Original Research Article

An Ultra Performance Liquid Chromatography-PDA Method for the Determination of a novel antipsychotic-Blonanserin in Bulk and its Tablet Dosage form

Running header: UPLC method for the estimation of Blonanserin

Abstract

To create a new ultra-high-resolution liquid chromatographic technology for estimating Blonanserin in APIs and tablets. The chromatographic separation was done using a DIKMA Endoversil (2.1 x 50mm, 1.7µm) column with a flow rate of 0.4 mL/min and an eluent monitored at 237 nm. The mobile phase was phosphate buffer, pH 4.2, and acetonitrile as a mobile phase (25:75) with a flow rate of 0.4 mL/min. The approach was validated in accordance with the principles set forth by the International Conference on Harmonization. Blonanserin was eluted with a retention time of 0.607 minutes in this procedure. Blonanserin calibration curve plots were shown to be linear for the concentration ranges of 1-75 µg/mL. The detection limit was 0.05 µg/mL, while the quantification limit was 0.25 µg/mL. Even though the current approach was determined to be effective in the analysis of Blonanserin in force degrading condition, the percent assay of the marketed dosage form was discovered to be 96.80%. The experiential evidences of all the study results revealed the suitability of the estimation of Blonanserin in API and tablet formulation.

Key Words: Blonanserin, UPLC, Method Development, Method Validation, ICH Guidelines.

1. INTRODUCTION

Blonanserin is a novel atypical antipsychotic[1] that belongs to a class of 4-phenyl-2-(1-piperazinyl) pyridines that acts as an antagonist at dopamine D2, D3, and serotonin 5-HT 2A receptors [2]. Blonanserin's safety and efficacy have been studied in schizophrenic and delirium patients, and it has been found to be effective and well tolerated in both conditions [3,4,5]. It has now emerged as a promising candidate for acute and maintenance therapy for schizophrenia, making it more widely accepted[2]. For the analysis of Blonanserin(chemical structure was cited in figure 1), having a stable, authentic, quick, and established analytical approach is critical.

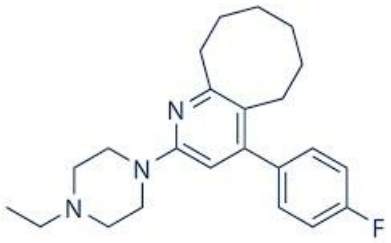

Figure 1. Chemical structure of Blonanserin

Blonanserin is not yet officially recognised by the I.P., B.P., USP, or any other pharmacopoeia, and there are only a few HPLC [6, 7], UV Spectrophotometric[8] methods for blonanserin analysis in pharmaceutical formulations, as well as a single bioanalytical LCMS/MS method for blonanserin and its metabolites in human plasma and urine[9,10, 11,12]. The mentioned HPLC procedures have their own set of constraints, such as detection limits, quantification and analysis times, and a low level of linearity. Blonanserin determination in bulk and tablet dosage form using ultraperformance Liquid chromatography has yet to be published. Ultra-performance liquid chromatography's advantages over high-performance liquid chromatography in terms of turnaround time, process dependability, method sensitivity, and drug specificity stimulate the use of LC techniques for a wide range of drug active chemical groups[13]. In order to comply with the International Conference on Harmonization (ICH) Guidelines Q2, the current research task aimed to eliminate all drawbacks and develop a fast, stability-indicating UPLC method for estimating isavuconazole in bulk drugs and capsule dosage forms, as well as a validation study (R1) [14].

2. MATERIALS AND METHODS

2.1.Chemicals and Reagents

Pharmaceutical grade working standards Blonanserin (99.93%) was procured from the by Hetero drugs limited, Hyderabad, India. The tablets (Valera- 5 mg Blonanserin, manufactured by Alkem pharmaceuticals, Sikkim, India) were purchased from the local market of Hyderabad, India. All required chemicals and reagents were purchased from Finer chemical Ltd, Fisher Scientific and Merck.

2.2.Instrumentation Conditions

The Blonanserin was analysed using Ultra performance liquid chromatography (UPLC) Acquity Waters, PDA detector. Software: Empower 2, equipped with auto sampler and PDA detector.The analytical column DIKMA Endoversil (2.1 x 50mm, 1.7µm) UPLC with the flow rate 0.4 ml/min (isocratic) was utilised. The analytical balance 0.1mg sensitivity (Afcoset ER-200A), pH meter (Adwa – AD 1020).

2.3.Preparation of 0.05 M phosphate buffer

About 6.8043 grams of potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 4.7 with orthophosphoric acid.

2.4.Preparation of mobile phase

Accurately measured 250 ml (25%) of phosphate buffer and 750 ml of HPLC grade acetonitrile (75%) were mixed well and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.5.Standard Solution Preparation

To get a 1 mg/mL concentration, weigh 25mg of Blonanserin working standard into a 25ml clean dry volumetric flask, add 10ml acetonitrile, sonicate to dissolve fully, and make volume up to the mark with the mobile phase. Blonanserin working solution was made by pipetting 1 mL of the foregoing stock solutions into a 100 mL volumetric flask and diluting to the mark with diluent to get 10 g/mL of Blonanserin.

2.6.Assay of marketed dosage form

The commercial formulation "Elicia-4" was used to test the proposed formulation procedure. Twenty tablets were weighed and triturated to a fine powder; the weight equivalent to 10 mg (i.e. 21.25 mg) was carefully weighed and transferred to a 100 mL clean dry volumetric flask. 10 mL acetonitrile, sonicated for 10 minutes to completely dissolve and bring volume up to the mark with the mobile phase It is then filtered via a 0.44 micron injection filter, which is used to create the stock solution. Using a micropipette, pipette 0.15 ml of the aforementioned stock solution into a 10ml volumetric flask and dilute to the mark with diluent. The autosampler was filled with 10 micro liters of standard and sample solution, which was then injected into the chromatographic system in triplicate. Blonanserin peak regions were measured, and the assay % was determined.

3. Method Validation

3.1.Specificity

It was performed using placebo interference test of the Blonanserin sample solution using 500 mg of placebo, which is equivalent to one tablet dissolved in 100ml of mobile phase and the placebo solution was treat like a standard solution. The solution was injected to the chromatographic system to assess the possible interfering peaks.

3.2. System suitability

It was conceded [8] to rationalize whether analytical system is running properly. It was carried out by injecting the six replicates of standard solution of Blonanserin. The %RSD of the a range of optimised parameters like peak area, theoretical plates, retention time and asymmetric factor were calculated.

3.3.Accuracy

To validate the accuracy of the present method recovery study was conducted at different levels (50%, 100%, and 150%) of pure Blonanserin. The amounts of standard Blonanserin was added to a fixed concentration to Blonanserin tablet sample solution to attain the various levels. This study [9] were carried out three times and the percentage recovery as well as percentage mean recovery was calculated.

3.4.Intra day & Inter day precision

The precision of the method was evaluated by analysing the six sample solutions in triplicate (n=6) of 10µg/mL of Blonanserin solution. The intra- and inter-day precision was determined by analysing for six times on the same day (intra-day study) and repeated on the second and also third day study (inter-day study). The chromatograms were recorded, peak area and retention time of Blonanserin was determined and relative standard deviation (RSD) was calculated.

3.5.Detection and Quantitation limit

The limit of detection is defined as a concentration for which a signal-to - noise ratio of 3 was obtained and a signal-to - noise ratio of 10 was considered to be for the limit of quantification. The standard solution of Blonanserin was prepared by chronological dilution to 0.05 and 0.25µg/mL and injected into the chromatographic system.

3.6.Linearity

Standard Blonanserin solution was prepared as stated earlier in order to conduct the linearity analysis, different volumes of aliquot from the standard solution were diluted with mobile phase to achieve different concentrations in the 1-75µg/ ml range of Blonanserin. In view of concentration versus peak area, the calibration curve was plotted for Blonanserin and the data obtained were subjected to regression analysis.

3.7.Robustness

Robustness of the developed method was studied[11] by deliberately changing the chromatographic condition. Six sample solutions were prepared and analysed in triplicate utilising the optimised condition by purposely varying the analytical conditions like flow rate, mobile phase ratio, and detection wavelength at three different levels. All the optimised parameter was found within the limit. For the calculation of percentage RSD the tailing factor was considered.

4.Force degradation study of Blonanserin

The Blonanserin solution was subjected to a force degradation research under ICH-specified stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic stress. All sorts of degradation investigations were carried out in triplicate, and the mean peak area was assessed to calculate the results.

4.1.Acid degradation

The acid degradation analysis was conducted at 60^0C and 75% relative humidity using 1 M HCL using an environmental test chamber (Acamus Technologies, India). 0.5 ml of stock solution of Blonanserin (1 mg / mL) was administered in a 10 ml volumetric flask, 0.5 ml of 1 M HCL was applied to the flask and held for 16 hours in the environmental test chamber. The solution was neutralized using 1 M NaOH after the suitable stress time and the mobile phase volume was composed, injected into the UPLC device.

4.2.Alkaline degradation

The experiment was carried out in the same climatic room at 600°C and 58 percent relative humidity. In a 10 ml volumetric flask, 0.5 ml of stock solution was combined with 1 M 0.5 ml of 1M NaOH and stored for 16 hours. The solution was neutralized with 1 M HCL after the appropriate stress duration, and the volume was made up to the mark with mobile phase before being injected into the UPLC system.

4.3.Oxidative degradation

At 40^0C, 75% relative humidity, it was carried out in the same environmental chamber. To conduct the oxidation of the studied sample, 6 percent H_2O_2 was used. For this reason, 0.5 ml of stock solution was taken in a 10 ml volumetric flask and 0.5 ml of 6 percent H_2O_2 was applied to the flask and held for 16 hours at 60^0C, eventually forming the volume up to the mobile phase mark and injected into the UPLC system.

4.4.Thermal degradation

It was done in an environmental chamber at 40°C with a relative humidity of 75% and in an oven at 105°C. For dry heat thermolysis, 0.5 ml of stock solution was accurately placed in a 10 ml volumetric flask and stored in the chamber for 144 hours, and 1 mg of dry drug in solid form was placed in an oven at 110^oC for 2 days.

4.5.Photolytic degradation

This study was carried out during the daytime in sunlight (60000-70000 lux) for the duration of 48 hr. Accurately 0.5 ml of Blonanserin stock solution was taken in a 10 ml volumetric flask and the volume was made up to the mark with mobile phase and analyzed.

6. RESULTS AND DISCUSSION

6.1. Optimization of the present method

In order to discover the optimal method for estimating Blonanserin in API and capsule format, various UPLC chromatographic settings were tried. Several variables, including mobile phase composition, column type, mobile phase pH, and diluents, were changed throughout the early studies. In order to acquire a suitable mobile phase composition for method optimization, various solvent (methanol, water, acetonitrile) and buffer (format buffer, acetate buffer, phosphate buffer) proportions were tested. Finally, acetonitrile and phosphate buffer (75:25) were used as the mobile phase, with a flow rate of 0.4 mL/min. Blonanserin was eluted with a low retention time and an outstanding peak shape. Blonanserin retention time was 0.607 minute with PDA detection at 236 nm, which is significantly less for the elution to be considered quicker. In compliance with ICH guidelines, the established technique was validated. The optimised chromatogram is shown in Figure 2.

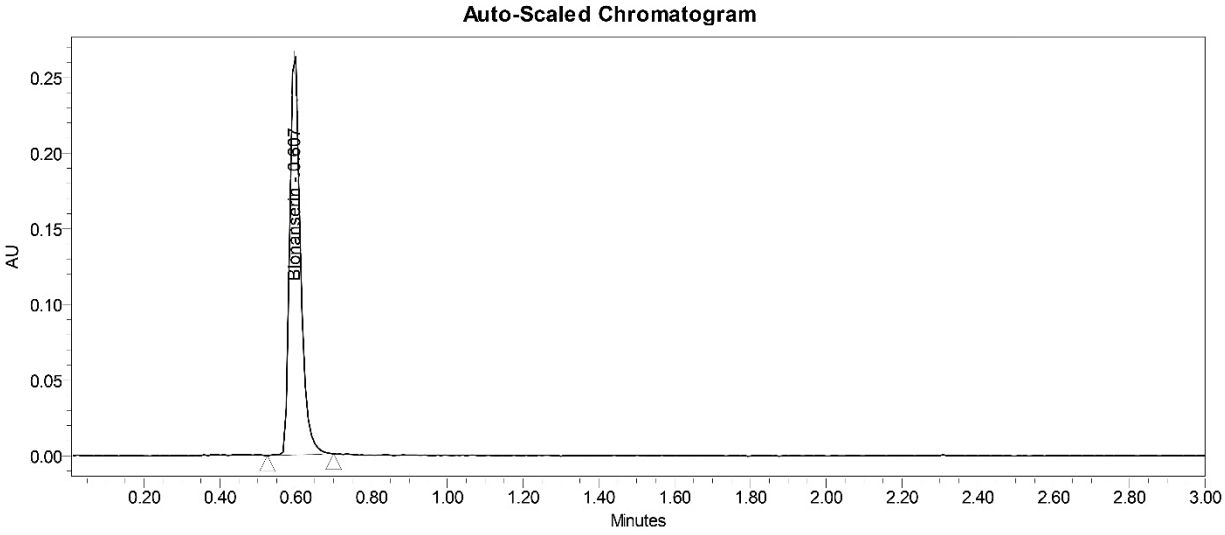

Figure 2. Optimized UPLC chromatogram of Blonanserin

6.2. Validation of the present method

A system suitability analysis was carried out to ensure the efficient functioning of the analytical measuring equipment by looking at many parameters (retention time, peak area, theoretical plate, tailing factor). The relative standard deviations of peak area, theoretical plates, tailing factors, and

retention duration were 0.76 %, 0.21 %, 1.87 %, and 1.23 %, respectively. It indicates the suitability of the system to carry out the present method. The results of the specificity analysis clearly showed that no excipient peaks were identified at the retention time of Blonanserin, proving the method's specificity for the present developed method. Standard quality control samples were used to determine the accuracy and precision of the established procedure. The linearity analysis was conducted in the concentration range of 1-75 µg/ml, and the correlation coefficient for the analyte Blonanserin listed in Table 1 was 0.998.

Table 1. Summary of the results of validation parameters

Parameters	Blonanserin
Linearity range (µg/ml)	1-75 µg/ml
Regression co-efficient	0.998
LOD µg/ml	0.05
LOQ µg/ml	0.25
% *Mean recovery (accuracy)	98.56
Intraday precision** (% RSD)	0.42
Inter-day precision** (% RSD)	0.89
% RSD of retention time* (robustness study)	0.61

*Average of three replicates. ** Average of six replicates

The acceptable correlation coefficient, which is close to 1, is used to create the regression line and find the linear curve in the method's linearity analysis. The results indicate the linearity of the developed method. The accuracy (mean percent recovery) was found to be 98.56, and the percent RSD was determined 1.21 which is less than 2%, The accuracy of the proposed method was confirmed after an accuracy study was conducted in accordance with the ICH criteria, and the % recovery was found to be within the permissible level, as discussed in the results and confirms the accuracy of the developed method. The percent RSD of the intraday and interday precision studies was 0.42 and 0.89, respectively, The precision (% RSD) of intraday and interday data was judged to be adequate and within acceptable bounds. The precision study's findings revealed that the suggested methodology was confirmed to be precise. Table 2 also included the results of the accuracy and precision investigation. Blonanserin has detection and quantitation limits of 0.05 µg/ml and 0.25 µg/ml, respectively, The acquired limit of detection and quantitation values demonstrated the proposed method's sensitivity. The method's robustness was tested by changing three parameters from the chromatographic conditions: mobile phase composition (2%), flow rate (0.1ml/min), and detection wavelength (2 nm), and the % RSD of the tailing factor, which was used as a tool parameter, was found to be 0.61 as shown in the table of validation parameters in

Table 1, indicating that the current established method for Blonanserin is robust, as no such significant changes were observed on the deliberate changes in the process parameters. The established optimized method for Blonanserin was used in succeeding validation experiments. The present method's applicability for the quantitative investigation of Blonanserin tablet dosage form is demonstrated by the percentage assay of 96.80 % in the marketed capsule dosage form. The assay result was found within the acceptance criteria. The chromatogram of the capsule dosage form is illustrated in Figure 3, and the results are listed in Table 2.

Table 2. Assay of Blonanserin marketed formulations

Blonanserin marketed formulation	Labelled claimed	Amount obtained*	Percentage purity of Blonanserin*
Blonanserin tablets (Valera) Contains Blonanserin 5 mg, by Alkem Pharmaceuticals limited, Sikkim, India.	5 mg	4.84 mg	96.80%

*average of three replicates

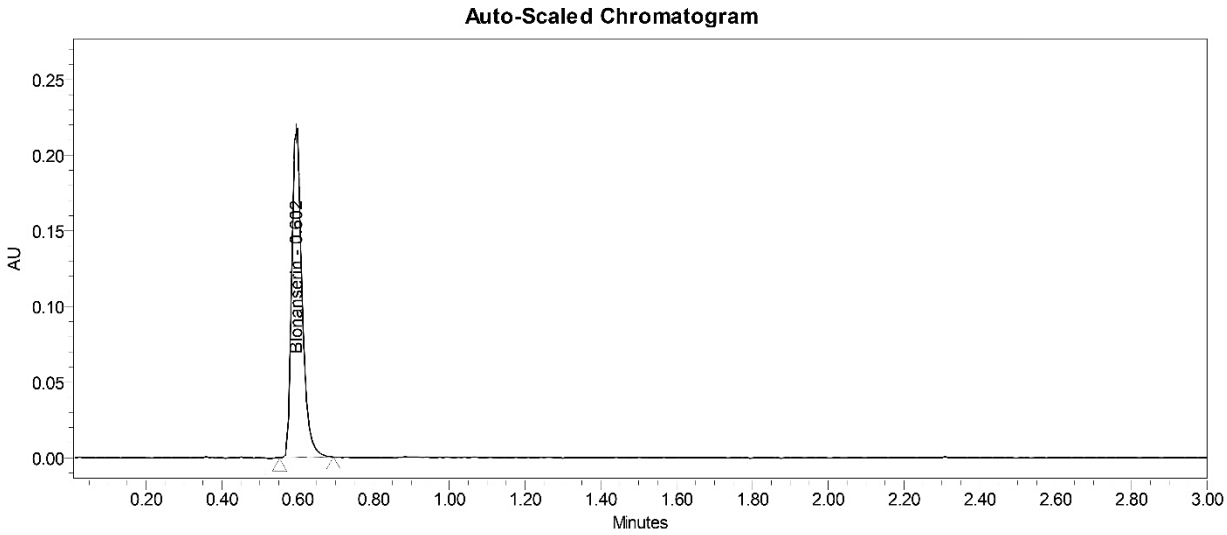

Figure 3. Assay of marketed dosage form of Blonanserin

Blonanserin degradation tests were carried out in a variety of stressful circumstances, including acid, alkali, oxidation, thermal, and photolytic environments. Almost all stressed conditions, with the exception of photolytic stressed conditions, showed degradation. Degradation is 7.20 % in acidic strained conditions, 6.92 % in alkaline stressed conditions, and 3.82 % in peroxide stressed conditions. The thermal degradation was found to be 5.38 %, with the results summarized in Table 3 and chromatograms illustrated in Figure 4. According to the results of Blonanserin force

degradation investigations, acidic and peroxide stressed conditions cause slightly higher degradation than other stressed conditions, however photolytic conditions cause no degradation. The results of the force degradation study indicates that in every stressful scenario, the chromatogram of Blonanserin was shown to be exceedingly specific.

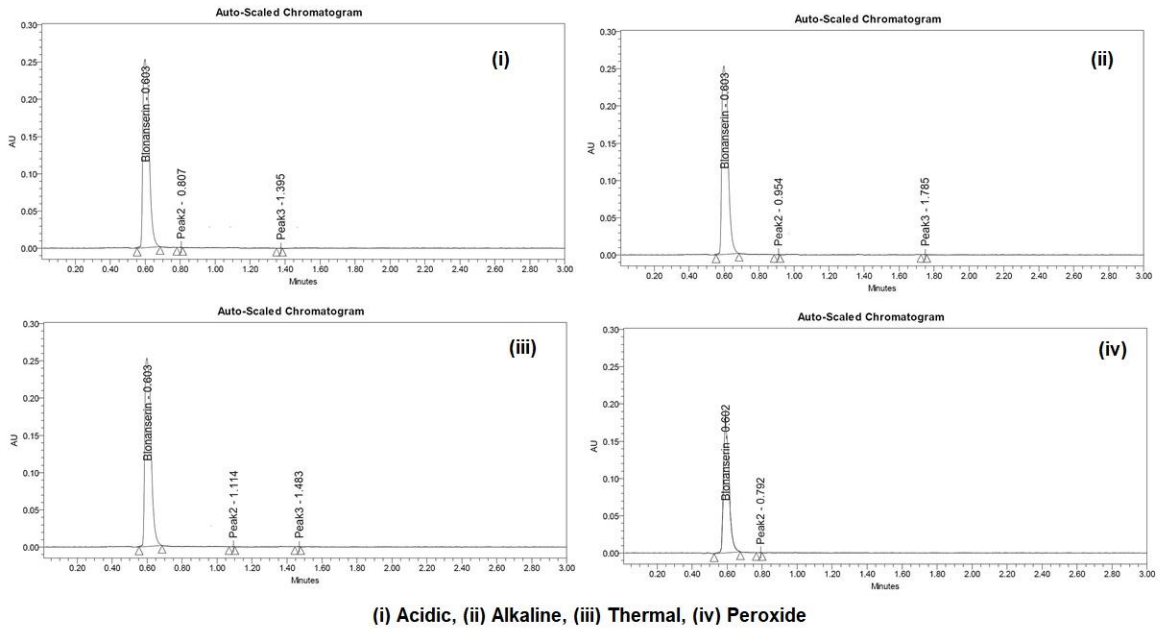

Figure 4. Force degradation chromatograms of Blonanserin in various stressed conditions.

Table 3. Degradation results for Blonanserin solution

Stressed condition	Blonanserin				
	Mean Area*	% Degraded	Purity Angle	Purity Threshold	Peak purity
Acid	317752	7.20	1.83	2.64	Passes
Base	402632	6.92	0.99	1.89	Passes
Peroxide	381739	3.82	0.83	1.66	Passes
Photo	454861	0.11	0.19	1.39	Passes
Thermal	309539	5.38	0.64	2.70	Passes

7.CONCLUSION

Based on the empirical evidence of the current established UPLC approach for Blonanserin estimation, the authors assert that the method is unique among the few HPLC methods currently available. The current UPLC method is referred to as "fast" because it drastically reduced overall analysis time to 0.607 minutes, which is the shortest time necessary for analysis. Because there was less deterioration in strained situations and excellent separation of Blonanserin from the other deteriorated peaks, the current approach is "stability suggesting." The validation parameters' findings were evaluated and confirmed to meet the ICH Q2B guidelines' acceptance criteria. As a result, the newly discovered approach can be used as an innovative, reliable, and validated method for routine analytical and quality control assays of Blonanserin in both bulk and tablet dosage forms.

ABBREVIATIONS

UPLC: Ultra performance liquid chromatography; **ICH:** International conference on harmonization; **PDA:** Photo diode array; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **SD:** Standard deviation; **RSD:** Relative standard deviation. **API**: Active Pharmaceutical ingredient.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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