



# **An Ultra Performance Liquid Chromatography-PDA Method for the Determination of a Novel Antipsychotic-Blonanserin in Bulk and its Tablet Dosage Form**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

To develop a new ultra-high-resolution liquid chromatographic technique for determining Blonanserin concentrations in APIs and tablets. A DIKMA Endoversil (2.1 x 50mm, 1.7µm) column with a flow rate of 0.4 mL/min and an eluent measured at 237 nm was used for the chromatographic separation. Phosphate buffer, pH 4.2, and acetonitrile (25:75) were used as the mobile phase, with a flow rate of 0.4 mL/min. The method was approved in accordance with the principles established by the International Conference on Harmonization. Blonanserin was eluted in this process with a retention time of 0.607 minutes. Blonanserin calibration curve plots were shown to be linear for the concentration ranges of 1-75 µg/mL. The quantification limit was 0.25 µg/mL, while the detection limit was 0.05 µg/mL. Despite the fact that the current method was found to be effective in the analysis of Blonanserin in a force degrading state, the percent assay of the marketed dosage form was found to be 96.80%. All of the study results' experience pieces of evidence demonstrated the usefulness of Blonanserin estimation in API and tablet formulation.

**Keywords:** *Blonanserin; UPLC; method development; method validation; ICH guidelines.*

## ABBREVIATIONS

*UPLC: Ultra performance liquid chromatography;*

*ICH: International conference on harmonization;*

*PDA: Photo diode array;*

*LOD: Limit of detection;*

*LOQ: Limit of quantitation;*

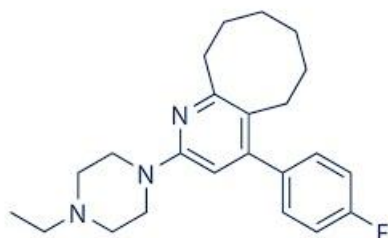
*SD: Standard deviation;*

*RSD: Relative standard deviation.*

*API: Active Pharmaceutical ingredient.*

## 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 D<sub>2</sub>, D<sub>3</sub>, and serotonin 5-HT<sub>2A</sub> receptors [2]. Blonanserin's safety and efficacy have been studied in schizophrenic and delirium patients, and it is effective and well-tolerated in both conditions [3,4,5]. Blonanserin can improve some types of cognitive associated with prefrontal cortical function in patients with first-episode and chronic schizophrenia. It has now emerged as a promising candidate for acute and maintenance therapy for schizophrenia, making it more widely accepted [2]. Therefore there is a need to have a reliable accurate and validated method for the estimation of Blonanserin using ultra fast technique. For the analysis of Blonanserin (chemical structure was cited in figure 1), having a stable, authentic, quick, and established analytical approach is critical.



**Fig. 1. Chemical structure of Blonanserin**

Blonanserin is not yet officially recognised by the I.P., B.P., USP, or any other pharmacopeia, 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 ultra-performance Liquid chromatography is yet to be studied. The benefits of ultra-performance liquid chromatography over high-performance liquid chromatography in terms of turnaround time, process dependability, method sensitivity, and drug specificity encourage the use of LC techniques to a wide range of drug active chemical groups [13]. 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), in order to comply with the International Conference on Harmonization (ICH) Guidelines Q2 [14].

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Pharmaceutical grade working standards Blonanserin (99.93%) was procured from 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

Blonanserin was analysed using Acquity Waters' PDA detector and Ultra Performance Liquid Chromatography (UPLC). Empower 2 is a software package that includes an auto sampler and a PDA detector. The DIKMA Endoversil (2.1 x 50 mm, 1.7 µm) UPLC analytical column was used, with a flow rate of 0.4 ml/min (isocratic). pH metre (Adwa – AD 1020), analytical balance with 0.1 mg sensitivity (Afcoset ER-200A).

### 2.3 Preparation of 0.05 M Phosphate Buffer

6.8043 grammes potassium dihydrogen orthophosphate was weighed and put to a 1000 mL beaker, where it was dissolved and diluted with HPLC water to 1000 mL. Orthophosphoric acid was used to bring the pH down to 4.7.

## 2.4 Preparation of Mobile Phase

250 mL of phosphate buffer (25%) and 750 mL of HPLC grade acetonitrile (75%) were accurately measured, thoroughly mixed, and degassed in an ultrasonic water bath for 10 minutes before being filtered through a 0.45 filter under vacuum filtration.

## 2.5 Standard Solution Preparation

25mg of Blonanserin working standard was weighted and transferred into a 25ml clean dry volumetric flask for the manufacture of a 1 mg/mL concentration of standard solution. 10 mL acetonitrile was added, sonicated to dissolve completely, and the mobile phase was added to bring the volume up to the mark. Pipetting 1 mL of the foregoing stock solutions into a 100 mL volumetric flask and diluting to the mark with diluent yielded 10µg/mL of Blonanserin working solution.

## 2.6 Assay of Marketed Dosage Form

The proposed formulation technique was tested using the commercial formulation "Elicia-4." Twenty pills 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 dissolve completely and bring volume up to the mobile phase's mark. The stock solution was then created by filtering it through a 0.44 micron injection filter. Pipette 0.15 ml of the aforementioned stock solution into a 10ml volumetric flask using a micropipette and dilute to the mark using diluent. The auto sampler 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.

## 2.7 Method Validation

### 2.7.1 Specificity

The Blonanserin sample solution was tested using a placebo interference test with 500 mg of a placebo, which is equivalent to one tablet dissolved in 100ml of mobile phase, and the placebo solution was treated like a standard solution. To see if there were any interfering peaks, the solution was put into the chromatographic apparatus.

### 2.7.2 System suitability

It was agreed to rationalise whether or not the analytical system is functioning correctly. It was done by injecting a standard solution of Blonanserin into each of the six replicates. The percent RSD of a range of optimal parameters was calculated, including peak area, theoretical plates, retention duration, and asymmetry factor.

### 2.7.3 Accuracy

A recovery study was undertaken at several levels (50 percent, 100 percent, and 150 percent) of pure Blonanserin to validate the accuracy of the present approach. To achieve the various levels, standard Blonanserin was added to a Blonanserin tablet sample solution at a predetermined concentration. This study was repeated three times, with the % recovery and percentage mean recovery calculated each time.

### 2.7.4 Intra day & Inter day precision

The precision of the procedure was tested by analysing six sample solutions of 10g/mL Blonanserin solution in triplicate (n=6). The intra- and inter-day precision were established by analysing six times on the same day (intra-day study) and then repeating the process on the second and third days of the study (inter-day study). Blonanserin's peak area and retention time were determined, and the relative standard deviation (RSD) was estimated using the chromatograms.

### 2.7.5 Detection and Quantitation limit

The limit of detection is defined as a concentration for which a signal-to-noise ratio of 3 was obtained, whereas the limit of quantification was set as a signal-to-noise ratio of 10. Blonanserin standard solutions were generated by dilution to 0.05 and 0.25 g/mL and then injected into the chromatographic apparatus.

### 2.7.6 Linearity

To conduct the linearity study, several amounts of an aliquot from the standard solution were diluted with mobile phase to achieve different concentrations of Blonanserin in the 1-75g/ml range. Blonanserin's calibration curve was plotted based on concentration against peak area, and the data acquired was subjected to regression analysis.

### 2.7.7 Robustness

The robustness of the new approach was investigated<sup>11</sup> by changing the chromatographic condition purposely. Six sample solutions were produced and analysed in triplicate using the optimised conditions, which included adjusting the flow rate, mobile phase ratio, and detection wavelength at three distinct levels. All of the parameters that were optimised were determined to be within the limit. The tailing component was taken into account while calculating the percentage RSD.

### 2.7.8 Force degradation study of Blonanserin

The Blonanserin solution was put through its paces under ICH-mandated stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic stress. All types of deterioration tests were done in triplicate, and the mean peak area was calculated to calculate the results.

### 2.7.9 Acid degradation

An environmental test chamber was used to conduct the acid degradation analysis at 60°C and 75% relative humidity using 1 M HCl (Acamus Technologies, India). In a 10 ml volumetric flask, 0.5 ml of Blonanserin (1 mg / mL) stock solution was added, followed by 0.5 ml of 1 M HCl, and the flask was left in the environmental test chamber for 16 hours. After the appropriate stress time, the solution was neutralised with 1 M NaOH, and the mobile phase volume was composed and injected into the UPLC equipment.

### 2.7.10 Alkaline degradation

The experiment was conducted in the same climatic room with a temperature of 60°C and a relative humidity of 58 percent. 0.5 ml of stock solution was mixed with 1 M 0.5 ml of 1M NaOH in a 10 ml volumetric flask and kept for 16 hours. After the necessary stress time, the solution was neutralised with 1 M HCl, and the volume was made up to the mark with the mobile phase before being injected into the UPLC system.

### 2.7.11 Oxidative degradation

The experiment was carried out in a controlled environment with a temperature of 60°C and a relative humidity of 58 percent. 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 neutralised with 1 M HCL after the required stress time, and the volume was made up to the mark with the mobile phase before being injected into the UPLC system.

### 2.7.12 Thermal degradation

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

### 2.7.13 Photolytic degradation

This study took place during the day in direct sunlight (60000-70000 lux) over 48 hours. Blonanserin stock solution was accurately 0.5 mL in a 10 mL volumetric flask, which was then filled to the mark with mobile phase and examined.

## 3. RESULTS AND DISCUSSION

### 3.1 Optimization of the Present Method

Various UPLC chromatographic settings were attempted to find the best method for estimating Blonanserin in API and capsule form. Several variables were adjusted throughout the early investigations, including mobile phase composition, column type, mobile phase pH, and diluents. Various solvent (methanol, water, acetonitrile) and buffer (format buffer, acetate buffer, phosphate buffer) proportions were investigated in order to get an appropriate mobile phase composition for method optimization. Finally, with a flow rate of 0.4 mL/min, acetonitrile and phosphate buffer (75:25) were utilised as the mobile phase. Blonanserin was eluted with an excellent peak shape and a short retention duration. Blonanserin retention time was 0.607 minutes with PDA detection at 236 nm, which is much less than what is needed to deem the elution to be faster. The established technique was validated in accordance with ICH criteria. Fig. 2 depicts the optimised chromatogram.

### 3.2 Validation of the Present Method

A system suitability analysis was performed to assure the analytical measuring equipment's efficient operation by looking at a variety of criteria (retention time, peak area, theoretical

plate, tailing factor). Peak area, theoretical plates, tailing factors, and retention length all had relative standard deviations of 0.76 percent, 0.21 percent, 1.87 percent, and 1.23 percent, respectively. It demonstrates the system's suitability for carrying out the current technique. The specificity analysis revealed that no excipient peaks were detected at the Blonanserin retention period, demonstrating the method's specificity for the current developed approach. To determine the accuracy and precision of the described technique, standard quality control samples were used. The linearity analysis was carried out in the concentration range of 1-75 µg/ml, with a correlation coefficient of 0.998 for the analyte Blonanserin (Table 1).

In the method's linearity analysis, the acceptable correlation coefficient, which is close to 1, is used to generate the regression line and identify the linear curve. The results show that the developed approach is linear. The accuracy (mean percent recovery) was found to be 98.56 percent, and the percent RSD was found to be 1.21 percent, which is less than 2%. The proposed method's accuracy was confirmed after an accuracy study was conducted using ICH criteria, and the percent recovery was found to be within the acceptable threshold, as discussed in the results, confirming the developed method's accuracy. The intraday and interday precision investigations had % RSDs

of 0.42 and 0.89, respectively. Intraday and interday data were deemed to have a precision (percent RSD) of within the acceptable limits. The findings of the precision study revealed that the suggested methodology was found to be precise. The results of the accuracy and precision inquiry were also presented in Table 2. The detection and quantitation limits for blonanserin are 0.05 g/ml and 0.25 g/ml, respectively. The proposed method's sensitivity was demonstrated by the obtained limit of detection and quantitation values. The method's robustness was tested by changing three chromatographic conditions: mobile phase composition (2%), flow rate (0.1ml/min), and detection wavelength (2 nm), and the percent RSD of the tailing factor, which was used as a tool parameter, was found to be 0.61 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. In subsequent validation studies, the known optimised procedure for Blonanserin was applied. The percentage assay of 96.80 percent in the marketed capsule dosage form demonstrates the applicability of the present method for the quantitative study of Blonanserin tablet dosage form. The assay result was confirmed to be within acceptable limits. Figure 3 shows the chromatogram of the capsule dosage form, with the results provided in Table 2.

**Table 1. Summary of the results of validation parameters**

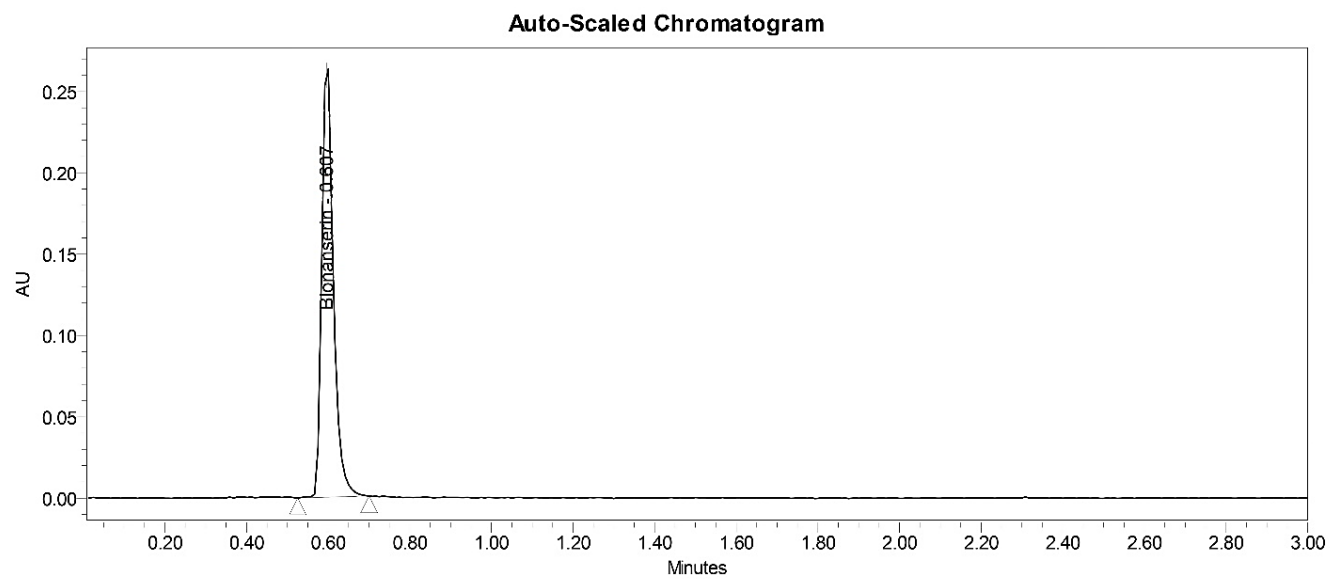
Parameters	Blonanserin
Linearity range (µg/ml)	1-75 µg/ml
Regression co-efficient	0.998
LOD µg/ml	0.05
LOQ µg/ml	0.25
% *Mean recovery (accuracy)	98.56
Intraday precision** (% RSD)	0.42
Inter-day precision** (% RSD)	0.89
% RSD of retention time* (robustness study)	0.61

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

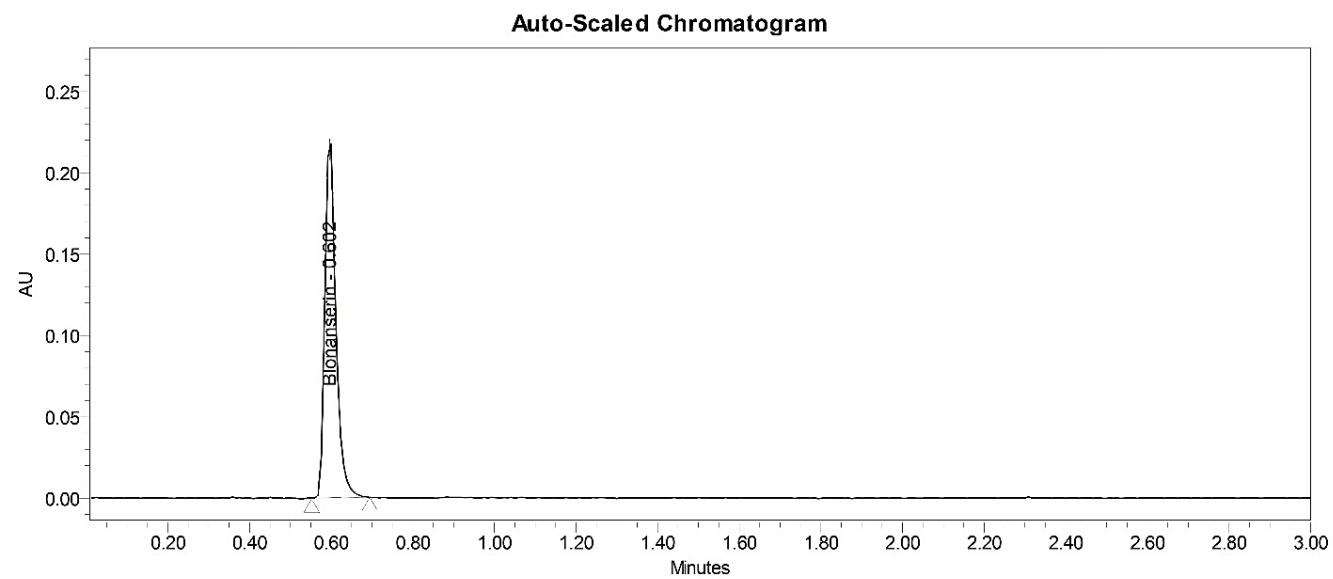
**Table 2. Assay of Blonanserin marketed formulations**

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%

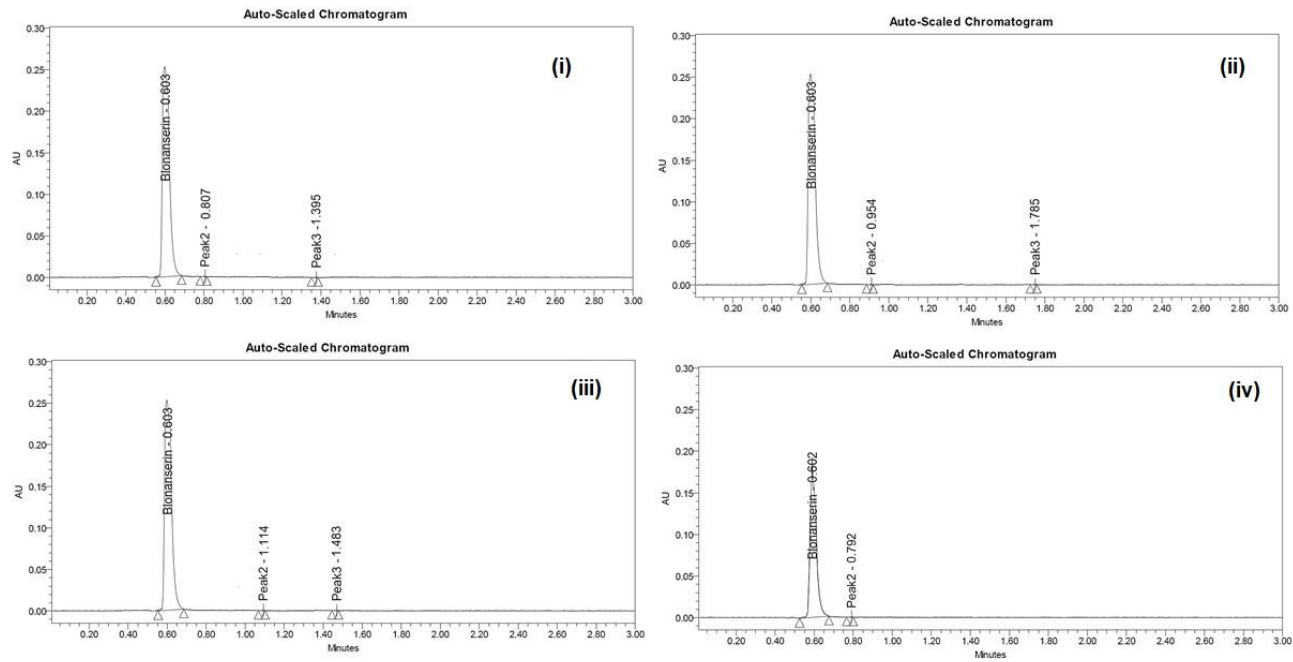
\*average of three replicates



**Fig. 2. Optimized UPLC chromatogram of Blonanserin**



**Fig. 3. Assay of the marketed dosage form of Blonanserin**



(i) Acidic, (ii) Alkaline, (iii) Thermal, (iv) Peroxide

**Fig. 4. Force degradation chromatograms of Blonanserin in various stressed conditions**



**Table 3. Degradation results for Blonanserin solution**

Stressed condition	Blonanserin		Purity Angle	Purity Threshold	Peak purity
	Mean Area*	% Degraded			
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

Blonanserin degradation studies were conducted in a variety of demanding conditions, including acid, alkali, oxidation, thermal, and photolytic. With the exception of photolytic stressed situations, almost all strained conditions degraded. In acidic strained settings, degradation is 7.20 percent, 6.92 percent in alkaline stressed conditions, and 3.82 percent in peroxide stressed conditions. The thermal deterioration was found to be 5.38 percent, as shown in Table 3 and the chromatograms in Fig. 4. Blonanserin force degradation experiments show that acidic and peroxide stressed environments induce slightly more degradation than other stressed conditions, whereas photolytic conditions had no effect. The findings of the force degradation investigation reveal that the chromatogram of Blonanserin is extremely specific in every stressed circumstance.

#### 4. CONCLUSION

The authors claim that the current established UPLC methodology for Blonanserin estimate is unique among the few HPLC methods now available, based on empirical evidence. The present UPLC approach is referred to as "quick" because it significantly reduced overall analysis time to 0.607 minutes, the least time required for analysis. The current technique is "stability indicating" since there was less deterioration in stressful settings and excellent separation of Blonanserin from the other deteriorated peaks. The outcomes of the validation parameters were examined and confirmed to meet the acceptance criteria of the ICH Q2B standards. As a result, the newly developed method can be used as a unique, dependable, and validated method for routine Blonanserin analytical and quality control tests in both bulk and liquid form.

#### DISCLAIMER

The products used for this research are commonly and predominantly used 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 the personal efforts of the authors.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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