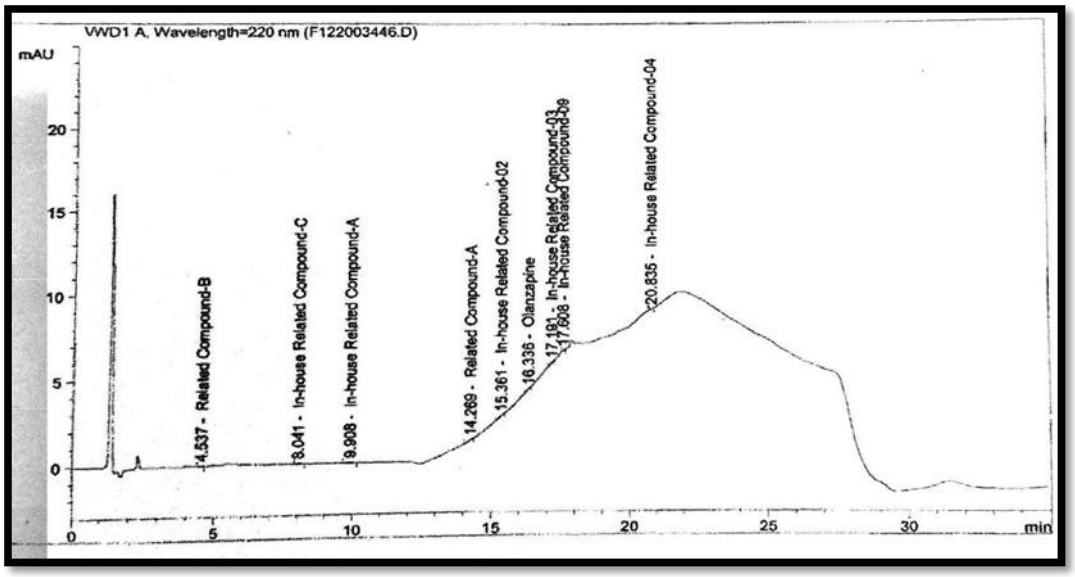
Mobile phase ratio	:	Acetonitrile: buffer: methanol (48:50:70% v/v)
Column	:	Intersil C8-3, (250 mm x 4.6 mm), 5 µm
Column Temperature	:	35°C
Wavelength	:	220 nm
Flowrate	:	1.5mL/min
Injection volume	:	20µL
Run time	:	35 minutes



Optimized Chromatogram

Limit of detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value.



Chromatogram showing LOD solution

RESULTS FOR THE LOD

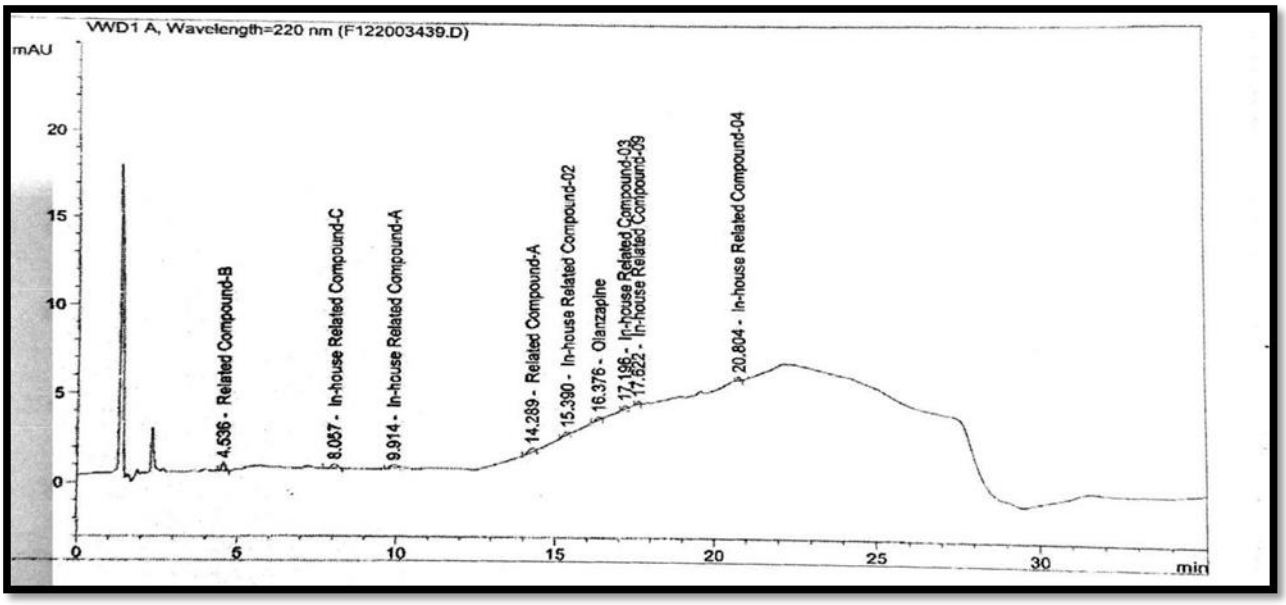
Acceptance Criteria:

- The signal-to-noise ratio of each component should be between 3.0 and 5.0.

The results are between the limits. Hence Method is detected.

Limit of quantitation:

Thequantitationlimitofindividualanalyticalproceduresisthelowestamountofanalytein a sample that can be quantitatively determined.



Chromatogram showing LOQ solution

Acceptance Criteria:

- The signal-to-noise ratio of each component should not be more than 10.0.
- The results are between the limits. Hence the Method is quantitated.

CONCLUSION

A new method was established for the estimation of Olanzapine of Related substances by RP-HPLC method. The Chromatographic conditions were successfully developed for the estimation of Olanzapine by using Intersil C8-3, (250 mm x 4.6 mm), 5 µm particle size column, the flow rate was 1.5 mL/min, mobile phase ratio was Acetonitrile: water (48:70 v/v), detection wavelength was 220 nm.

The instrument used was Agilent 1200 series, separation module, Empower software-version-3. The Retention time was found to be 35 minutes. The system suitability parameters for Olanzapine Form-1 such as theoretical plates and tailing factor were found to be within the limits. The analytical method was validated according to the ICH guidelines.

The Linearity study for Olanzapine Form-1 was found in the concentration range within the limits and the Correlation coefficient of the impurities was found to be 0.998, 0.999, 0.999, and 0.998, respectively. % RSD for repeatability was 0.143. The precision study was precise and repeatable. The accuracy of the components was found to be within the limits of 85-115%. LOD and LOQ of the signal-to-noise ratio are within the limits.

Hence the suggested RP- HPLC method can be used for the routine analysis of Olanzapine Form-1 of related substances in the Pharmaceutical dosage forms.

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