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Research Article

METHOD DEVELOPMENT AND VALIDATION OF OLANZAPINE BY UPLC METHODS FOR PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The aim of current research was to develop and validate a new very rapid, sensitive, Ultra Performance Liquid Chromatography (UPLC) technique for the estimation of Olanzapine in dosage form, as there is no official monograph & no analytical method by UPLC. Different chromatographic column and different mobile phase composition were tried to optimise the method. Liquid chromatography (LC) was carried out on a Waters Acquity UPLC with a photodiode array detector (DAD/PDA). The output signal was monitored and processed using empowers 2 software. The chromatographic column used was Acquity UPLC BEH C-18 (100-mm, 2.1-mm, and 1.7-µm) particle sizes. The flow rate of the mobile phase was 0.36 mL/min. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. 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 %. 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.

1. 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 injection-spectrophotometry [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 C18 column (100 × 2.1 mm, 1.8µm) UPLC column. The proposed method was validated as per the guidelines suggested by ICH [12].

2. MATERIAL AND METHODS

2.1. Instrumentation

An Ultra High performance liquid chromatography (UPLC) system consisted of Waters Acquity with PDA detector and data-handling system Empower-2 and all pH measurements were performed on a pH meter (Metrohm).

2.2. Reagents and Standards

All chemicals used were of analytical reagent grade and HPLC grade acetonitrile (Merck, Ltd, Mumbai) was used. Distilled water filtered through 0.2 µm filter (Millipore) was used to prepare solutions.

2.3. Preparation of Standard Solution of Olanzapine

Forty (40) mg Olanzapine working standard was weighed accurately and transferred in a 100 mL volumetric flask, added sufficient quantity of diluent, sonicated for 10 min and volume was made up to the mark with diluent to make a 400 µg/mL solution. An aliquot of 1 mL of this solution was diluted to 10 mL with diluent yielding 40 µg/mL solution.

2.4. Preparation of Sample solution

Forty (40) mg of test sample was weighed accurately and transferred into 100 mL calibrated flask. Added about 70 mL of diluents and sonicated for about 15 mins with intermediate shaking. Diluted to volume with diluents and mixed well. An aliquot of 1 mL of the solution was diluted to 10 mL with diluent yielding 40 µg/mL solution.

In case of dosage form, twenty tablets were weighed accurately and ground into a fine powder using pestle and mortar. A quantity of tablet powder equivalent to 40 mg of OLN was accurately weighed into a 100 mL calibrated flask, 60 mL of diluent solution was added and content was shaken for 20 min; then, the volume was diluted to the mark with the diluent and mixed well. An aliquot of 1 mL of resultant solution was diluted to 10 mL with diluent yielding 40 µg/mL solution.

2.5. Preparation of Impurity Stock solutions of each impurity

Two (2.0) mg of Standard Impurities 1-5 (Impurity-1, Impurity 2,Impurity3,Impurity-4,Impurity-5) of Olanzapine were weighed separately and transferred to a series of 100 mL volumetric flasks, added sufficient quantity of diluent, sonicated for 10 min and volume was made up to the mark with diluent. (Concentration: 20 µg/mL) Impurity- 1, 3 and 5 are process related and Impurity-2 & 4 is degradation related impurities of Olanzapine.

2.6. Preparation of Impurity mix solution

Impurity mixed solution was prepared according to the concentration of each individual impurity. From stock solutions of impurities, 1 mL stock solution of impurity-1 to 5 each was transferred to 100 mL of amber volumetric flask. Contents of the flask were sonicated for 10 min. and made up the volume. Thus the concentration of Impurity 1 to 5 is 0.2 µg/mL.

All the information related to concentration of impurities is shown in Table 1

2.7. Parameter-wise method development plan

2.7.1. Molecular weight

Molecular weight of olanzapine is 312.43. From the exhaustive literature it is concluded that for high molecular weight drug, the reverse phase is suitable for separation of drug and their impurities.

Table 1: Concentration of all Impurities present in Impurity mix solutions

Material	Actual wt. taken (mg)	Diluted up to (mL)	Purity	Volume pipette out (mL)	Diluted up to (mL)	Final Conc. (µg/mL)
Impurity 1	2.145	100	98.6	1.0	100	0.2
Impurity 2	2.152	100	99.0	1.0	100	0.2
Impurity 3	2.210	100	98.4	1.0	100	0.2
Impurity 4	2.041	100	99.97	1.0	100	0.2
Impurity 5	2.242	100	99.93	1.0	100	0.2

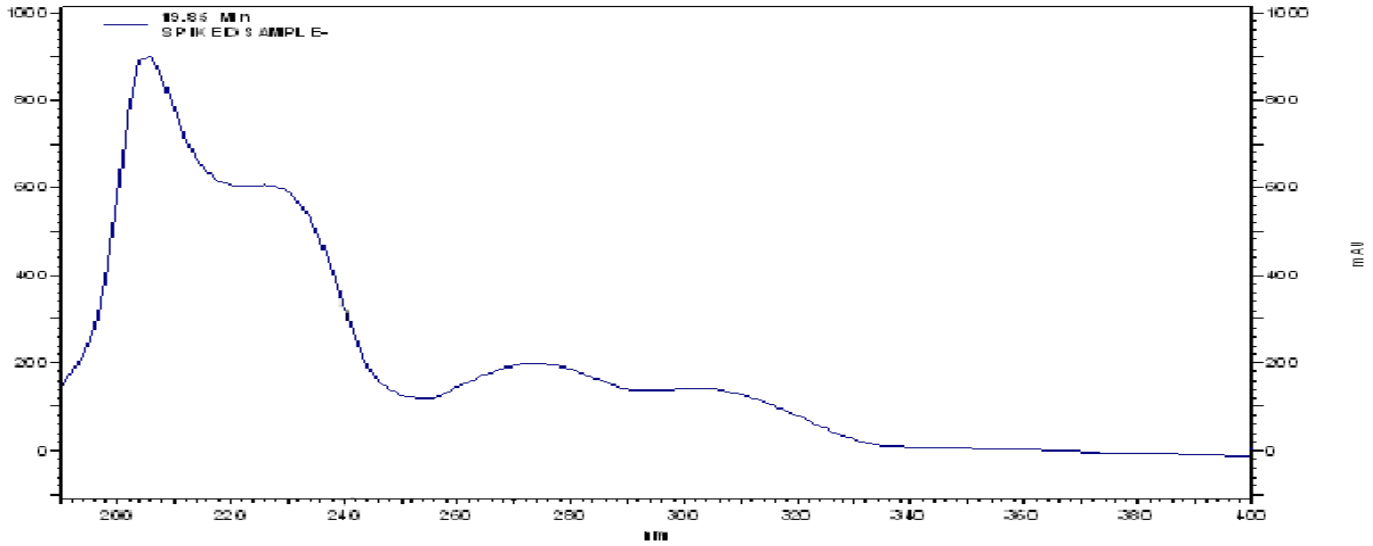

Fig. 1: UV Spectrum of Olanzapine spiked sample

2.7.2. Column selection

Slightly polar column (Aquity UPLC BEH) was taken in order to decrease the run time and increase resolution.

2.7.3. Detector selection

Since the method was developed for the separation of olanzapine (UV active compound) with their impurities and degradants, the PDA detector was chosen to identify the peak of similar polarity.

2.7.4. Selection of wavelength

The standard olanzapine spiked with impurities solution was scanned in the range of 200-400 nm to identify the λ max of the drug. The λ max was found to be 270 nm and this λ max was selected for the analysis.

2.7.5. Mobile phase selection

In mobile phase preparation following buffer and solvent was taken.

2.7.5.1. Preparation of Buffer Solution

4.8 g of Sodium dihydrogen ortho phosphate was weighed accurately and dissolved in about 1000 mL of Ultra pure water (MilliQ water) & added 2 mL triethylamine. pH was adjusted to 6.8 with dilute ortho phosphoric acid. Filtered the solution through 0.2 µm membrane filter paper.

2.7.5.2. Preparation of Mobile Phase A

Mixed 500 mL of buffer, 200 mL of Acetoniltrile and 300 mL of Methanol, sonicate and degassed for 10 minute.

2.7.5.3. Preparation of Mobile Phase B

Acetonitrile and Ultra pure water (90:10) was mixed and sonicated and degassed for 10 min. Mobile phase A and B were filtered through 0.2 µm membrane filter paper.

3. RESULTS AND DISCUSSION

3.1. Method development

The method development for related substances in bulk drug and pharmaceutical dosage form by UPLC was carried out. Different chromatographic column and different mobile phase composition were tried to optimise the method. Following UPLC method trials were run to resolve the previously mentioned impurities.

Trial-1: Initially, a mobile phase composed of ammonium dihydrogen orthophosphate (ADP) solution (20 mM) and acetonitrile (1:1) flowing at a rate of 0.36 mL/min over Inertsil ODS-3 (50-mm×2.1-mm, 2-µm particles) column was employed for separation of impurities. The gradient system used is given in Table 2.

Inference: The mobile phases were allowed to run for equilibration of column. A 1.0 µL of Impurity mix solution was injected and observations were recorded. Observations of Trial- 1 were not satisfactory, hence decided to take further trial.

Trial-2: The organic modifier *i.e* Triethylamine (TEA) in concentration of 0.02 % was added but symmetrical peak could not be obtained. In order to obtain symmetrical peak with good resolution, Zorbax column

was used and the following gradient system were run as shown in Table 3

Inference: The column was equilibrated with mobile phase and different gradient systems were run. The peaks of low resolution were obtained. Hence further trials were needed to select a suitable mobile phase as well as column.

Trial 3: In order to obtain peak with better resolution, the column (Zorbax) was changed with aquity BEH C18, and the buffer Ammonium dihydrogen ortho-phosphate was replaced with sodium dihydrogen ortho-phosphate and the column was equilibrated with mobile phase and following gradients were run (Table 4).

The chromatogram so obtained was recorded as shown in Fig 2

***Inference*:** The well resolved peaks of olanzapine and impurities were obtained. Therefore the column Aquity BEH C18 and mobile phase using sodium dihydrogen ortho phosphate was selected for the analysis.

Table 2: Gradient program for elution

Time	%MP-A	%MP-B	Flow	Observation
0	65	35	0.36 mL/min	Highly asymmetric peak
30	65	35		

Table 3: Gradient program for elution

Time	% MP-A	% MP-B	Flow	Observation
0.01	87	13		
5	87	13	0.36 mL/min	Improper resolution b/w impurity peaks.
10	55	45		
20	55	45		
25	87	13		

Table 4: Gradient program for elution

Time	% MP-A	% MP-B	Flow	Observation
0.01	90	10		
2	90	10		
4	55	45	0.36 mL/min	Perfect resolution
6	90	10		
8	90	10		

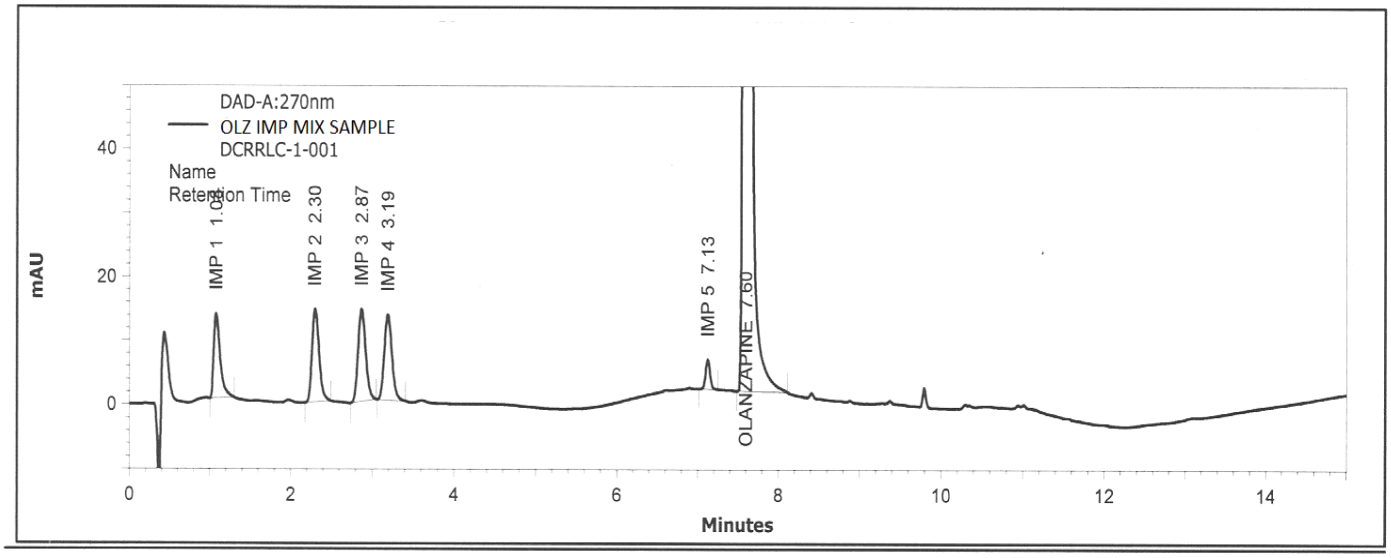

Fig. 2: Chromatogram showing well resolved peaks of Olanzapine and its impurities

3.2. Optimized Chromatographic conditions

Liquid chromatography (LC) was carried out on a Waters Acquity UPLC with a photodiode array detector (DAD/PDA). The output signal was monitored and processed using empowers 2 software. The chromatographic column used Acquity UPLC BEH C-18 (100-mm, 2.1-mm, and 1.7-µm) particle sizes. The flow rate of the mobile phase was 0.36 mL/min. The column temperature was maintained at 27°C, and the detection was monitored at a wavelength of 270 nm. The injection volume was 1.0 µL. A 1.0 µL injection of Diluent (Blank) and standard olanzapine solution were injected in system and chromatogram was recorded which is depicted in Figs. 3 & 4.

3.3. Chromatogram of spiked solution

A 1.0 µL injection of olanzapine spiked with impurities was injected and the following well resolved chromatograph of olanzapine with their impurities was obtained (Fig. 5)

From above chromatogram (Fig. 5) RT of olanzapine and all impurities and RRT of all impurities were noted and are given in Table 5

From the above graph it was also observed that in case of system suitability study the resolution between Impurity-5 and olanzapine was considered because, all other 4 impurities were well separated and impurity-5 eluted out very close to olanzapine peak. This was the reason for considering the impurity- 5 and olanzapine for system suitability studies.

Table 5: RT and RRT of all impurities and Olanzapine

Material	RT	RRT
Impurity- 1	1.08	0.14
Impurity- 2	2.3	0.3
Impurity- 3	2.87	0.37
Impurity- 4	3.19	0.42
Impurity- 5	7.13	0.93
Olanzapine	7.6	NA

NA: not applicable

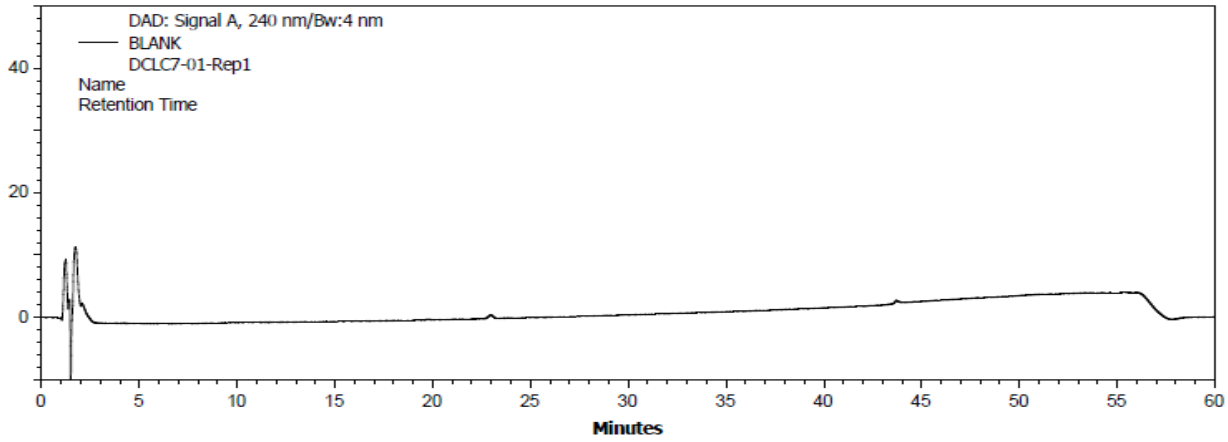

Fig. 3: Chromatogram of Diluent (Blank) solution

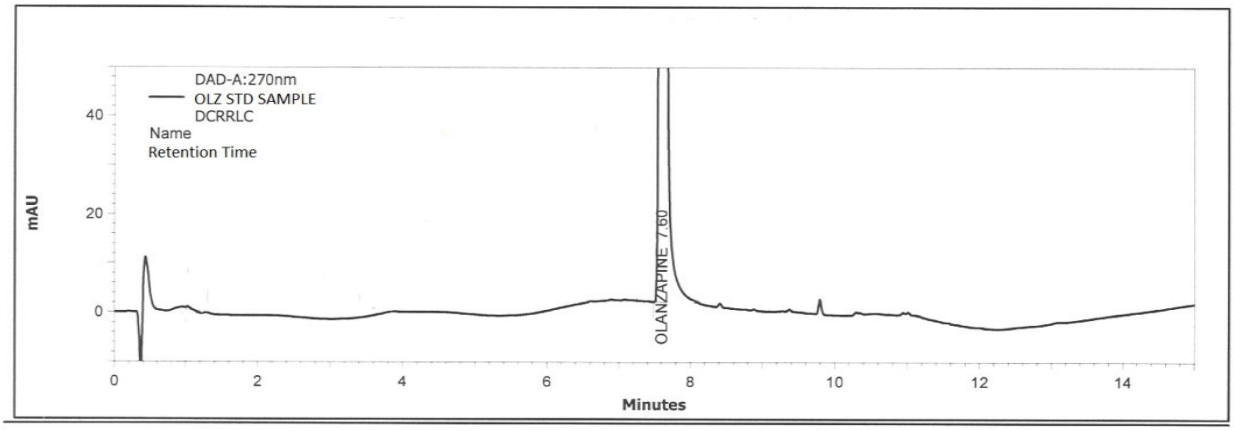

Fig. 4: Chromatogrm of standard OLN solution

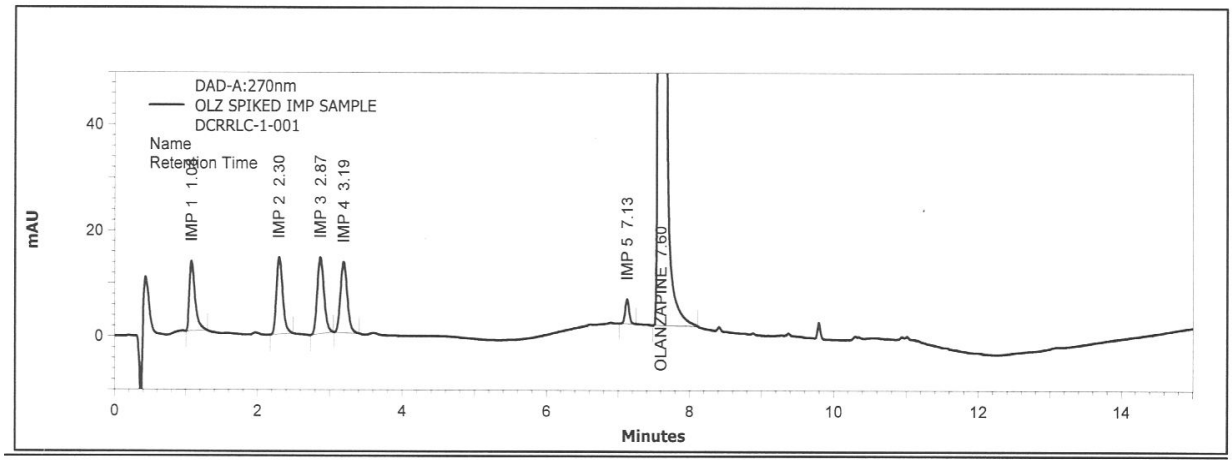

Fig. 5: Chromatogram of Olanzapine Spiked sample

3.4. Validation of developed method

When a method is developed it is necessary to validate to confirm whether the method is suitable for its intended purpose or not.

3.4.1. Method validation

The main objective of Method validation is to demonstrate the reliability of a particular method for quantitative determination of a particular analyte. The validation of proposed method was performed by impurity addition method. Though the related substances were very nicely resolved but to strengthen the method of analysis, the method was validated as per ICH guideline Q2 (R1). The following parameters were chosen to validate the analytical method of Olanzapine.

Table 6: Method Validation injection sequence

S. NO.	Description	No. of Injection
1.	Blank	1
2.	System suitability solution	1
3.	Blank	1
4.	Sample solution	1

3.4.2. Accuracy

The accuracy expresses the closeness of agreement between the true value and the observed value. The accuracy of the related substances for olanzapine was evaluated in triplicate (n = 3) at the three different concentrations (50 %, 100 % and 150 %) of drug product, and the recovery was calculated for each added (externally spiked) concentration. For all impurities, the recovery was determined in triplicate for (50 %, 100 % and 150 %) of the analyte concentration (40 µg/mL) of drug product, and the recovery of the impurities was calculated.

% Impurity was calculated using following formula-

$$\textbf{\% Impurity} = \frac{A_t * W_s * 1 * 25 * A_v * P}{A_s * 100 * 100 * W_t * L}$$

Where At Area of impurity in test sample, As Mean area of Olanzapine in diluted standard, Ws Weight of Standard taken (mg), Wt Weight of tablet powder taken(mg), Av Average weight of tablet (mg), P Potency of standard and L Label claim in mg.

In case of bulk drug

% Impurity = (Rv/ Rs) x 100

Where Rv Peak response of impurity from sample solution and Rs Peak response of OLN standard solution.

% Recovery was calculated using following formula-

% Recovery = [(Impurity Estimated - Amt. Contributed)/ Impurity Added] x 100

From the observations obtained the % Impurity and % Recovery of each impurity was calculated using the formulae and respectively and results of recovery studies are shown below in Table 8-12.

From the above results the Mean % Recovery of Olanzapine related substance at each level was found to be in range of 85 % to 115 %.

Inference: According to acceptance criterion (Mean % Recovery of Olanzapine related substance/Impurity at each level should be in range of 85% to 115%) and from results it can be concluded that method passes recovery study.

Table 7: Data sheet for AUC of Impurities

Spiked sample	Impurity-1	Impurity- 2	Impurity-3	Impurity-4	Impurity-5
50 %	37823.67±288.63	48144±344.21	34018.33±327.97	47098.67±526.18	7291±218.42
100 %	78608.33±1672.79	92958.33±202.64	67943.67±1511.63	91658.67±160.24	17894±214.78
150 %	118453.3±744.65	144282.3±583.51	96468.67±1976.61	156136±1408.93	24025±45.57

n= 3 (Mean±Std. Dev)

Table 8: Data showing Recovery studies for Impurity 1

Sample	Spike Level	Mean µg/mL Added	Mean µg/mL Recovered	Mean % Recovery
1	50%	1.82	2.001±0.02	109.5±0.85
2	100%	3.65	4.158±0.09	113.8±2.56
3	150%	5.48	6.26±0.03	114.1±0.71

n=3

Table 9: Data showing Recovery studies for Impurity 2

Sample	Spike Level	Mean µg/mL Added	Mean µg/mL Recovered	Mean % Recovery
1	50%	2.93	3.026±0.03	102.9±0.65
2	100%	5.87	5.850±0.06	99.5±0.54
3	150%	8.81	9.082±0.02	103.0±0.86

n=3

Table 10: Data showing Recovery studies for Impurity 3

Sample	Spike Level	Mean µg/ml Added	Mean µg/ml Recovered	Mean % Recovery
1	50%	2.38	2.142±0.02	90.0±0.71
2	100%	4.76	4.278±0.06	89.7±0.82
3	150%	7.15	6.072±0.08	85.5±0.73

n=3

Table 11: Data showing Recovery studies for Impurity 4

Sample	Spike Level	Mean µg/ml Added	Mean µg/ml Recovered	Mean % Recovery
1	50%	3.13	2.96±0.02	94.5±0.82
2	100%	6.27	5.76±0.07	92.0±0.65
3	150%	9.41	9.82±0.03	104.4±0.45

Table 12: Data showing Recovery studies for Impurity 5

Sample	Spike Level	Mean µg/mL Added	Mean µg/mL Recovered	Mean % Recovery
1	50%	1.96	1.83±0.02	93.5±0.76
2	100%	3.92	4.50±0.01	114.6±0.84
3	150%	5.89	6.04±0.05	102.6±0.64

n=3

3.4.3. Precision

Precision means that all measurements of an analyte should be very close together. The precision of an analytical procedure is usually expressed as the variance, standard deviation, relative standard deviation or coefficient of variation of a series of measurements. According to the ICH, precision should be performed at two different levels repeatability and intermediate precision. Repeatability should be determined from a minimum of nine determinations covering the specified range of the procedure (for example, three levels, three repetitions each) or from a minimum of six determinations at 100% of the test or target concentration.

3.4.3.1. System precision

System suitability solution was prepared as described in 13 & 14. The solution was injected in six replicate and

the area and resolution of olanzapine and impurities were noted. The %RSD was calculated for olanzapine and impurity-5 in the chromatogram obtained with six replicate of system suitability solution.

Inference: The % RSD was calculated for olanzapine and impurity-5 and it was found to be 0.3 & 1.2 respectively which was less than 5. The low % RSD showed that system suitability.

3.4.3.2. Repeatability

The repeatability of the method for the related substances was determined by a six fold analysis of 40 µg/mL of olanzapine spiked with 0.2 µg/mL of each of five impurities. A 1.0 µL injection of each sample was injected in system and chromatograms were recorded. The % RSD of AUC and RRT of impurities were calculated and are recorded.

Table 13: Data showing the system precision with respect to area counts and between Olanzapine and Impurity-5

Injection	Area counts	
	Olanzapine	Impurity-5
1	92814	18094
2	92871	17921
3	93190	17667
4	92749	17545
5	92271	17726
6	93044	17569
Mean	92823.16	17753.66
S.D	314.99	214.35
% RSD	**0.3**	**1.2**

Table 14: Data sheet for AUC of Impurities.

Sample	Impurity- 1	Impurity- 2	Impurity-3	Impurity- 4	Impurity- 5
1	79800	92814	66585	91813	18094
2	79329	92871	67674	91494	17921
3	76696	93190	69572	91668	17667
4	75886	92749	69648	91311	17545
5	78749	92271	69856	93814	17726
6	77451	93044	71638	91614	17569
Avg.	77985.16	92823.16	69162.16	91952.33	17753.66
SD	1551.56	314.99	1781.66	927.56	214.35
% RSD	**2.0**	**0.3**	**2.6**	**1.0**	**1.2**

Table 15: Data showing RRT of Impurities

Sample	Impurity- 1	Impurity- 2	Impurity- 3	Impurity- 4	Impurity- 5
1	0.131	0.288	0.359	0.408	0.940
2	0.131	0.290	0.359	0.408	0.940
3	0.132	0.288	0.358	0.407	0.940
4	0.132	0.290	0.360	0.410	0.939
5	0.133	0.289	0.361	0.408	0.938
6	0.133	0.290	0.361	0.409	0.940
Avg.	0.1320	0.2892	0.3597	0.4083	0.9395
SD	0.0009	0.0010	0.0012	0.0010	0.0008
% RSD	**0.7**	**0.3**	**0.3**	**0.3**	**0.1**

RRT of impurities were calculated with respect to olanzapine.

4. CONCLUSION

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 4.6 mm, 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 C18 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.

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