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A simple and validated reverse phase HPLC method for the determination of Blonanserin in pharmaceutical dosage forms

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ABSTRACT

A Simple and rapid reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for quantitative determination of blonanserin in bulk drug samples and formulations. Blonanserin was analysed by using column (Phenomanex Luna C_{18}, 4.6 mm × 25 cm, 5 microns), with mobile phase consisting of water : acetonitrile : TFA (65:35:0.05 v/v). The flow rate was set 1.0 mL/min and and analysis was performed at wavelength 237 nm using Photo Diode Array (PDA) detector at ambient temperature. The method was validated. The retention time for blonanserin was around 4.9 minutes. The calibration curves were linear (r≥0.9996) over a concentration range from 10.0 to 150.0 µg/mL. Limit of detection (LOD) and Limit of quantitation (LOQ) were 5.08 µg/mL and 15.39 µg/mL respectively. The developed method was successfully applied to estimate the amount of blonanserin in tablet formulations.

Keywords: RP-HPLC, PDA, Blonanserin, Pharmaceutical dosage forms, Validation.

INTRODUCTION

Blonanserin (2-(4-ethyl-l-piperazinyl)-4-(4-fluorophenyl)-5, 6, 7, 8, 9, 10 hexahydrocyclo-octa[b]-pyridine ;) is a novel antipsychotic agent, having dopamine D_2 and serotonin 5-HT_{2A} receptor antagonist properties [1-6]. It is one of the second- generation antipsychotic agents, together with risperidone and olanzapine, it is effective in the treatment of both positive and negative symptoms of schizophrenia without extra-pyramidal symtoms, but has original properties of affinity higher for the dopamine D_2 receptor than for the serotonin 5-HT_{2A} receptor[1-4,6,7]. On the other hand, blonanserin is much less potent in adrenergic-α_1, histamine H_1 and muscarinic M_1 antagonist activities [6]. Such a pharmacological profile shows that blonanserin is more specific to the dopamine D_2 and serotonin 5-HT_{2A} receptors with fewer side effects; its excellent effects on schizophrenia have been reported in many reports [7-10]. There is a possibility that this drug gain popularity for treatment of schizophrenia throughout the world.

Blonanserin is not yet official in I.P., B.P. and U.S.P. Extensive survey revealed that not a single UV or HPLC method is however reported for blonanserin. So the need was felt to devlop simple, economical, rapid, precise and accurate method to analyze the drug by HPLC method.

EXPERIMENTAL SECTION

Chemicals and Materials

Blonanserin working standard (purity, 99.80%) used from Cadila Healthcare Ltd., Ahmedabad, India. Blonanserin tablets were obtained from Cadila Healthcare Ltd., Ahmedabad, India. Each tablet was lebeled contain 4 mg of Blonanserin. Tri flouro acetic acid of Analytical grade (Merck India Ltd.). Methanol and Acetonitrile were used of HPLC grade (Spectrochem, India).High pure water was prepared by using Millipore Milli Q plus purification system.

Instrumentation

Quantitative HPLC was performed on a high pressure liquid chromatography (Shimadzu HPLC class 10AT) with four LC-10AT pumps, PDA detector (SPD-M10A VP), an SIL-10AD series auto sampler and C_{18} bonded stainless steel column (Phenomanex luna C_{18}, 250 mm × 4.6 mm ID, partical size 5µ). HPLC system was equipped with data acquisition and processing software "CLASS-VP series" (Shimadzu).

HPLC Conditions

Detector : 237 nm
Injection volume : 10 µL
Flow rate : 1.0 mL/ min
Temperature : 30° C
Mobile phase : Water: Acetonitrile: TFA (65:35:0.05)
Diluent : Methanol

Mobile phase preparation

0.5 ml of Triflouroacetic acid (TFA) was added to 1000 ml Milli-Q water. Then resulting solution was Mixed with Acetonitrile (65:35:0.05) was used as mobile phase. Mobile phase was sonicated for 5 minutes.

Standard preparation

An accurately weighed quantity of blonanserin working standard about 40 mg was transferred into 100 ml volumetric flask. About 50 ml of diluent added and sonicate to dissolve. The solution was cooled to the room temperature and made up to mark with diluents. 5 ml of stock solution of blonanserin was pipette out and transferred to 25 ml volumetric flask and made volume up to mark with diluent.

Sample preparation

Weigh accurately tablet powdered equivalent to about 4 mg blonanserin and transferred into 250 ml volumetric flask. Approximately 125 ml was added and sonicate for 20 minutes. The solution was cooled to the room temperature and make up to volume with same. Filtered through 0.45 µ Millipore PVDF filter; and filtrate was collected after discarding first few ml.

Method Validation

The method was validated for the parameters like Specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, system

suitability parameters were also calculated. To demonstrate specificity in the presence of excipients used in formulation, blonanserin was spiked in drug product; chromatogram was observed and compared with that of raw material. To evaluate the linearity, the LOD and LOQ of the method in reference drug, different serial dilutions (10, 20, 40, 80, 100, 120 and 150 μg/mL) were prepared from the standard stock solution. The samples were injected (10 μL) and signals from the samples were recorded at 4.9 ±0.2 minute which were compared with those of blank. LOD and LOQ values were calculated as signal-to noise ratio of 3:1 and 10:1 respectively. To determine accuracy of the method, working standard of blonanserin was prepared in triplicate at three concentration levels (50, 100 and 150 μg/ml) and analyzed. Repeatability of the method was checked by analyzing six replicate samples of blonanserin (at the 100% concentration level) and calculating relative standard deviation (%RSD).

RESULTS AND DISCUSSION

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996] and [USP 2002] have recommended the accomplishment of accuracy tests, precision, specificity, linearity of the method.

System Suitability

The % CV of peak area and retention time for the drug was within 2% indicating the suitability of the system (Table 1). The efficiency of the column was expressed by number of theoretical plates for the 6 replicate injections was 10729.57±0.035% (mean ±%CV)and the USP tailing factor was 1.23±0.010% (mean ±%CV).

Table 1. System suitability study of blonanserin

Injection	Retention time, min	Peak area	Plate count	Tailing factor
1	4.79	1896876	10324.43	1.20
2	4.82	1975620	10223.05	1.22
3	4.83	1943923	10922.38	1.26
4	4.84	1949428	10963.09	1.26
5	4.86	1939589	10990.35	1.23
6	4.88	1946249	10954.12	1.22
Mean	4.84	1941947.5	10729.57	1.23
% RSD			1.3	
RSD= Relative standard deviation				

Accuracy/recovery

The data presented in Table 2 show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50,100, and 150% levels were within the acceptable criteria. Excellent recovery and low relative standard deviation value showed that the method is suitably accurate for potency assay of Blonanserin.

Linearity and Range

The plot of peak area responses against concentration is shown in Figure 1. It can be seen that plot is linear over the concentration range of 10 to 150 μg/mL for Blonanserin with a correlation coefficient (r^2) 0.9996. The results of linearity, limit of detection and limit of quantification were presented in Table 3.

Table 2. Method Accuracy for blonanserin

Levels	Drug Added	Drug recovered	% Recovery	Mean % recovery	% RSD
50%	10.28	10.41	101.3	101.5	0.7
50%	9.98	10.07	100.9		
50%	10.28	10.51	102.2		
100%	19.96	19.98	100.1	99.6	1.3
100%	19.96	20.08	100.6		
100%	20.16	19.77	98.1		
150%	30.04	29.99	99.8	99.1	0.8
150%	30.14	29.61	98.2		
150%	29.94	29.74	99.3		
RSD= Relative standard deviation					

Table 3. Characteristics of the Analytical Method Derived from the Standard Calibration Curve

LOD µg/mL	LOQ µg/mL	Linearity µg/mL Range n=(7)	Correlation co-efficient µg/mL	Residual std. regression σ	Slope of regression (S)
5.08	15.39	10 to 150	0.99965	36011.73088	23384.75520
LOD= Limit of detection, LOQ= Limit of quantification					

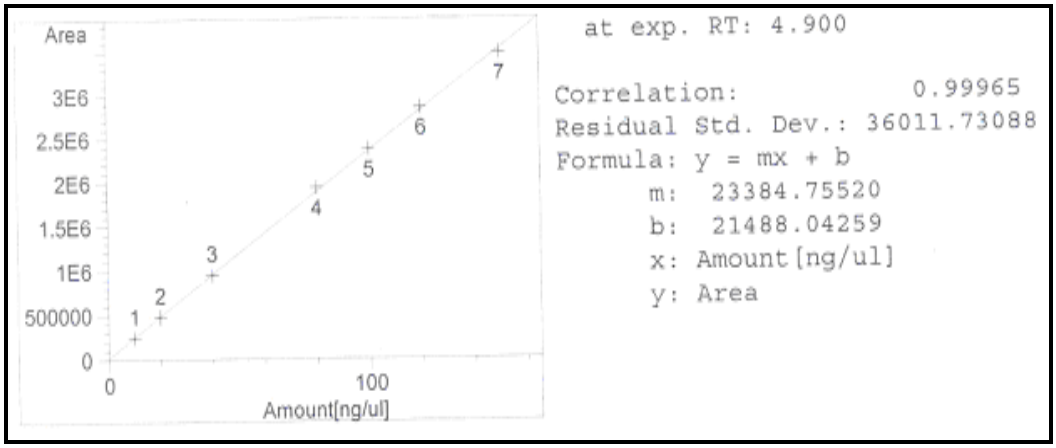

Figure: - 1 Standard Calibration Curve

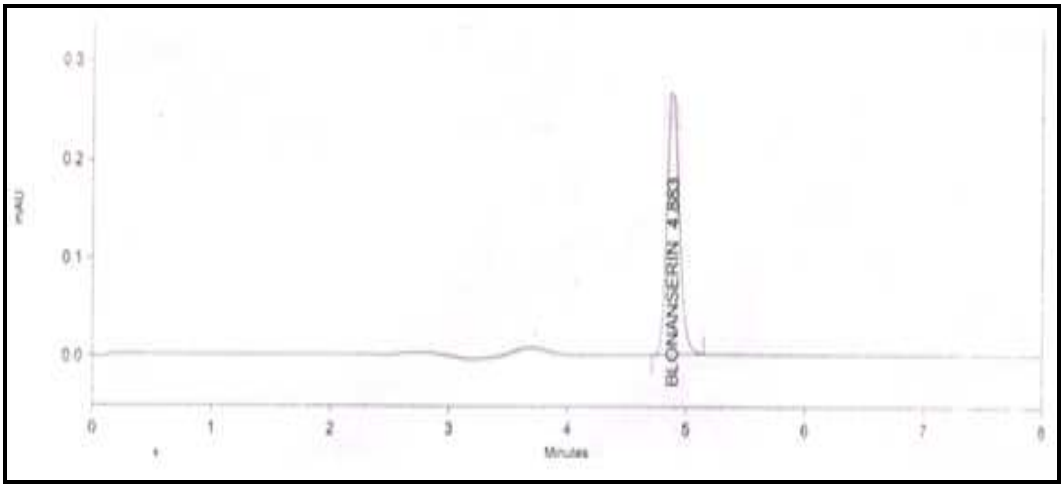

Figure 2: - Chromatogram for Test solution

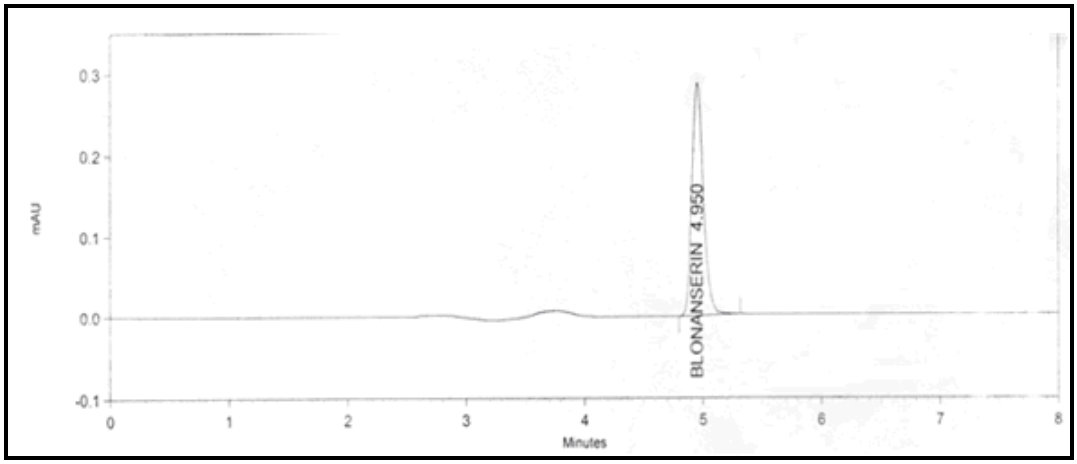

Figure 3: - Chromatogram for Standard solution

Method Precision

The relative standard deviation for six replicate injections was less than 2.0 %, which met the Acceptance criteria established for the method. The results obtained were presented in Table 4.

Table 4. Method Precision

Concentration (µg/mL) (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
80.0	4.9 ± 0.2	99.3 ± 0.500	1.2

Specificity

There was no interference from sample placebo and peak purity of Blonanserin was 0.99960. It indicating that developed analytical method was specific for its intended purpose. Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Figure-2). The excipiants used in different formulation products did not interfere with the drug peak and thus, the method is specific for Blonanserin. To further confirm the specificity of the method, UV scans of spiked drug were taken in the range 200-400nm and no significant change was found by comparing the absorbance of pure drug and spiked drug at the analytical wavelength of drug.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 24 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 h at room temperature.

Method robustness

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. The content of the drug was not adversely affected by various changes.

Limits of Detection and Quantitation

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as

a concentration that gives a signal-to-noise ratio of 2:1 or 3:1 (USP 2004, ICH Q2B guidelines, 1996, 1997, FDA, and Guidance for Industry 2000 [11-13]. The lower limit of detection for blonanserin is 5.08 µg/ml in reference material and formulation. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal to- noise ratio of 10:1 can be taken as LOQ of the method (USP 2004). The LOQ values were found to be 15.39 µg/ml for raw material, formulation.

CONCLUSION

A rapid, specific isocratic HPLC method has been developed for the determination of Blonanserin using a PDA detector. The method was validated for accuracy, precision, linearity, specificity & stability, LOD & LOQ and robustness. The method uses a simple mobile phase composition, easy to prepare with little or no variation. The rapid run time of 8 min and the relatively low flow rate allows the analysis of large number of samples with less mobile phase that proves to be cost-effective. Efficient UV detection at 237 nm was found to be suitable without any interference from excipients or solvents. The calibration curves were linear ($r \geq 0.9996$) over a concentration range from 10.0 to 150.0 µg/mL. The relative standard deviations were <2% and average recovery was 100.06%. LOD and LOQ were 5.08 ng/mL and 15.39 ng/mL respectively. Stability experiments indicated that the solutions prepared were stable for a period of 24 hours and the results obtained in this period were reliable. The proposed HPLC method is fast, precise, accurate, sensitive, and efficient and can be used in routine analysis in quality control laboratories.

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