Original Research

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR RELATED SUBSTANCE IN OLANZAPINE BY HPLC

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ABSTRACT:
Olanzapine antipsychotic activity is likely due to a combination of antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Antagonism at D2 receptors relieves positive symptoms while antagonism at 5HT2A receptors relieves negative symptoms of schizophrenia.
A simple and reproducible RP-HPLC procedure was developed and validated as per ICH guidelines for the determination of related substances present in Olanzapine. After development of the method it was validated for specificity, system suitability, accuracy, linearity, precision, ruggedness and robustness. The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits, hence it is concluded that the system is suitable to perform assay.

Keywords: Olanzapine, Hplc, Phosphoric acid, Electronic balance.

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INTRODUCTION

Olanzapine antipsychotic activity is likely due to a combination of antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Antagonism at D2 receptors relieves positive symptoms while antagonism at 5HT2A receptors relieves negative symptoms of schizophrenia(**1**).Olanzapine binds to alpha(1), dopamine, histamine H1,muscarinic and serotonin type 2 (5-HT_2) receptors.Oral formulation: acute and maintenance treatment of schizophrenia in adults, acute treatment of manic or mixed episodes associated with bipolar I disorder.Intramuscular formulation: acute agitation associated with schizophrenia and bipolar I mania in adults(**2,3**).

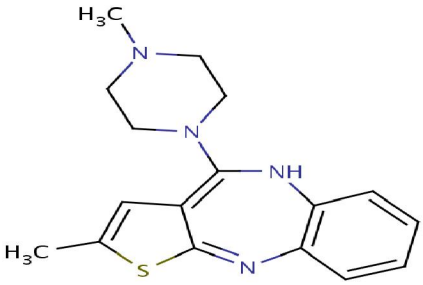

Figure 1: Structure of Olanzapine

MATERIALS AND METHODS

Chemicals, Reagents and Samples

The chemicals, equipment and samples which were used in the present study was given in the following Tables.1-3

Table 1: List of chemicals.

S.No	Name of the solvent	Grade	Make	Assay/ Purity
1	Sodium dihydrogen orthophosphate	GR	Merck	99.0
2	Sodium pentane sulfate	GR	Sigma -Aldrich	98.5
3	Ortho phosphoric acid	GR	Merck	88.0
4	Acetonitrile	HPLC	Rankem	99.8
5	Water	Milli-Q	--	--

Table 2: List of equipments.

S.No	Name of the instrument	Make	Model
1	HPLC	Waters	2695 model pump with 2998 PDA detector
2	HPLC	Waters	2695 model pump with 2489 UV detector
3	Electronic balance	Stratories	CP224S
4	pH meter	Eutech	pH510
5	UV	SHIMADZU	2400 PC series

Table 3: List of the samples.

S.No	Name	Grade	Assay/Purity (%)
1	Related compound-A	USP	100.0
2	Related compound-B	USP	100.0
3	Related compound-C	USP	100.0
4	Olanzapine-standard	IH	100.0

Chromatographic conditions

The chromatographic conditions used in the present study were given in the following Table 4.

Table 4: Chromatographic conditions.

Column	Zorbax RX-C 8, (150X4.6) mm,5µ
Detection wavelength	UV at 220 nm
Sample cooler	5°C
Flow	1.50 mL/minute
Injection Volume	20 µL
Run time	35 minutes.
Column oven température	35°C
Elution	Gradient
Mobil phase A	buffer:acetonitrile= 60:40
Mobil phase B	buffer:acetonitrile= 25:75

Preparation of the buffer solution

Weighed and transferred about 5.0 g of sodium pentane sulfonate in 1500 mL of milli-Q water, 2mL of phosphoric acid was added and sonicated to dissolve. The pH adjusted to 2.50 with 1N sodium hydroxide solution. Filtered through 0.45-micron porosity membrane and degassed.

7.2.4. Mobile phase-A: The buffer and acetonitrile mixed in the ratio of 60:40 (v/v).

7.2.5. Mobile phase-B: The buffer and acetonitrile mixed in the ratio of 25:75 (v/v).

7.2.6. Preparation of the diluent

Weighed and transferred about 5.0 g of sodium pentane sulfonate in 1500 mL of milli-Q water, 2mL of phosphoric acid was added and sonicated to dissolve. The pH adjusted to 2.50 with 1N sodium hydroxide solution. This buffer and acetonitrile were mixed in the ratio of 25:75 (v/v).

7.2.7 Preparation of stock solution

7.2.8 Stock solution-1

Weighed and transferred about 10.0 mg of Olanzapine standard into 100 mL volumetric flask, dissolved in 20 mL of diluent and made upto mark with diluent.

7.2.9 Stock solution-2

Weighed and transferred about each 10.0 mg of related compound-A standard and related compound-B standard into 100 mL volumetric flask, dissolved in 20 mL of diluent and made upto mark with diluent.

7.2.10 Preparation of standard solution preparation

2.0 mL of the stock solution-1 was transferred into 100 mL volumetric flask and made up to the volume with diluent.

7.2.11 Stock solution-3

Weighed and transferred about each 10.0 mg of Related compound-C standard into 100 mL volumetric flask and dissolved in 20 mL of diluent and make upto mark with diluent.

7.2.12 Preparation of Identification solution

In the above stock solutions each 2.0mL of stock solution-1, stock solution-2 and stock olution-3 were transferred into a 50.0 mL volumetric flask and made up to mark with diluent. Further diluted from 1.0 mL to 10 mL with diluents.

7.2.13 Sample preparation

Weighed and transferred about 10.0 mg of sample into a 25 mL volumetric flask, dissolved in diluent and made up to the mark with diluent.

Procedure

The blank solution, standard solution, identification solution and sample solutions were injected and the retention times were recorded for the Olanzapine and Related compounds.

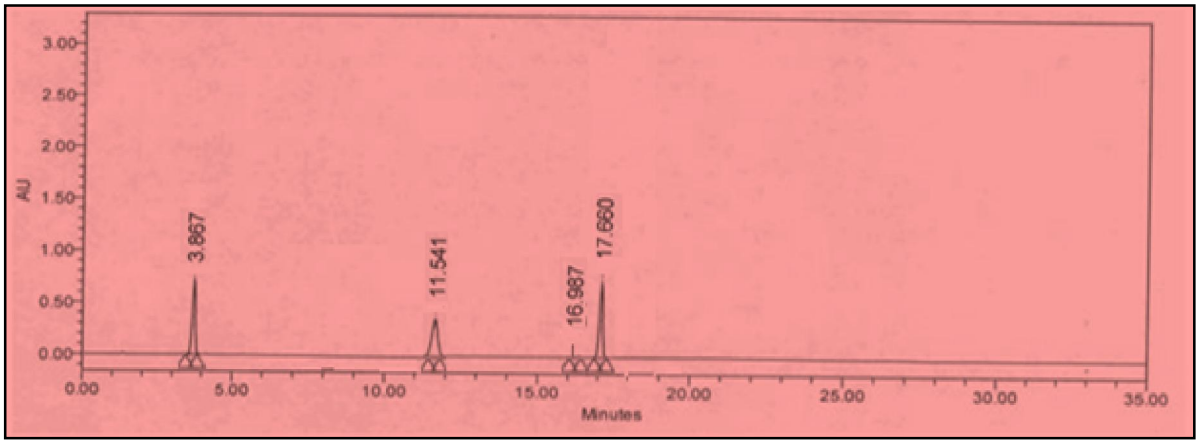

Figure 2 : Typical Chromatogram of identification solution

S. No.	Impurity Name	RT
1	Related compound-B	3.867
2	Related compound-A	11.541
3	Related compound-C	16.987
4	Olanzapine	17.660

RESULTS AND DISCUSSION

System suitability

The system suitability & standard solution was prepared and analysed as per the proposed method for resolution between Related compound-A & Olanzapine peak in system suitability solution, the tailing factor for Olanzapine peak and the % RSD of four replicate injections in standard solution of Olanzapine peak to demonstrate system suitability for studying of each validation parameter(**4,5**).

Table 5: Observations of System suitability

Validation Parameter	Resolution between Related compound-A and Olanzapine	Tailing factor for Olanzapine peak	The % RSD for Olanzapine
Specificity	22.03	1.02	0.96
LOD, LOQ & Linearity	23.17	0.98	0.33
Accuracy	25.06	0.99	0.24
Method Precision	23.66	1.00	0.69
Intermediate precision	22.01	1.01	0.37
Solution stability study	24.08	1.00	0.18

Selectivity/specificity

Each known specified impurity solution was prepared individually [i.e., Related compound-A, Related compound-B and Related compound-C] and a solution of all known specified impurities spiked with the Olanzapine at 1.0% level and finally Olanzapine sample was also prepared. All these solutions were analyzed by using the PDA detector(**6**).

Table 6: Retention time, RT ratio and peak purity values for Olanzapine and its specified impurities

Peak name	Retention time (minutes)	RT ratio	Peak purity		USP resolution
			Purity angle	Purity threshold	
Related compound-B	3.865	0.219	0.174	1.115	--
Related compound-A	11.551	0.655	0.293	1.312	27.51
Related compound-C	16.972	0.963	0.110	1.172	19.00
Olanzapine	17.631	1.000	0.105	1.105	3.22

Accuracy

The accuracy was performed on samples spiked with known amounts of each specified impurity. The inherent amount of the individual impurity was taken into account.

The following concentrations were performed:

Table 7: Percentage recoveries for Related compound-A

Level	Theoretical conc.(mg/mL)	Measured conc.(mg/mL)	% Recovery	Average	SD	% RSD
50.0 %	0.000202	0.000186	92.08	92.57	0.8545	0.92
	0.000202	0.000189	93.56			
	0.000202	0.000186	92.08			
100.0 %	0.000404	0.000389	96.29	96.70	0.5164	0.53
	0.000404	0.000393	97.28			
	0.000404	0.000390	96.53			
120.0 %	0.000485	0.000508	104.74	104.54	0.5443	0.52
	0.000485	0.000509	104.95			
	0.000485	0.000504	103.92			
250.0 %	0.001010	0.001022	101.19	100.99	0.3464	0.34
	0.001010	0.001016	100.59			
	0.001010	0.001022	101.19			

Precision

The repeatability expresses the precision under the same operating conditions over a short interval of time. It expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample.

Table 8: Results for precision of the method

Preparation No.	Related compound-B	Related compound-A	Related compound-C	Unspecified impurity	Total impurities
1	0.11	0.09	0.10	0.03	0.33
2	0.12	0.10	0.10	0.03	0.35
3	0.12	0.10	0.10	0.03	0.35
4	0.11	0.09	0.10	0.03	0.33
5	0.12	0.10	0.11	0.03	0.36
6	0.11	0.09	0.10	0.03	0.33
Average (% w/w)	**0.12**	**0.10**	**0.10**	**0.03**	**0.34**
SD	**0.0055**	**0.0055**	**0.0041**	**0.0000**	**0.0133**
% RSD	**4.58**	**5.50**	**4.10**	**0.00**	**3.91**

Limit of detection (LOD) The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantitated. The limit of detection was determined as the lowest concentration for which the response is approximately two times greater than the baseline noise (**7**).

Table 9 : Limit of detection for Olanzapine and its specified impurities

Component name	Related compound-B	Related compound-A	Related compound-C	Olanzapine
Conc. (mg/mL)	0.000008	0.000006	0.000014	0.000030
LOD with respect to sample conc.(%)	0.002	0.0020	0.004	0.008
Signal to Noise ratio	6.0:1	4.5:1	4.0:1	5.8:1
Reported LOD (%)	**0.002**	**0.002**	**0.004**	**0.008**

Limit of quantitation (LOQ)

The Limit of quantitation (LOQ) values was determined from the same experiment as mentioned in the limit of detection section. Based on the limit of detection, roughly three folds of limit of detection solution was prepared and analyzed for the determination of limit of quantitation(**8**).

Table 10:Limit of quantitation for Olanzapine and specified impurities

Component name	**Related compound-B**	**Related compound-A**	**Related compound-C**	**Olanzapine**
Conc. (mg/mL)	0.000025	0.000021	0.000045	0.000101
LOQ w.r.t sample conc.(%)	0.006	0.005	0.011	0.025
Signal to Noise ratio	11.0:1	11.5:1	11.0:1	12.7:1
Reported LOQ (%)	**0.01**	**0.01**	**0.01**	**0.03**

Linearity

The linearity of the HPLC method was demonstrated for Olanzapine and each specified impurity solutions ranging from LOQ to 250.0 % of the specification limit.

Table 11: Linearity data for Related compound-B

Levels	**Conc.(mg/mL)(w.r.to potency)**	**Avg peak area of Related compound-B**
LOQ level	0.000025	2499
30.0% level	0.000121	10010
50.0 % level	0.000202	16845
100.0 % level	0.000404	34008
120.0 % level	0.000485	37964
200.0 % level	0.000808	64656
250.0 % level	0.001010	83308

Range

Range of the method was determined from the linearity data.

Table 12 :Range of the method from accuracy and linearity data

Name of the Component	Range of conc. (mg/mL)	Range of conc. % with respect to sample concentration
Related compound-B	0.000025 mg/mL to 0.001010 mg/mL	6.25% to 252.5%
Related compound-A	0.000021 mg/mL to 0.001010 mg/mL	5.25% to 252.5%
Related compound-C	0.000045 mg/mL to 0.001000 mg/mL	11.25% to 250.0%
Olanzapine	0.000111 mg/mL to 0.001010 mg/mL	27.75% to 252.5%

CONCLUSION

A simple and reproducible RP-HPLC procedure was developed and validated as per ICH guidelines for the determination of related substances present in Olanzapine. After development of the method it was validated for specificity, system suitability, accuracy, linearity, precision, ruggedness and robustness. The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits, hence it is concluded that the system is suitable to perform assay. The method was found to be specific because it did not show any interference with standard and blank. The linearity studies were performed for the standard and found to be linear. It was evaluated by the visual inspection of the plot of peak area Vs concentration. From the linearity studies, the specified range was found. The precision was checked and found to be within limits, hence the method is precise. The accuracy has been determined and % recovery was calculated and found to be within limits. The ruggedness of the method was checked on different systems and by different columns and standard was able to give same results which indicate that the method is rugged. The robustness of the method was checked by changing flow rate and temperature, and standard was able to give system suitability parameters within limit, which indicates that the method is robust. Therefore it was concluded that the proposed method can be used for routine analysis of determination of related substances present in Olanzapine.

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