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Innovative Method Development Comprehensive Separation of Impurities and Validation for a novel Antipsychotic Drug Blonanserin

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ABSTRACT

Blonanserin an antipsychotic novel drug used for the treatment of schizophrenia has antagonist properties for dopamine D2 and serotonin 5-HT2. On the other hand, it lacks adrenergic-α1, muscarinic M1, and histamine H1 antagonist activities. Clinical studies demonstrated in Japan had shown to be more effective for treating negative as well as positive schizophrenic symptoms. This drug was accepted and approved worldwide in the treatment of schizophrenia. A new HPLC method was developed and validated for the estimation of Impurities of Blonanserin (BNS) to ensure that the methodology meets the requirements of the target analysis application. Active and efficient chromatographic separation was achieved on a Zorbax Bonus RP EP C18 column having a particle size of 5μm, with dimensions of 250mm $\times$ 4.6 mm, mobile phase containing pH 2.4 buffer and Organic, with 1.0 ml/min flow rate, column oven temperature at 30^{o}C and the eluent detection at 245 nm. The method shows well-separated impurities, is specific without interference from blank solution with resolution more than 1.2 between any of the impurity, correlation coefficient more than 0.99 showing good linearity; mean recovery ranging from 97% to 105% and is very sensitive at lower detection and quantification limits. This method was well developed and has been applied successfully to monitor and estimate impurities in Blonanserin.

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INTRODUCTION

Blonanserin, a new second-generation antipsychotic drug (Garcia *et al.*, 2009) used to treat schizophrenia, was approved in Korea and Japan by PMDA in 2008. The drug is now made more acceptable world wise as a promising antipsychotic drug for schizophrenia treatment. Blonanserin binds and acts as an antagonist and inhibits the serotonin 5-HT2A receptors, D2 and D3 receptors of Dopamine (Oka *et al.*, 1993). It is very effective in treating patients with schizophrenia, which is equivalent to risperidone and haloperidol for positive symptoms and is also higher than haloperidol for the development of negative symptoms (Ghosh

et al., 2012). Blonanserin, a heterocyclic compound belongs to the chemical series of 4-phenyl-2-(1-piperazinyl) pyridines, IUPAC nomenclature 1-ethyl-4- [4- (4-fluorophenyl) -5H, 6H, 7H, 8H, 9H, 10H-cycloocta [b] pyridin-2-yl] piperazine, metabolized mainly by CYP3A4 (Figure 1). The piperazine ring of BNS undergoes N-de-ethylation and N-oxidation as well as hydroxylation of the cyclooctane ring. The literature review of Blonanserin reveals that this molecule is not yet official in IP, BP, USP, or any pharmacopeia. In line with regulatory requirements and The International Conference of Harmonization (ICH), the FDA endorse identification, quantification, qualification and also laid control over impurities in pharmaceutical drug substances and their formulation. It has been found that there is no analytical method developed for the determination of impurities in Blonanserin. It is very important that there should be a simple, stable, sensitive, accurate, authentic, rapid and reliable method to determine the impurities of Blonanserin drug substance. Therefore, it was considered that Blonanserin impurities should be determined to ensure the quality, effectiveness, and safety of the final pharmaceutical drug. There is recognition of the UV Spectrophotometric method (Modi *et al.*, 2011), HPLC (Modi and Chandrul, 2011; Zhou *et al.*, 2013) bioanalytical LCMS / MS method of Blonanserin and its human plasma and urine metabolites (Saruwatari *et al.*, 2010; Ogawa *et al.*, 2010), HPLC-FDA (Wen *et al.*, 2012; Matsuda *et al.*, 1997), GC-MS (Hattori *et al.*, 2010). The reported HPLC method has its limitations related to LOD, LOQ and analysis time. To date, there is no indicative, well-separated, fast analysis method available so far for the estimation of Blonanserin impurities. Keeping this fact in mind, the purpose of the present study is designed to develop a sensitive, fast, reliable method, demonstrating the stability of the analysis and separation of impurities in the Blonanserin pharmaceutical drug substances. This research demonstrates the novelty of the work by reduced runtime, well-separation of impurities, detection at lower LOD and LOQ levels, and proved the stability in different conditions.

MATERIALS AND METHODS

Reagents and chemicals

In the present research, the following materials were used Blonanserin, Acetonitrile (Merck, HPLC Grade), Orthophosphoric acid (Qualigens, HPLC Grade), Triethylamine (Qualigens HPLC Grade), Tetrahydrofuran (Sigma Aldrich, HPLC Grade), Water (Millipore water system).

Apparatus and Conditions for Chromatography

Chromatography was performed on Waters, Alliance 2695 HPLC system connected with 2998 PDA detector with Empower software Version3. Zorbax Bonus RP EP C18 column having a particle size of 5μm, with dimensions of 250mm $\times$ 4.6 mm, mobile phase containing pH 2.4 buffer and Organic with 1.0 ml /min flow rate, column oven temperature at 30^{o}C was used for separation and detected at 245nm using a PDA detector. The impurities and Blonanserin were separated in gradient mode of elution with Triethylamine (1ml in 1000ml water), pH adjustment was made to 2.4 with H_3PO_4 (orthophosphoric acid) and a mixture of tetrahydrofuran and acetonitrile (6:94). Mobile phase A (75:25) and Mobile phase B (25:75) with the 10 μl injection volume. Water and acetonitrile mixed in an equal ratio is used as a diluent. The gradient elution program was designed as (0$\rightarrow$5, 95:5, 5$\rightarrow$30, 75:25, 30$\rightarrow$35, 5:95, 35$\rightarrow$40, 5:95, 40$\rightarrow$45, 95:5, 45$\rightarrow$48, 95:5).

Resolution, Standards and sample preparation

The resolution solution was prepared by accurately weighing and dissolving the Blonanserin sample solution containing all the impurities to get the concentration of 0.5mg/ml solution. The request was to have a resolution of a minimum of 1.2 between impurity B and C peaks and other impurities to confirm their relative retention times.

The stock solution of Blonanserin standard was prepared by weighing an accurate amount of reference standard in a diluent, dissolved to get a concentration of 0.25ppm solution (equivalent to 0.05% w.r.t test sample solution).

Blonanserin test solution was prepared with diluent and diluted to an appropriate volume to get the concentration of 0.5mg/ml.Optimization of the Method.

Optimization of the Method

Several experimental trials have been taken to optimise the developed method by changing buffer concentration, pH, organic solution, and the ratio of buffer and organic solution. Finally, the mobile phase was optimized with gradient mode of elution, Zorbax Bonus RP EP C18 column with length 250 mm, internal diameter 4.6 mm and particle size 5μm, 30^{o}C temperature, the flow of mobile phase at 1.0ml/min and detected at 245nm.

System Suitability

System suitability parameter, which is an essential part of method parameters, is measured for verifying the system, method and column performance. Blank, Resolution solution, peak identification solution, Reference standard solution was prepared and

Table 1: System suitability results

S.No	Parameter	%RSD of BNS standard solution from 6 injections	Resolution between Imp-B and Imp-C
1	System suitability	2.1	1.4
Acceptance criteria		Should not be more than 5	Should not be less than 1.2

Table 2: Specificity results

S.No	Peak names	RT	Purity angle	Purity threshold
1	Impurity-A	3.1	1.93	3.21
2	Impurity-B	14.9	2.63	3.36
3	Impurity-C	15.6	1.82	2.79
4	BNS	17.2	0.08	2.51
5	Impurity-D	24.0	2.35	3.22
	Acceptance criteria	All impurities should resolve and peak purity should pass		

Table 3: Forced Degradation results

Condition	Purity angle of BNS	Purity Threshold of BNS	Imp-A (%W/W)	Imp-B (%W/W)	Imp-C (%W/W)	Imp-D (%W/W)	Total Imp	Assay	Mass Balance
0.5N HCl at 50^{O}C for 20 hours	0.61	1.98	0.006	0.01	0.004	0.01	0.07	99.2	99.6
0.5N NaOH at 50^{O}C for 20 hours	1.21	2.73	0.003	0.011	0.001	0.01	0.05	100.5	100.4
1%H_2O_2 at room temperature for 1hour	0.95	2.36	0.002	0.018	0.015	0.007	24.8	76.5	99.8
Heat at 50^{O}C for 20 hours	0.37	2.91	0.008	0.013	0.006	0.01	0.09	99.1	99.4

Table 4: Linearity

Level		Imp-A	
	Conc (μg/ml)		Avg area counts (μV*Sec)
LOQ	0.02715		523.9
50%	0.12360		3438.1
80%	0.19833		5487.8
90%	0.22290		6127.3
100%	0.24690		6986.6
120%	0.29340		8334.8
150%	0.37290		10288.8
Slope	28436.4872		
Intercept	-149.4289		
C.C	0.9994		
R.Square	0.9987		
Level		Imp-B	
	Conc (μg/ml)		Avg area counts (μV*Sec)
LOQ	0.02763		1184.2
50%	0.14527		4354.1
80%	0.23648		6905.3
90%	0.26745		7749.4
100%	0.29083		8728.4
120%	0.35697		10345.7
150%	0.44143		12834.5
Slope	28230.8627		
Intercept	320.6004		
C.C	0.9996		
R.Square	0.9991		
Level		Imp-C	
	Conc (μg/ml)		Avg area counts (μV*Sec)
LOQ	0.03688		1438.2
50%	0.12995		4416.7
80%	0.20587		6997.5
90%	0.23952		7962.9
100%	0.25481		8799.4
120%	0.31629		10657.4
150%	0.38716		13140.4
Slope	33433.8834		
Intercept	129.3221		

Continued on next page

Table 4 continued

Level	Imp-A	
	Conc (μg/ml)	Avg area counts (μV*Sec)
C.C	0.9996	
R.Square	0.9992	
Level	BNS	
	Conc (μg/ml)	Avg area counts (μV*Sec)
LOQ	0.03866	1154.6
50%	0.12846	4367.8
80%	0.20738	6911.7
90%	0.22693	7847.3
100%	0.25567	8651.8
120%	0.30369	10608.9
150%	0.37116	13234.5
Slope	36034.3154	
Intercept	-346.5956	
C.C	0.9992	
R.Square	0.9984	
Level	Imp-D	
	Conc (μg/ml)	Avg area counts (μV*Sec)
LOQ	0.03768	1186.6
50%	0.12518	4058.9
80%	0.20786	6544.7
90%	0.22234	7339.1
100%	0.25936	8152.7
120%	0.30567	9815.1
150%	0.37123	12249.9
Slope	32729.0351	
Intercept	-100.8811	
C.C	0.9990	
R.Square	0.9980	

RESULTS AND DISCUSSION

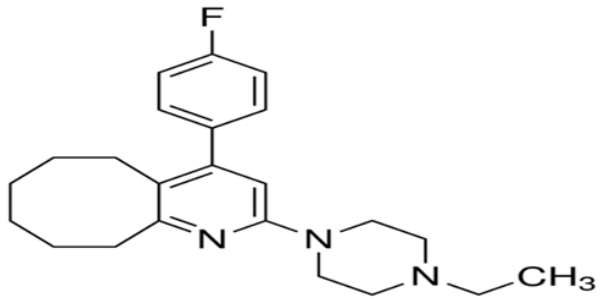

Figure 1: Chemical Structure of Blonanserin

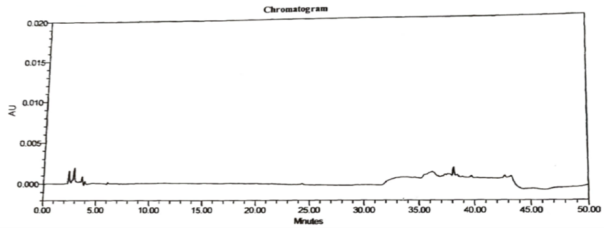

Figure 2: Typical chromatogram of Blank

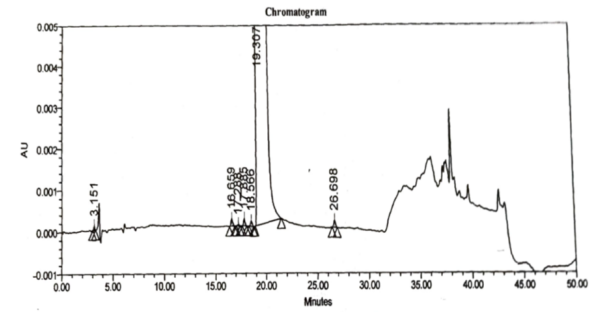

Figure 3: Typical chromatogram of Control sample

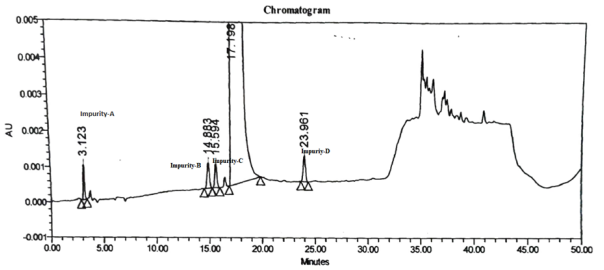

Figure 4: Typical chromatogram of spiked sample

analysed as per the method. Results of system suitability parameters such as %RSD of the standard and resolution between Imp-B and Imp-C, results of Suitability of the system are compiled in Table 1.

Specificity

A research study was conducted to detect interference. A blank solution was injected three times, According to the test method. A spiked sample and

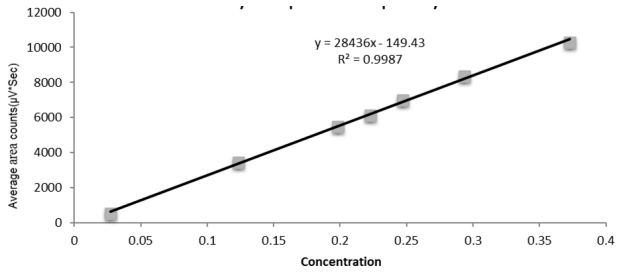

Figure 5: Linearity of Impurity-A

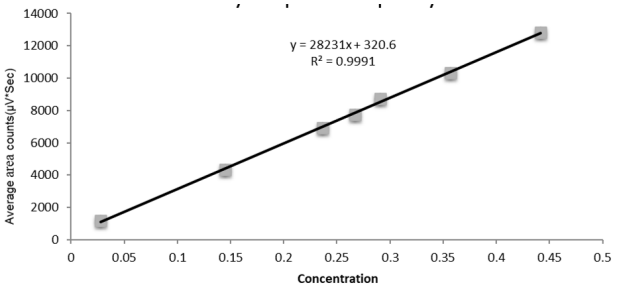

Figure 6: Linearity of Impurity-B

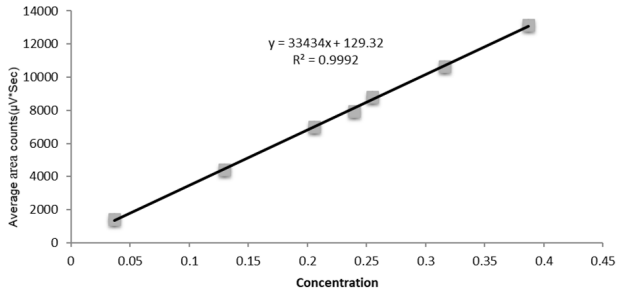

Figure 7: Linearity of Impurity-C

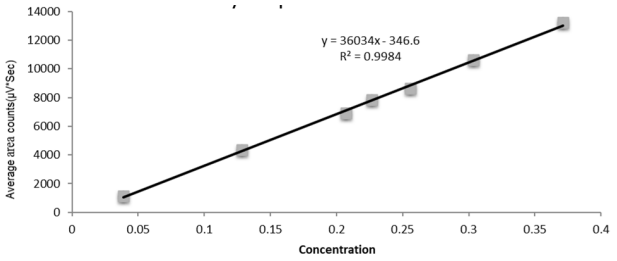

Figure 8: Linearity of BNC

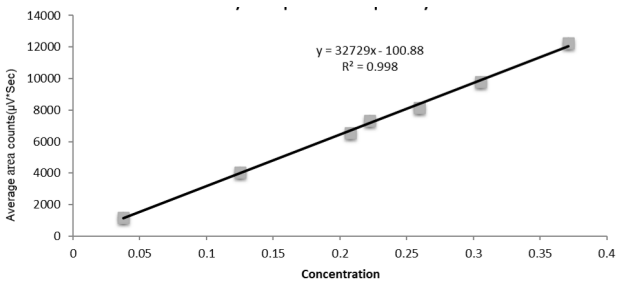

Figure 9: Linearity of Impurity-D

Table 5: Recovery of Impurity-A

Amount Added(mcg/ml)	Amount Added(mcg/ml)	Amount Added(mcg/ml)	%Recovered	Mean Recovery	%RSD
LOQ-1	0.00002547	0.00002730	107.18	100.27	5.97
LOQ-2	0.00003272	0.00003173	96.97		
LOQ-3	0.00002957	0.00002858	96.65		
50% of Rec-1	0.1358	0.1398	102.95	103.18	4.09
50% of Rec-2	0.1313	0.1301	99.09		
50% of Rec-3	0.1292	0.1389	107.51		
100% of Rec-1	0.2913	0.2861	98.2	100.33	3.08
100% of Rec-2	0.2823	0.2792	98.90		
100% of Rec-3	0.2346	0.2437	103.88		
120% of Rec-1	0.3389	0.3375	99.59	100.59	1.01
120% of Rec-2	0.3810	0.3872	101.63		
120% of Rec-3	0.3675	0.3705	100.57		

Table 6: %Recovery of Impurity-B

Amount Added(mcg/ml)	Amount Added(mcg/ml)	Amount Added(mcg/ml)	%Recovered	Mean Recovery	%RSD
LOQ-1	0.00002744	0.00002841	103.53	98.77	4.29
LOQ-2	0.00003171	0.00003026	95.43		
LOQ-3	0.00002957	0.00002879	97.36		
50% of Rec-1	0.1869	0.1796	96.09	97.70	2.83
50% of Rec-2	0.1593	0.1531	96.11		
50% of Rec-3	0.1583	0.1597	100.88		
100% of Rec-1	0.2654	0.2738	103.2	104.67	1.26
100% of Rec-2	0.2558	0.2692	105.24		
100% of Rec-3	0.2446	0.2583	105.60		
120% of Rec-1	0.3286	0.3363	102.34	102.70	2.49
120% of Rec-2	0.3268	0.3279	100.34		
120% of Rec-3	0.3375	0.3558	105.42		

Table 7: %Recovery of Impurity-C

Amount Added(mcg/ml)	Amount Added(mcg/ml)	Amount Added(mcg/ml)	%Recovered	Mean Recovery	%RSD
LOQ-1	0.00002693	0.00002586	96.03	97.55	1.46
LOQ-2	0.00002525	0.00002496	98.85		
LOQ-3	0.00002591	0.00002533	97.76		
50% of Rec-1	0.1869	0.1796	96.09	97.70	2.83
50% of Rec-2	0.1593	0.1531	96.11		
50% of Rec-3	0.1583	0.1597	100.88		
100% of Rec-1	0.2654	0.2738	103.2	104.67	1.26
100% of Rec-2	0.2558	0.2692	105.24		
100% of Rec-3	0.2446	0.2583	105.60		
120% of Rec-1	0.3184	0.3081	96.77	99.59	3.23
120% of Rec-2	0.3133	0.3099	98.91		
120% of Rec-3	0.3201	0.3300	103.09		

Table 8: %Recovery of Impurity-D

Amount Added(mcg/ml)	Amount Added(mcg/ml)	Amount Added(mcg/ml)	%Recovered	Mean Recovery	%RSD
LOQ-1	0.00002485	0.00002503	100.72	98.39	2.29
LOQ-2	0.00002596	0.00002498	96.22		
LOQ-3	0.00002544	0.00002499	98.23		
50% of Rec-1	0.1363	0.1355	99.41	101.66	2.74
50% of Rec-2	0.1423	0.1491	104.78		
50% of Rec-3	0.1385	0.1396	100.79		
100% of Rec-1	0.2592	0.2582	99.6	99.61	0.51
100% of Rec-2	0.2588	0.2591	100.12		
100% of Rec-3	0.2553	0.253	99.10		
120% of Rec-1	0.3555	0.3421	96.23	97.57	1.19
120% of Rec-2	0.3483	0.34199	98.19		
120% of Rec-3	0.3363	0.3306	98.31		

Table 9: Effect of Robustness

Parameters	%RSD		Resolution		Imp-A (%w/w)		Imp-B (%w/w)		Imp-C (%w/w)		Imp-D (%w/w)	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Flow(ml/min)	1.5	0.4	1.5	1.7	0.05	0.06	0.04	0.06	0.05	0.05	0.05	0.05
Wave length (nm)	2.7	0.9	1.9	2.3	0.06	0.05	0.05	0.05	0.05	0.04	0.05	0.05
Column Temperature(^{o}C)	1.7	1.1	1.3	1.5	0.05	0.06	0.05	0.04	0.05	0.05	0.04	0.05
Buffer (pH)	2.7	1.8	2.4	2.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

individual impurities were prepared and injected. The blank chromatogram did not show any interference at BNS and impurities retention time. This indicates that the blank does not interfere with the BNS and related impurities (Figures 2, 3 and 4). The results are tabulated in Table 2. All forced degradation samples were stressed by 0.5N HCl, 0.5N NaOH, 1%H_2O_2, Heat and analyzed as per the method and the resulted are tabulated in Tables 2 and 3 .

Linearity

The results obtained between concentration and Analyte peak area infers that a very good correlation exists. The calibration plot, which is linear, was obtained over the tested measured values, i.e., from LOQ to 150% for BNS and its impurities (Figures 5, 6, 7, 8 and 9), the correlation coefficient obtained was greater than 0.99 and tabulated in Table 4.

Accuracy

The recovery of BNS and impurities (in percentage) was determined using a sample solution containing all impurities spiked at LOQ, 50%, 100%, and 150% of the sample concentration. Percentage acquisition of the recovery sample is calculated and tabulated in Tables 5, 6, 7 and 8.

Percentage recovery for each impurity should be between 90% and 110%.

Robustness

Robustness is a deliberate varied chromatographic condition for parameters like flow (±0.1%), wavelength (±2nm), column oven temperature (±2^{o}C) and Buffer pH (±1), the resolution between critical pairs was more than 1.2, illustrating the robustness of the method as mentioned in Table 9.

CONCLUSIONS

A gradient method was developed for the separation and estimation of Impurities in Blonanserin active pharmaceutical ingredients. The validated method is linear, precise and accurate, selective and specific and shows ruggedness. The method can be effectively used to monitor impurities for stability analysis of controlled samples in Blonanserin API.

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Conflict of interest

The authors report that they have no conflict of interest in this work.

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