

**DEVELOPMENT AND VALIDATION OF NEW RP-UPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF OLANZAPINE IN TABLET DOSAGE FORM**RAJA ABHILASH PUNUGOTI\*<sup>1,3</sup>, VENKATESHWAR RAO JUPALLY<sup>2,3</sup><sup>1</sup>S.V.S.School of Pharmacy, Bhemaram, hanamkonda. <sup>2</sup>TallaPadmavathi College of Pharmacy, Urus, Warangal. <sup>3</sup>Department of pharmacy, Acharyanagarjuna university, Guntur. Email: abhilashmpfarm@gmail.com.

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**ABSTRACT**

**Objective:** to develop and validate a new very rapid, sensitive, reverse phase Ultra Performance Liquid Chromatography (RP-UPLC) technique for the estimation of Olanzapine in dosage form, as there is no official monograph & no analytical method by UPLC.

**Methodology:** Chromatographic separation was achieved on a Waters Acquity HSS T-3 C<sub>18</sub> column (100 × 2.1 mm, 1.8µm) using an isocratic method with mobile phase composed of Potassium di-hydrogen phosphate: methanol in the ratio 60:40 v/v. The flow rate was 0.8 ml/min, temperature of the column was maintained at ambient and detection was made at 228 nm. The run time was as short as 6 min. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

**Results:** The developed method was linear for Olanzapine from 10 - 50 µg/ml and the linear regression obtained was > 0.999. Precision, evaluated by intra- and inter-day assays had relative standard deviation (R.S.D) values within 1.5 %. Recovery data were in the range 98.2% to 100.9% with R.S.D. values < 1.5 %.

**Conclusion:** The method is precise, accurate, linear, robust and fast. The short retention time of 2.433 min allows the analysis of a large number of samples in a short period of time and, therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

**Keywords:** Olanzapine, UPLC, new method development, validation

**INTRODUCTION**

Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Though high-performance liquid chromatography (HPLC) is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (API's) and dosage forms, it is often a slow technique because of the complexity of some of the samples, it could still be improved. A new category of separation technique, ultra-performance liquid chromatography (UPLC), has proven to be one of the most promising developments in the area of fast chromatographic separations with its unique characteristics of high chromatographic resolution, speed, and sensitivity analysis. UPLC, by Using sub-2 µm particles and mobile phases at high linear velocities, and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity, and speed of analysis can be obtained. Analysis of operation cost and sample throughput found UPLC cost advantageous over HPLC. In the present work, this technology has been applied to the method development and validation study of related substance and assay determination of olanzapine [1].

Olanzapine (OLP), chemically known as (2-methyl-4- (4-methyl-1-piperazinyl)-10H-thieno-[2, 3b] [1, 5] benzodiazepine (Fig 1), is a typical antipsychotic drug used in the treatment of schizophrenia and other psychotic syndromes [2].

The literature provides some of the references on the estimation of olanzapine tablets by non-aqueous titrimetry and UV-spectrophotometry [3], visible spectrophotometry [4-6], and flow injectionspectrophotometry [7]. Several HPLC methods [8-11] have also been reported. The reported HPLC methods are more time consuming, complex mobile phase mixtures, use high flow rate of analysis, lack of sensitivity and peak symmetry. However there were no reports available on the estimation of olanzapine by UPLC method. It is, therefore, felt necessary to develop a new rapid method for the determination of olanzapine by UPLC method. Hence a reproducible RP UPLC method was developed for the quantitative

determination of olanzapine tablets by using Waters Acquity HSS T-3 C<sub>18</sub> column (100 × 2.1 mm, 1.8µm) UPLC column. The proposed method was validated as per the guidelines suggested by ICH [12].

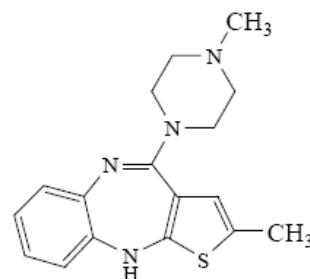


Fig.1. structure of olanzapine.

**EXPERIMENTAL DETAILS****Materials and Reagents**

Olanzapine Working Standard was procured from Teena laboratories, Hyderabad, India. Commercially available Olanzapine purchased from local pharmacy. Methanol HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

**Chromatographic conditions**

Chromatography separation was performed on Waters Aquity UPLC with photodiode array detector. The output signal was monitored and processed using empowers software. The chromatographic column used Waters Acquity HSS T-3 C<sub>18</sub> (100 × 2.1 mm, 1.8µm). The mobile phase of Potassium di-hydrogen phosphate: methanol in the ratio 60:40 v/v at a flow rate of 0.8 ml/min. The detection was monitored at the Wavelength of 228 nm. The injection volume was 20.0 µL and the chromatographic runtime of 5 min was used.

### Preparation of solutions

**Preparation of Phosphate buffer:** Weigh 7.0 grams of Potassium di hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with milli pore water. Adjusted the pH to 4.0 with ortho phosphoric acid.

**Preparation of mobile phase:** Mix a mixture of above buffer 600mL (60%) and 400 mL of methanol (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45  $\mu$  filter under vacuum filtration.

### Preparation of the Olanzapine Standard & Sample Solution:

#### Standard Solution Preparation

Accurately transferred 10mg of Olanzapine Working standard into a 10 mL volumetric flask and about 7 mL of Diluent added then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further pipetted 5 ml of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45 $\mu$ m filter. Further pipetted 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 $\mu$ m filter.

#### Sample Solution Preparation

Accurately transferred the sample equivalent to 10 mg of Olanzapine into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45 $\mu$ m filter. Further pipetted 5 ml of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45 $\mu$ m filter. Further pipetted 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 $\mu$ m filter.

### Method validation

#### Precision

The precision of the method was evaluated by carrying out six independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

#### Intermediate Precision/Ruggedness

**Intra-day precision:** The precision of the assay method was evaluated by carrying out six independent assays Olanzapine (50,100, 150% i.e. 5.0, 10.0, 15.0  $\mu$ g/ml.) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

**Intermediate precision (inter-day):** Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the six samples of Olanzapine against qualified reference standard. The percentage of RSD of six assay values was calculated. The %RSD for the area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

#### Accuracy

Recovery of the assay method for Olanzapine was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was injected thrice (n=3) into UPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

#### Linearity

Test solutions were prepared from stock solution at 5 concentration levels (10, 20, 30, 40 and 50  $\mu$ g/ml). The peak area vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

### Limit of Detection (LOD) Limit of Quantification (LOQ)

LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

### Robustness

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g, flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

## RESULTS

### Method development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Acquity BEH C18, 50mm  $\times$  2.1 mm, 1.7  $\mu$ m column used for the elution, but the peak eluted before 4.5 minutes with a tailing factor of 2. Experiment with Phenyl 100mm  $\times$  2.1 mm, 2  $\mu$ m column ended with inconsistent retention time and peak fronting. Buffers like sodium dihydrogen orthophosphate, dipotassiumhydrogenorthophosphate, and disodium hydrogen orthophosphate did not yield desired results. Use of ion pair reagents also did not yield the expected peak.

At 60:40 (buffer: solvent) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 60:40 (buffer: solvent) in an isocratic mobile phase flow rate. The typical chromatogram obtained for Olanzapine from final UPLC conditions are depicted in Figure 2.

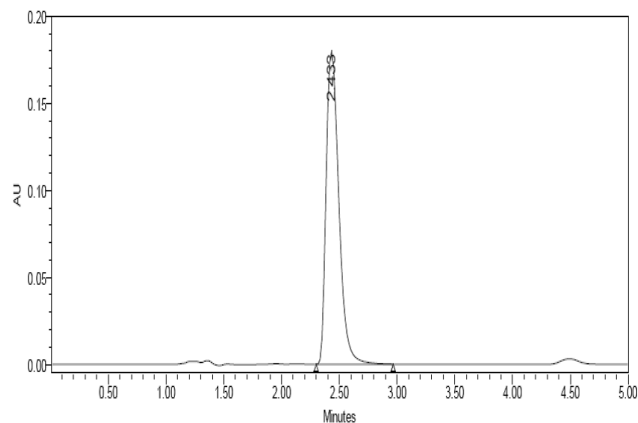


Figure 2: typical chromatogram of Olanzapine by proposed method

### Method validation

Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ, robustness and sensitivity as follows.

#### System suitability

The system suitability results for the proposed UPLC method are Tailing factor Obtained from the standard injection is 1.3. Theoretical Plates Obtained from the standard injection is 9287.2. The results proved that the optimized UPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

### Precision

The % R.S.D. of Olanzapine assay during the method precision was found to be 0.4%, indicating good precision of the method. The results are summarized in table 1.

**Table 1: Results of precision**

Injection	Area
Injection-1	693078
Injection-2	693338
Injection-3	695080
Injection-4	694843
Injection-5	695336
Average	694335
Standard Deviation	1047.5
%RSD	0.15%

### Intermediate Precision

The % R.S.D. of Olanzapine assay during the intermediate precision was found to be 0.08%, the value is well within the generally acceptable limits. The results are summarized in table 2.

**Table 2: Results of intermediate precision**

Injection	Area
Injection-1	696977
Injection-2	696531
Injection-3	696214
Injection-4	695535
Injection-5	696708
Average	696393
Standard deviation	553.8
%RSD	0.08%

### Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for Olanzapine were 0.024 and 0.07 µg/ml, respectively. Since the LOQ and LOD values of Olanzapine are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

### Accuracy

Percentage recovery of Olanzapine samples ranged from 98.2% to 100.9% and the mean recovery is 99.8%, showing the good accuracy of the method. The result is shown in Table 3.

**Table 3: Results of Accuracy**

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	688287	5.68	5.78	103.20%	100.70%
100%	1378200	10	9.97	99.70%	
150%	2065480	14.05	13.93	99.30%	

### Linearity

The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 10 - 50 µg/ml for three times, and the correlation coefficient obtained was 1.000, thus indicating excellent correlation between peak areas and concentrations of the analyte.

### Robustness

In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 10 -50 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% and change in the Mobile phase ±10%. The results are summarized in table 4.

**Table 4: Results of Robustness**

Chromatographic changes	USP Plate Count	USP Tailing
<b>Flow rate(ml/min)</b>		
0.6	4187.6	1.5
0.8*	4184.7	1.3
1.0	4084.7	1.4
<b>Change in organic composition in the mobile phase</b>	4194.5	1.5
10% less	3156.0	1.3
60:40( Buffer: methanol)*	3097.0	1.4
10% more		
<b>UV wavelength(nm)</b>		
226	4205.0	1.5
228*	3648.3	1.3
230	3354.2	1.4

\* optimized parameters

3.2.8 Application of the developed method to commercial Olanzapine tablets

When the developed method was used to analyze a commercial brand of Olanzapine tablet formulation, the mean recovery of five replicates was 99.69 % with % R.S.D. of 0.52. The % recovery value indicates non-interference from the excipients present in the dosage form.

### DISCUSSION

#### Method development and optimization

The main aim of the developed method was to achieve separation and quantification of Olanzapine using an isocratic mobile phase with UPLC system. Developing a UPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Olanzapine was adequate at 228 nm. The initial trial was conducted using HPLC and chromatographic separation was obtained on a Waters symmetry C18 column (150 x 4.6mm, particle size 5µm). Olanzapine is an acid labile compound and to avoid any degradation, a mobile phase with basic pH was selected. The mobile phase was optimized in the ratio of Potassium di-hydrogen phosphate: methanol in the ratio 60:40 v/v at a flow rate of 0.8 ml/min. While developing the UPLC method, basic chromatographic conditions such as the column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the UPLC column, its stability at the higher pH was taken into consideration to preserve the long life of the column. Most commercial C<sub>18</sub> columns are not stable at high pH on the longer run, thus shortening their life span. Waters Acquity HSS T-3 C18 column (100 × 2.1 mm, 1.8µm) column was found to be more suitable and stable at this pH. The peak was sharp and acceptable. The flow rate also is scaled down from 2.0 to 0.8 ml/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for Olanzapine, which eluted at around 2.433 min giving a total run time of 5 min.

### CONCLUSION

The new, isocratic RP-UPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation

more than that achieved with conventional HPLC. The short retention time of 2.433 min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of Olanzapine in tablet formulations.

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