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EXPERIMENTAL DESIGN BASED OPTIMIZATION OF HPLC METHOD FOR DETERMINATION OF BLONANSERIN IN *IN-VITRO* HUMAN SERUM SAMPLE, FORCED DEGRADED SAMPLE AND PHARMACEUTICAL FORMULATIONS

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Keywords:

Blonanserin, Box-Behnken, Plan of Experiments, Human Blood Serum, Formulation Analysis, Stability Studies

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ABSTRACT: Paper presents a novel high performance liquid chromatography method development, optimization, validation and application of determining blonanserin in bulk drug, its formulation, human serum and in the presence of its degradation products. Selection of important factors which may influence separation were concluded: percentage of acetonitrile in the mobile phase, the percentage of orthophosphoric acid in aqueous phase and flow rate. Factors selected were altered in accordance to Box-Behnken design plan and investigation in factors having significant influence on separation were executed. Finally, the choicest values from solution offered of investigated factors were 43 % of acetonitrile in the mobile phase, 0.03% orthophosphoric acid in water and flowrate of 0.75 ml/min. The method validation was performed according to International Conference on Harmonisation guideline acceptance criteria for robustness, linearity, selectivity, precision and accuracy. Quantitative and qualitative system responses have not significantly affected by little variations in chromatographic parameters, which demonstrates robustness of the method. Limits of quantification and detection for blonanserin was calculated. Method shows excellent linearity, which was confirmed by r^2 = 0.9991 over the concentration range of 5 – 50 µg/ml. Accuracy was calculated by recovery experiment and found to be in range 99.44–100.76 %. Precision was carried out at different levels: analysis repeatability, injection repeatability and intermediate precision and was found well under acceptable criteria. Furthermore, the applicability of method was analysed in real time sample analysis of blonanserin in its commercial pharmaceutical preparations and *in- vitro* human serum samples.

INTRODUCTION: Schizophrenia is a neuropsychiatric disorder generally occurs in early adulthood or late adolescence and results occupational or social dysfunction with increase rate of mortality [1].

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Blonanserin, chemically represented as (2- (4-ethylpiperazin-1-yl) -4- (4-fluorophenyl) -5, 6, 7,8, 9, 10-hexahydrocycloocta [b] pyridine; **Fig. 1)** belongs to a series of phenyl-2-(1-piperazinyl) pyridines and antagonizes dopamine D2, D3 and serotonin 5-HT2A receptors [2].

It is antipsychotic second-generation drug prescribed in treatment of schizophrenia having an experimental solubility of 10 mg/ml in methanol and 7.66 as its PKa value [3–5]. It displays higher affinity compared to haloperidol [6] and risperidone [7] for D2 receptors.

In lower consumption of reagents and saves time, it also permits development of mathematical model which allows to evaluate the interaction between the significant factors [9]. Multivariate optimization allows us to study effect of all variables simultaneously as there may be chance that the level of one variable may be depends on other, and that's in turn limitation of univariate optimization. By using multi-variation experimental designs such as Box-Behnken design the optimized conditions were attained [10].

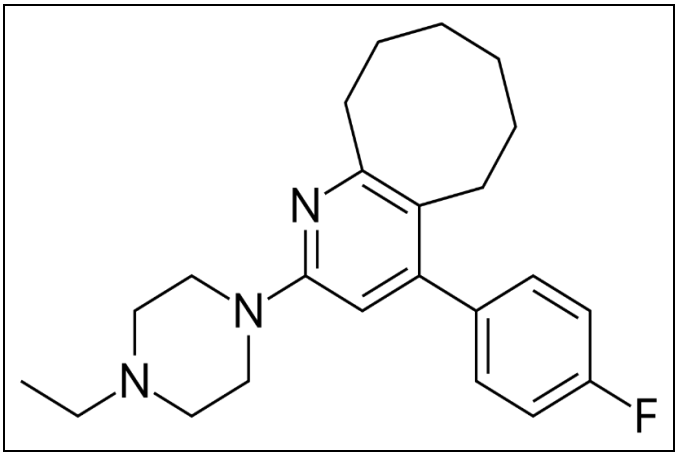


FIG. 1: CHEMICAL STRUCTURE OF BLONANSERIN

To date procedures for determination of blonanserin [11–15] along with is degradant product [16–19] and in biometrics [20–23] using HPLC had been reported. In addition to this GC method for residual solvent [24, 25] determination in blonanserin and blonanserin determination in human plasma [26] also been reported. Caiyan et al., Modi et al., Xiang ping et al., Ping and Patel et al. presented an analytical procedures using HPLC with limited scope for determination of only blonanserin and not its degradation product, moreover except Modi et al. all of them used high concentration of phosphate buffer (10 – 25 mM). Huawei et al., Ahir et al. and Mondal et al. had cited the stability indicating assay methods while Kalariya et al. provides a distinct advantage of degradant characterization but all of them uses high quantity of organic solvent in mobile phase (65- 90 %) also Huawei et al. uses ion paring agent which made the respective analytical methods costly.

In addition to this Kalariya et al. stated that blonanserin is thermally stable. The Mondal et al. analytical procedure is specific stability indicating assay method [27]. Of these published methods not a single one can be used for determination of blonanserin in both formulation and human serum also none of them used quality by design approach for optimization.

On reviewing of literature, we learnt there is need and indeed objective of the presented study is to conduct stress degradation studies according to ICH [28–30] prescribed condition along with the development and desirability based optimization of a selective stability indicating assay method for blonanserin and its degradation products estimation using high performance liquid chromatography moreover, the validation of developed method and its application in analysis of blonanserin in its tablet formulations and in in-vitro human serum sample.

The scientific novelty of stated work includes qualitative and quantitative estimation of blonanserin and its degradation product formed after exposure to heat and use of newer, less complex, cost efficient solvent system using acetonitrile and orthophosphoric acid in aqueous medium. Furthermore, it's a singular approach where analytical method optimization was carried out using Derringer and Suich [31] desirability function after performing multivariate analysis using Box-Behnken plan of experiment for determination of blonanserin along with its degradants also same method was used to estimate blonanserin in tablet formulation and in-vitro human serum sample.

MATERIAL AND REAGENTS: HPLC grade acetonitrile and methanol were purchased from Merck Specialties Private Limited (Mumbai, India); Type I water was obtained from Millipore water purification system (Milford, MA, USA). The purchase of ortho -phosphoric acid was carried out from Merck Specialties Private Limited (Mumbai, India) while sodium hydroxide, hydrochloric acid, hydrogen peroxide solution was acquired from Loba Chemie Pvt. Ltd. (Mumbai, India) and all of these materials were of analytical grade. Blonanserin was certified to have a purity of 99.9% and the commercial blonanserin tablets Blonitas® 8 mg (Intas Pharmaceuticals, Dehradun, India) and Elicia® 8 mg (Zydus Cadila Healthcare Ltd, Gujrat, India) were purchased from local market.

Instrumentation and chromatographic conditions: HPLC analysis of blonanserin was performed using a Shimadzu semi-preparative HPLC instrument (Shimadzu Corporation, Tokyo, Japan) with a LC-6AD/7A Pump, connected with an SPD-M20A photo diode array detector (PDA) which is set at 330 nm for UV detection and controlled using CBM-20A system controller. The injection was performed on a manual Rheodyne injector (Model 7725i, Oak Harbor, WA, USA) fitted with a 20µl loop and a 20 µl Hamilton syringe. The instrument was connected to a Hewlett-Packard® compatible PC, an HP® Inkjet printer and bundled with Shimadzu Lab Solution Lite® release 5.52 software on which analysis and data collection were performed. Chromatographic separation was achieved on a Inertsil® ODS-4 (3µm, 4.6 X 150 mm) column (GL Sciences Inc., Japan) maintained at ambient temperature 25^{O}C. Based on experimental design, the mobile phase 43% v/v acetonitrile: 57% v/v water (0.03% ortho phosphoric acid) pumped into an isocratic elution system at a flow rate of 0.75 ml/min. The mobile phase was previously filtered using a 0.2 µm filter and degassed using sonicator.

Methods:

Standard preparation: Blonanserin stock solution of concentration 1 mg/ml was prepared by dissolving 10.01 mg (corrected for purity 10 mg) of blonanserin in methanol. The solution was sonicated for 15 minutes and filtered through 0.2 µm nylon filter. A concentration of 10µg/ml for analysis was prepared by diluting 100 µl of the blonanserin stock solution with 9900 µl of the methanol: water (50: 50, v/v) which was used as diluent throughout the study.

Forced degradation studies: Forced decomposition studies were carried out at drug concentration of 1mg/ml. All stress samples were prepared using co- solvency except in acidic condition. Acidic and basic hydrolysis were performed in 0.5 M hydrochloric acid and sodium hydroxide at 70°C for 7 hrs, after that solution were carefully neutralized. Oxidative study was carried out at room temperature in 0.3 % hydrogen peroxide for 12 hrs. Stress study in neutral condition, drug which initially dissolved in 0.5 ml methanol and further diluted up to 10 ml using water was refluxed for 48 hrs.

For photo and thermal degradation, two portion of blonanserin powder were spread as thin film in two separates petri dishes. One portion was kept in oven at temperature of 105°C for 12 hrs while another portion was exposed to a UV lamp producing UVA radiation for 7 days. All solution was further diluted using diluent to obtain concentration of 10µg/ml.

Preliminary study and method optimization: Development of a new HPLC method was carried out using mobile phase composition containing acetonitrile and water (0.03% ortho phosphoric acid) and C18 column. The diluent for preparing working standard was varied from acetonitrile, water, methanol, mobile phase, composition of acetonitrile water, methanol water and the composition of water and methanol in ratio of 1:1 was finalised as diluent. Optimization and for that experiments were performed according to the plan generated by Box- Behnken design. To evaluate interaction and quadratic effects of flow rate, % content of acetonitrile on mobile phase and % content of ortho- phosphoric acid in aqueous part of mobile phase on retention time and tailing factor a three level design was applied. Design Expert® 10.0 software, Trial version (Stat Ease Inc., Minneapolis, MN, USA) is used to perform the experimental design and statistical analysis.

Assay of tablet dosage form: A commercially available blonanserin tablets Blonitas® and Elicia® were weighted and 20 tablets were triturated. Sample equivalent to 10 mg of blonanserin from each formulation were taken in a two different 10 ml volumetric flask in which methanol was added, resultant was vortexed and subjected to sonication for 15 minutes. After sonication it were made to mark using methanol, vortexed and filtered. Appropriate dilutions with diluent of both formulations were carried to obtained solution having concertation 10µg/ml and analysed according to optimized chromatographic conditions.

Sample preparation for serum (*In- vitro* study): Serum was procured from local blood bank and stored at -20^{o}C. After attaining room temperature, the serum was spiked at a concentration 10 µl/ml of blonanserin stock solution. The spiked serum the underwent protein precipitation using acetonitrile

which was added at three-fold volume of spiked serum, further it was centrifuged at 10, 000 rpm for 15 min. The supernatants from treatment were separated and evaporated using nitrogen gas at 40°C; after evaporation it was reconstituted using diluent and analysed in HPLC system.

Method validation: Validation of method was carried out in alignment with the ICH guideline [30].

Robustness: Intended but small variation in selected chromatographic parameters was made to evaluate the robustness of method. Blonanserin at the concertation of 10µg/ml was injected at changed chromatographic conditions and mean peak area and retention time were monitored.

Linearity and range: Linearity is studied over the concentrations range of 5- 50 µg/ml at eight different concentrations. The working solution of eight different concentrations for preparing calibration curve in range of 5- 50 µg/ml were prepared by diluting stock solution. Three replicate injections of each concentration were made every day to determine the linearity of blonanserin over the concentration range. Peak area verses drug concentration was plotted using linear least square regression and evaluated for inter day linearity.

Precision and accuracy: Precision was studied at three level: analysis repeatability was determined by preparing and injecting six sample solution; whereas intermediate precision was tested with same procedure on different chromatographic system on another day by another analyst with different set of reagents. Injection repeatability was tested with ten injection of the same sample solution. Accuracy of the method was evaluated by performed by adding known concentration of blonanserin to the tablet sample. Accuracy and precision were determined by analysing the mean, standard deviation and relative standard deviation.

Limit of detection and quantification: The limit of detection (LOD) was defined as the lowest concertation of blonanserin resulting in a signal to noise ratio of 3:1 and limit of quantification (LOQ) was expressed as a signal to noise ratio of 10:1.

RESULTS AND DISCUSSION: Method development was performed on the basis of trial and error basis. At start methanol and water at different composition was tried; this approach at any tried composition unable to separate blonanserin and its degradation. Also use of different salts i.e. ammonium acetate, ammonium formate, ammonium phosphate at concertation's ranging from 0.5-40 mM and pH 2.9- 5.8 doesn't provide required separation. The use of acetonitrile and water (0.05%, v/v ortho phosphoric acid) allows separation of blonanserin and its degradation. At the point the tailing factor is high and resolution is very low between peaks. The use of basic c18 column of small particle size allowed control in peak tailing under acceptable limit.

The diluent for preparing working standard was varied from acetonitrile, water, methanol, mobile phase, composition of acetonitrile water, methanol water and the composition of water and methanol in a ratio of 1:1 was finalized as diluent as it also helps in controlling tailing factor. Further optimization and for that experiments were performed according to the plan generated by Box-Behnken design. To evaluate interaction and quadratic effects of flow rate, % content of acetonitrile on mobile phase and % content of ortho- phosphoric acid in aqueous part of mobile phase on retention time and tailing factor a three level design was applied. Design Expert® 10.0 software, Trial version (Stat Ease Inc., Minneapolis, MN, USA) is used to perform the experimental design and statistical analysis.

Preliminary study: As discussed formerly, blonanserin is a typically new anti-psychotic substance on which there are few papers published dealing with its investigation by use of high performance liquid chromatography methods. After review of literature and studying physiochemical parameter, structure a theoretical consideration was developed and thereafter preliminary study was carried on. Method development was performed on the basis of trial and error basis. At start methanol and water at different composition was tried; this approach at any tried composition unable to separate blonanserin and its degradation. Also use of different salts i.e. ammonium acetate, ammonium formate, ammonium phosphate at concertation's ranging from 0.5-40 mM and pH 2.9- 5.8 doesn't provide required separation.

The use of acetonitrile and water (0.05%, v/v ortho phosphoric acid) allows separation of blonanserin and its degradation. At the point the tailing factor is high and resolution is very low between peaks. The use of basic c18 column of small particle size allowed control in peak tailing under acceptable limit. The diluent for preparing working standard was varied from acetonitrile, water, methanol, mobile phase, composition of acetonitrile water, methanol water and the composition of water and methanol in a ratio of 1:1 was finalized as diluent as it also helps in controlling tailing factor. Further on, experimental design was adapted for studying experimental parameter and achieving optimum chromatographic conditions.

On first the chromatographic factors which could influence chromatographic separation were sorted out. During preliminary development the flow rate, concentration of ortho phosphoric acid in aqueous part of mobile phase and percentage organic content of mobile phase were recognised as three important factor for profound analysis during optimization. The factors and their studied levels are tabulated in **Table 1**. Factors range was decided while exploring the experimental condition and it imitate the range in which acceptable system suitability parameter of product under investigation was expected.

TABLE 1: FACTORS AND THEIR LEVELS

Factors	Factors' levels		
	-1	0	+1
Flow Rate, ml/min	0.7	0.8	0.9
Ortho- Phosphoric Acid in Water, %	0.03	0.05	0.07
Acetonitrile in mobile phase, %	40	43	46

The randomization and plan of experiment had been carried out; it was generate using Box-Behnken design. The plan generated by Box-Behnken design and is based on three- level incomplete factorial design, in which effect of them were studied on responses namely retention time and tailing factor. Thirteen experiments plus central point replication are required for investigation of three factors, so as 17 experiments were performed randomly.

Method optimization: Desirability function was applied, in order to find optimal chromatographic conditions [32]. Derringer and Such developed this function in 1980 [33] and applied in areas of industry and sciences. Review paper by Candioti et al. [34] had explained how to carry out these experiments and described its application with many examples. Shortly, when more responses have been pursued, then optimization will be achieved by changing multiple responses into single and merging the singular responses into composite function.

Broadly speaking, the Derringer's desirability function is specified as the geometric base, weighted of the individual desirability functions and expressed as:

$$D = \left(\pi_{i=1}^{n} d_i^{ri}\right)^{1/n}$$

In above expression D is global desirability function, i is response, r_i is relative importance and d_i is the partial desirability function of respective n responses. Relative importance varies from most important (r_i = 5) to least important (r_i = 1). In present work, for two responses weights equal to 1 was chosen. A non-zero value of desirability function, D implies that responses exists in desirable range and therefore D value of 1, signifies the combination of factors is globally optimal [35].

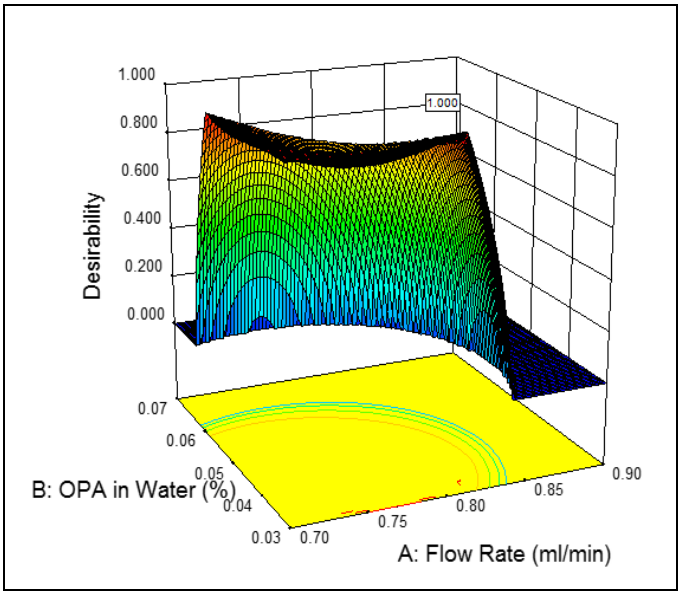


FIG. 2: 3D GRAPHICAL REPRESENTATION OF D= 1 [OPA IN WATER, %, FLOW RATE, ML/MIN]; CONTENT OF ACETONITRILE IN MOBILE PHASE IS 43 %

In said study, for both responses optimum values were in defined range. The chosen weights and upper and lower values of responses are given in **Table 2**.

It was examined that all factors were important and hence they were followed in optimization of procedure. Further, optimization was carried out and surface response graph was retrieved for maximum desirability function (D=1) is presented in **Fig. 2**. The factors value generating higher in case maximum desirability value and forecasted response in relation to optimum conditions are tabulated in **Table 3**. The experimental and predicted values of responses was in good agreement when blonanserin was injected at optimal chromatographic conditions (**Table 3**).

TABLE 2: LOWER AND UPPER VALUES OF SELECTIVITY FACTOR IN OPTIMIZATION

Response	Goal	Lower	Upper	Weights
Retention Time, Rt	Maximization	3.90	4.40	1
Tailing Factor, T	In range	1.01	1.50	1

TABLE 3: OPTIMUM CHROMATOGRAPHIC CONDITIONS AND COMPARISON OF EXPERIMENTAL AND PREDICTED VALUES

	Flow Rate, ml/min	Ortho- Phosphoric Acid in Water, %	Acetonitrile in mobile phase, %	Rt	T
Optimum Conditions	0.75	0.03	43		
Desirability Value (D) = 1.000					
Predicted Values				4.147	1.428
Experimentally Obtained Values				4.001	1.448

Method validation: Method validation was carried out in reference to the guideline laid down by ICH. The system suitability of developed- optimized method is tabulated in **Table 5**.

TABLE 5: SYSTEM SUITABILITY DATA (N=6)

Parameter	Mean	SD	RSD (%)	SEM	Required Limit
Retention Time (Rt)	4.001	0.047	1.156	0.018	RSD ≤ 2 %
Capacity Factor (K')	2.311	0.099	4.285	0.037	K'>2
Theoretical Plate (USP; N)	2942.857	281.047	4.550	106.226	N> 2000
Tailing Factor (T)	1.448	0.033	2.650	0.013	T≤ 2

Robustness: In reference to ICH Q2 (R1) guideline, the robustness study should be done at development phase depends upon the nature of study. In HPLC, column temperature, flow rate, pH values, mobile phase composition and different columns, are factors that should be considered during the robustness testing 30. In current study robustness was determined using one factor at a time approach. Investigated factors and their changes were: Flow rate 0.75 ± 0.05 ml/min and percent content of acetonitrile in mobile phase 43 ± 1 %. Changes (expressed in %) of peak area and retention time, in comparison with those obtained under optimal chromatographic conditions, were followed. The deliberated changes in factor under investigation affected peak area and retention was tabulated in **Table 4**. The low % RSD value suggests that method is robust and there are no significant changes in quantitative and qualitative chromatographic response.

TABLE 4: ROBUSTNESS OF THE DEVELOPED RP- HPLC METHOD (10 µG/ml, n=3)

	Flow Rate, ml/min		Acetonitrile in Mobile Phase, %	
	0.7	0.8	42	44
Mean Area	914268	915868	916318	913818
% RSD	1.141	1.017	0.423	0.204
Retention time, Rt	4.124	4.060	4.012	4.172
% RSD	0.530	0.348	0.416	1.547

Limit of Detection, Quantification and Linearity: The linear regression data for the calibration curves (n=3) of blonanserin at 330 nm (**Table 6**) shower a good linear relationship over a concentration range of 5- 50 µg/ml with respect to the peak area. No significant difference was observed in the slope of standard curves (ANOVA, $p > 0.05$). The observed equation of the straight line was found as; $y = 53485 x + 25012$ with R^2 value of 0.9991.

Linearity data showed concertation interval of blonanserin in which the intensity of the detector response is proportional to the concentration of the analysed substance. The LOD and LOQ obtained were 0.02 and 0.06 µg/ml respectively.

TABLE 6: LINEAR REGRESSION DATA FOR THE CALIBRATION CURVE (N=3)

Statistical Parameter	Data
Linearity range (µg/ml)	5- 50
Correlation coefficient (r^2 ±SD)	0.9991 ± 0.0005
Slope of Curve ±SD	53485 ± 398.178
Confidence limit of slope [a]	0.985810/ 1.012385
SEM of Slope	229.888
Interception of Curve ±SD	25012 ± 6772.592
Confidence limit of intercept [a]	-0.390073 / 0.438413
SEM of intercept	3910.158
Limit of Detection (µg/ml)	0.02
Limit of Quantitation (µg/ml)	0.06

Accuracy and precision: Accuracy and precision are presented in **Table 7**. Recovery values and relative standard deviation (RSD) were within specified limit, which proved the suggested method is accurate and precise for estimation of blonanserin.

TABLE 7: PRECISION AND ACCURACY OF PROPOSED RP-HPLC METHOD

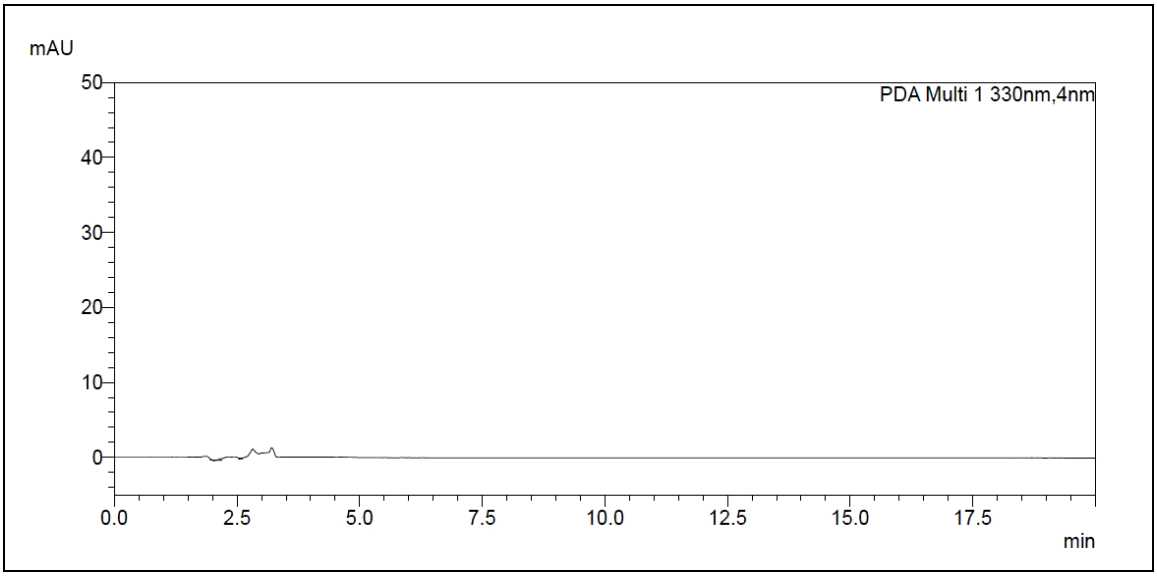
		Mean recovered concentration, µg/ml	Recovery %	SD	RSD, %	SEM
Precision						
Injection repeatability[a]		10.12	101.12	0.070	0.640	0.193
Analysis repeatability[b]		10.04	100.40	0.650	1.647	0.231
Intermediate[c]		10.03	100.30	0.270	2.696	0.151
Accuracy[d]						
Levels	80 %	18.12	100.66	-	1.073	0.426
	100 %	19.89	99.44	-	1.738	0.774
	120 %	22.17	100.76	-	1.334	0.427

[a] n= 10 RSD< 1%; [b] n= 6 RSD< 2%; [c] n= 6 RSD< 3%; [d] n= 3 Recovery: 98-102%

Application of the developed, optimized method: Analysis of forced degraded samples: An analytical procedure which can able to separate all the degradation products from the major peak of sample is known as stability indicating assay method. The model chromatograms of diluent, blonanserin and blonanserin under acidic, basic, oxidative and thermal stress condition are presented in **Fig. 2**. **(a), (b), (c), (d), (e)** and **(f)** respectively. Blonanserin under acidic condition showed degradants peak at retention time, Rt 1.892, 6.228, 7.937 and 9.668; whereas under basic stress condition Rt 3.550, 5.817, 7.283 and 9.570 shows degradants peak. Oxidation of blonanserin with hydrogen peroxide yields two degradants at Rt 6.218 and 9.134 while dry heat thermal degradation results in three degradants at Rt 3.213, 6.658 and 7.906. Stress sample under water hydrolysis and photolysis shows no degradation. In all the above case degradants peak does not interfere with each other and blonanserin peak, suggests that method is selective stability indicating assay method [27]. **Table 8** summarizes the degradation of blonanserin in tablet formulation.

TABLE 8: SUMMARY OF FORCED DEGRADATION STUDIES OF BLONANSERIN

Exposure Conditions	Time (h)	% BLN Recovery	Degradation Products Rt Value (min) and Degradation (%)
Acid, 0.5 M, HCl, 70°C	7	66.84	1.892 (9.59); 6.228 (0.80); 7.937 (19.89); 9.668 (2.89)
Base, 0.5 M NaOH, 70°C	7	61.43	3.550 (8.97); 5.817 (7.12); 7.283 (14.59); 9.570 (7.89)
H_2O_2, (0.3% v/v), Room Temperature	12	48.91	6.218 (30.08); 9.134 (21.01)
H_2O, refluxed	48	100	Not Detected
UV (UVB Radiation)	7 days	100	Not Detected
Dry heat (105°C)	12	67.2	3.213 (9.66); 6.658 (17.73); 7.906 (5.41)


mAU
PDA Multi 1 330nm,4nm
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40
30
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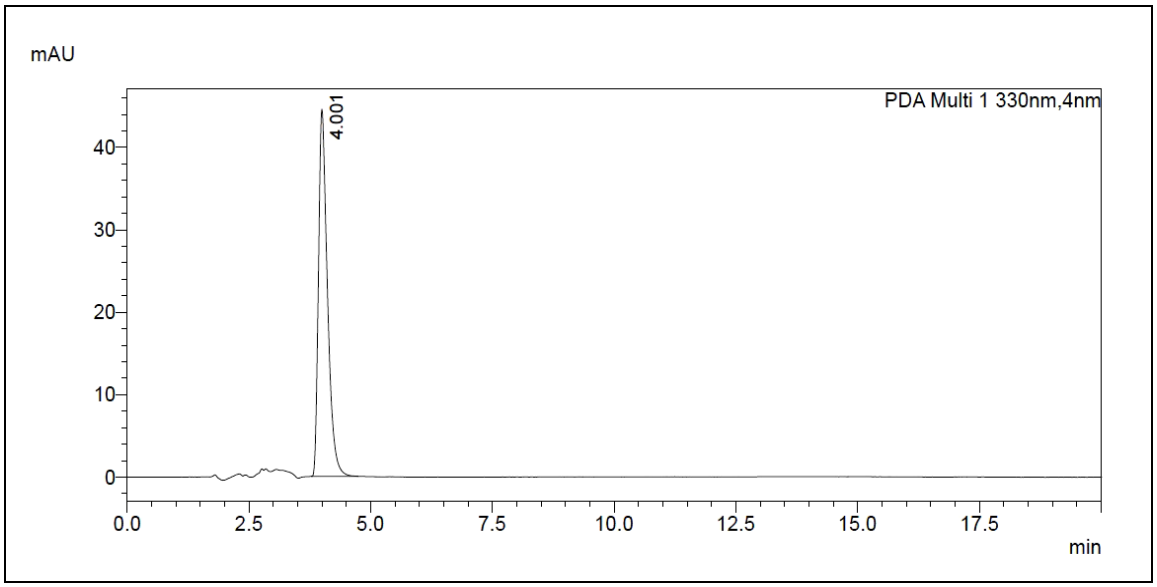
(a)


mAU
PDA Multi 1 330nm,4nm
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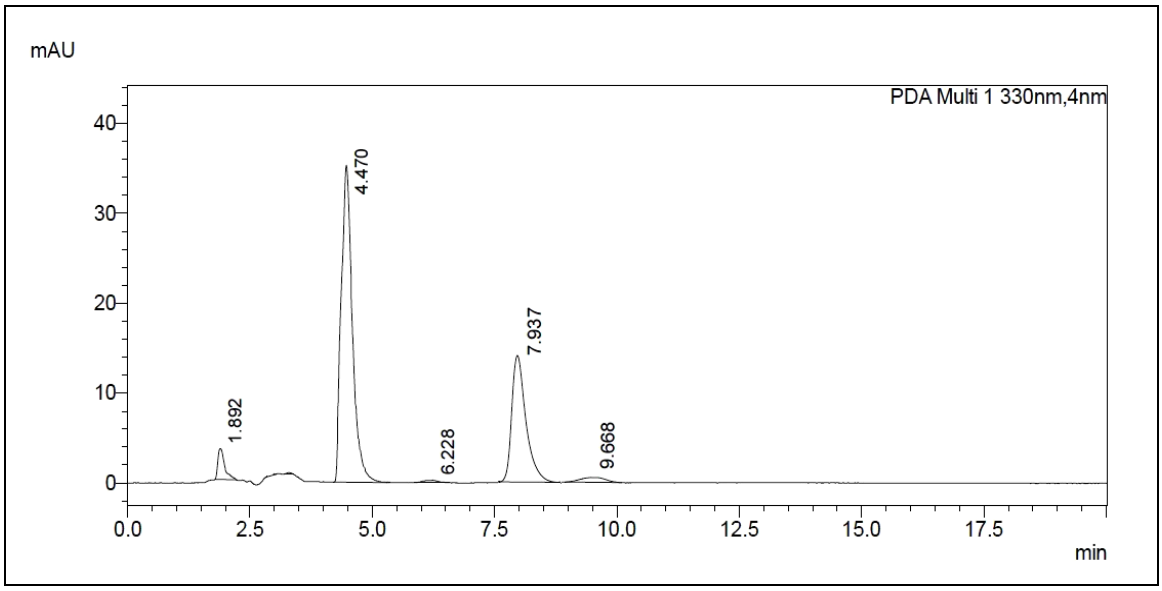
(b)


mAU
PDA Multi 1 330nm,4nm
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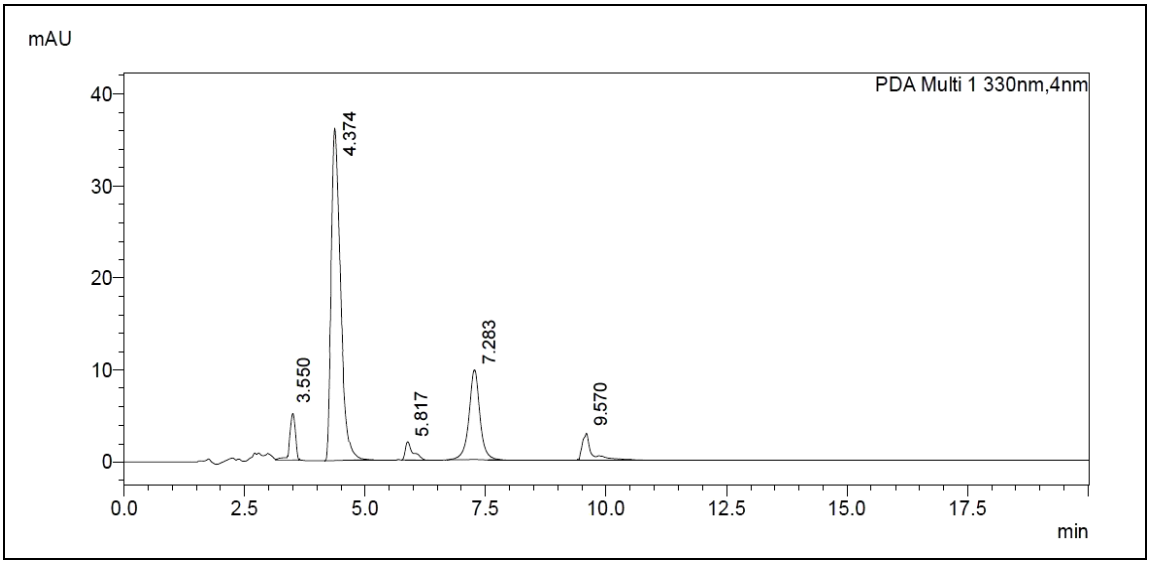
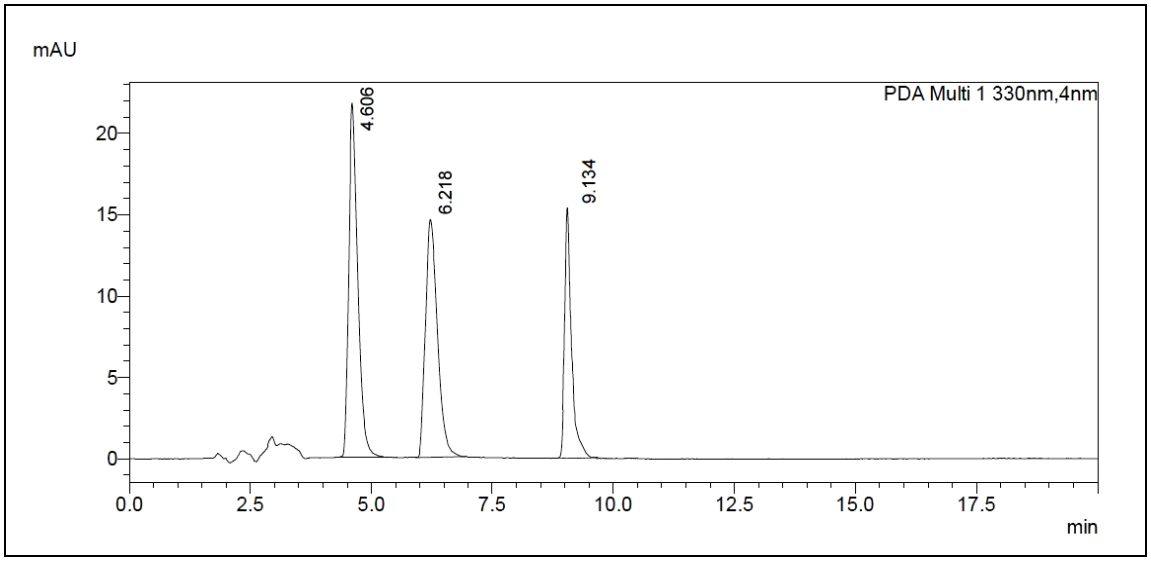
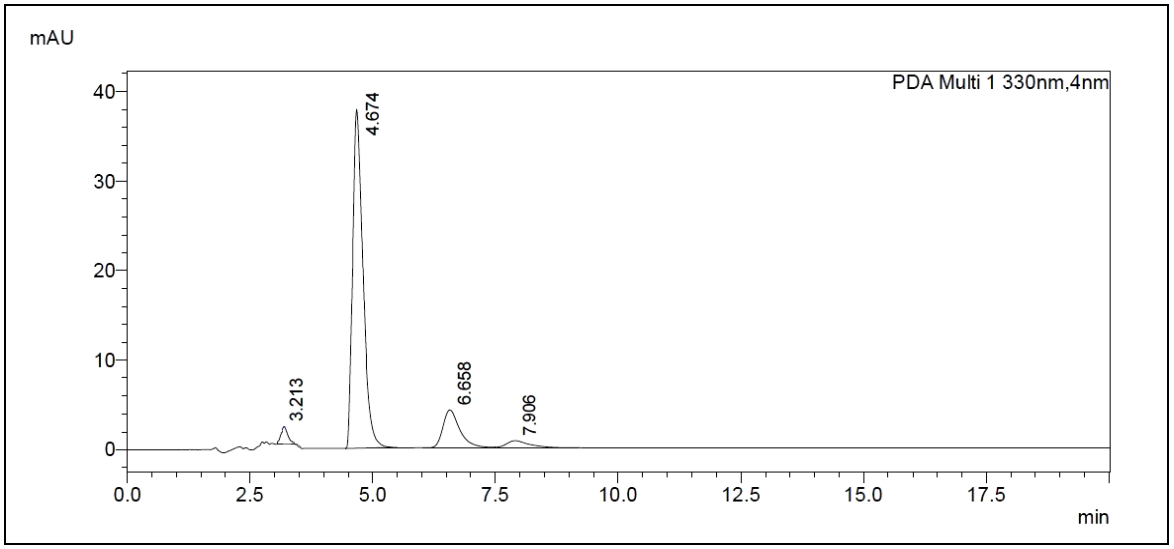
(c)



(d)



(e)



(f)

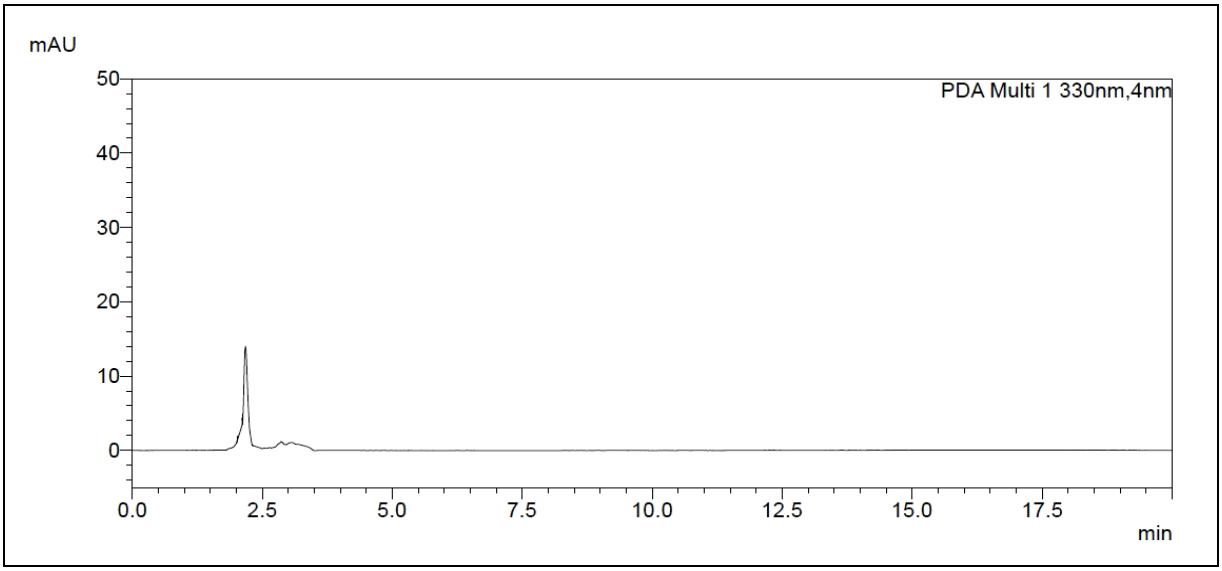
FIG. 3: CHROMATOGRAMS OF (A) DILUENT (B) BLONANSERIN (C) ACID HYDROLYSIS (D) BASIC HYDROLYSIS (E) OXIDATION AND (F) THERMAL DEGRADATION

Formulation analysis: Applicability in routine analysis of this method was tested by analysis of Blonitas® 8 mg and Elicia® 8 mg tablets. The content of blonanserin was found 101.30 and 101.10% respectively, which is within the specified limits (95 – 105 %) (see **Table 9**).

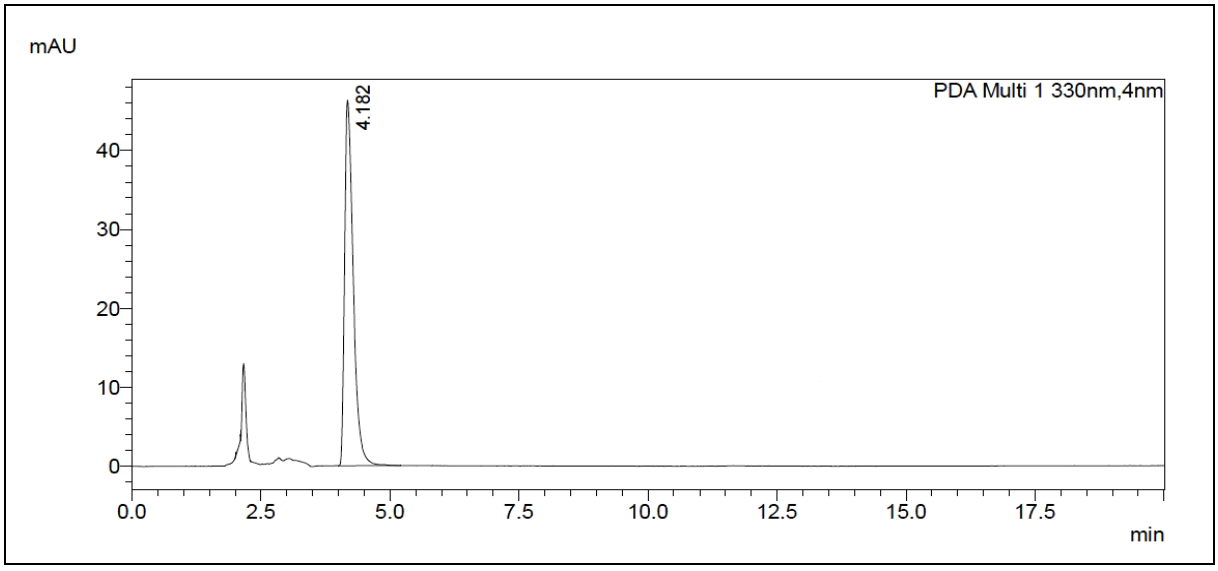
TABLE 9: DETAILED ANALYSIS REPORT OF BLONANSERIN TABLETS, FORTIFIED BLONANSERIN IN SERUM

Sample	Manufacturer	Theoretical concentration (µg/ml)	Concentration found (µg/ml) ± SD	% RSD	SEM	Drug Content, %
Blonitas® Tablet	Intas Pharmaceuticals, India	10	9.96 ± 0.150	1.442	0.184	99.63
Elicia ® Tablet	Zydus Cadila Healthcare Ltd, India	10	9.82 ± 0.281	2.779	0.115	98.22
Blonanserin in Serum	-	10	9.80 ± 0.004	0.041	0.002	98.02

***In-vitro* Recovery from Serum**: *In-vitro* study in human serum was carried out by using aforementioned procedure to check the applicability of method in analysis of serum sample. The data summarized in **Table 9** shows excellent recovery of blonanserin in human serum sample. **Fig. 4. (a)** and **(b)** shows chromatogram for blank serum and in-vitro analysis of blonanserin in human plasma.



(a)



(b)

FIG. 4: CHROMATOGRAMS OF (A) BLANK SERUM AND (B) BLONANSERIN FORTIFIED IN HUMAN SERUM

CONCLUSIONS: Optimization and validation of a new high performance liquid chromatography method blonanserin was presented. The Box-Behnken design of experiments was followed during method optimization to study factors which have significant impact on critical pair separation of blonanserin and its degradation products. Derringer's desirability function was used to determine optimal chromatographic conditions. Developed-optimized method was validated in according to the ICH guidelines for analytical validation and tested for robustness, linearity, accuracy and precision. Applicability of method was confirmed by analysis of blonanserin in forced degraded sample, commercially available formulation and *in-vitro* human serum sample. So, after study it was concluded that method was selective stability indicating assay method and used for quality control analysis of blonanserin in its tablet formulations, moreover it can be used for quantification of blonanserin in in-vitro human serum sample.

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REFERENCES:

1. T. R. Insel: Rethinking schizophrenia. Nature 2010; 468-187.
2. I. P. Stolerman: Encyclopedia of Psychopharmacology. Springer Berlin Heidelberg, Berlin, Heidelberg, 2010.
3. Chembldb. Compound Report Card : Blonanserin. can be found under https://www.ebi.ac.uk/chembldb/ index. php/compound/inspect/CHEMBL178803.
4. R. Ghosh, M. S. Bhatia and S. K. Bhattacharya: Blonanserin in management of Schizophrenia. Delhi Psychiatry Journal 2012; 15:406.
5. Cayman Chemicals: Product Information: Blonanserin 2014.
6. E. Garcia, M. Robert, F. Peris, H. Nakamura, N. Sato and Y. Terazawa: The efficacy and safety of blonanserin compared with haloperidol in acute-phase schizophrenia: A randomized, double-blind, placebo-controlled, multicentre Study. CNS Drugs 2009; 23: 615.
7. T. Tenjin, S. Miyamoto, Y. Ninomiya, R. Kitajima, S. Ogino, N. Miyake and N. Yamaguchi: Profile of blonanserin for the treatment of schizophrenia. Journal of Neuropsychiatric Disease and Treatment 2013; 9:587.
8. G. E. P. Box, J. S. Hunter and W. G. Hunter: Statistics for Experimenters: Design, Innovation, and Discovery. Wiley-Interscience, 2005.
9. R. E. Bruns, I. S. Scarminio, B. de and B. Neto: Statistical Design--Chemometrics. Elsevier, 2006.
10. B. G. M. Vandeginste and D. L. Massart: Handbook of Chemometrics and Qualimetrics: Part A. Elsevier, 1997.
11. S. Patel and A. Patel: Development and Validation of Reverse Phase High Performance Liquid Chromatography Method for Estimation of Blonanserin in Synthetic Mixture. International Journal of Pharmaceutics and Drug Analysis 2014; 2: 49.
12. Z. Caiyan and S. Yingbing: Determination of Blonanserin Tablets by HPLC. China Pharmacist 2010; 10.
13. G. Modi and K. Chandrul: A simple and validated reverse phase HPLC method for the determination of Blonanserin in pharmaceutical dosage forms. Journal of Chemical and Pharmaceutical Research 2011; 3: 274.
14. H. Ping: Determination of Blonanserin in Blonanserin tablets by HPLC. Journal of North Pharmacy 2013; 03.
15. Y. Xiang-ping, L. Bo-rong, W. Kang-rui and W. Pu-ha: Determination content of Blonanserin tablets by HPLC. West China Journal Pharmacy Sciences 2012; 05.
16. K. B. Ahir, A. S. Patel, H. S. Naik, D. N. Solanki, C. B. Gandhi and A. K. Bari: A Validated Stability Indicating Reverse Phase High Performance Liquid Chromatography Method for the Determination of Blonanserin in Bulk and Tablet Dosage Form. Pharmaceutical Analysis and Quality Assurance Journal 2014.
17. L. Huawei, C. Yun and B. Jiahe: Determination of the Content of Blonanserin and Its Related Substances by RP-HPLC. China Pharmacist 2011; 19.
18. P. D. Kalariya, P. N. Patel, M. Sharma, P. Garg, R. Srinivas and M. V. N. K. Talluri: Characterization of stress degradation products of blonanserin by UPLC-QTOF-tandem mass spectrometry. RSC Advances 2015; 5: 69273.
19. P. Mondal, S. Shobharani and R. Ramakrishna: A New Stability Indicating Quantitative RP-HPLC Method for Blonanserin-A Novel Antipsychotic Agent. Indo American Journal of Pharmaceutical Research 2014; 4: 1968.
20. T. Ogawa, H. Hattori, R. Kaneko, K. Ito, M. Iwai, Y. Mizutani, T. Arinobu, A. Ishii, O. Suzuki and H. Seno: Sensitive analysis of blonanserin, a novel antipsychotic agent, in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry. Analytical Sciences 2010; 26: 1099.
21. Y.-G. Wen, X.-J. Ni, M. Zhang, X. Liu and D.-W. Shang: Simultaneous determination of blonanserin and its metabolite in human plasma and urine by liquid chromatography-tandem mass spectrometry: application to a pharmacokinetic study. Journal of Chromatography B 2012; 903:46.
22. Y. Zheng, X. Hu, J. Liu, G. Wu, H. Zhou, M. Zhu, Y. Zhai, L. Wu and J. Shentu: Simple and sensitive analysis of blonanserin and blonanserin C in human plasma by liquid chromatography tandem mass spectrometry and its application. Journal of Analytical Methods in Chemistry 2014.
23. Y. Zhou, M. Liu, J. Jiang, H. Wang and P. Hu: Simultaneous determination of blonanserin and its four metabolites in human plasma using ultra-performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography B 2013; 939: 59.
24. L. Cai-xia, W. Chang-bin, Y. Hai-xia and X. Yu-ren: Determination of Residual Solvent in Blonanserin by Gas Chromatography with Headspace Sampling. Food Drug 2013; 04.

25. M. Peng, J. Liu, D. Lu and Y. J. Yang: Development and validation of a stability-indicating gas chromatographic method for quality control of residual solvents in blonanserin: A novel atypical antipsychotic agent. Journal of Chromatographic Science 2012; 50: 727.
26. H. Hattori, M. Iwai, T. Ogawa, Y. Mizutani, A. Ishii, O. Suzuki and H. Seno: Simple analysis of blonanserin, a novel antipsychotic agent, in human plasma by GC-MS. Forensic Toxicology 2010; 28: 105.
27. M. Bakshi and S. Singh: Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis 2002; 28: 1011.
28. International Conference on Harmonization: Q1A (R2): Stability Testing of New Drug Substances and Products. International Conference on Harmonisation, 2003; 1–9.
29. International Conference on Harmonisation: Specifications: Test Procedures and Acceptance criteria for new drug substances and new drug products. Chemical substances. 1999; 35.
30. International Conference on Harmonisation: Validation of Analytical Procedures : Text and Methodology Q2 (R1). 2005.
31. G. Derringer and R. Suich: Simultaneous-Optimization of Several Response Variables. Journal of Quality Technology 1980; 12: 214.
32. S. L. C. Ferreira, R. E. Bruns, H. S. Ferreira, G. D. Matos, J. M. David, G. C. Brandão, E. G. P. da Silva, L. A. Portugal, P. S. dos Reis, A. S. Souza and W. N. L. dos Santos: Box-Behnken design: An alternative for the optimization of analytical methods. Analytica Chimica Acta 2007; 597: 179.
33. N. R. Costa, J. Louren and Z. L. Pereira: Desirability function approach: A review and performance evaluation in adverse conditions. Chemometrics and Intelligent Laboratory Systems 2011, 107: 234.
34. L. V. Candioti, M. M. De Zan, M. S. Cámara and H. C. Goicoechea: Experimental design and multiple response optimizations. Using the desirability function in analytical methods development. Talanta 2014; 124: 123.
35. T. Sivakumar: An improved HPLC method with the aid of a chemometric protocol: simultaneous analysis of amlodipine and atorvastatin in pharmaceutical formulations. Journal of Separation Science 2007.

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