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Development of Analytical Method for the Validation of Blonanserin Tablet

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ABSTRACT
The objective of the work is to validate method of analysis for Blonanserin tablet on Reversed phase HPLC. The Blonanserin Sample was analyzed by reverse phase octadecylsilane (C18) column shimpack shimadzu stationary Phase, phosphate buffer, acetonitrile and methanol in ratio of 40:40:20 with pH 4 adjusted with orthophosphoric acid, run with flow rate of 1ml/min. Quantification was achieved with UV-Visible Detector at wavelength of 237nm at ambient temperature. The retention time of Blonanserin was found to be 9.8 min. The calibration curve was linear over a 32 ppm to 48 ppm concentration of sample. Limit of detection and limit of quantification were found to be 1.49µg/ml and 4.53µg/ml. Not any chromatographic interference found from expedients in chromatogram. Thus, the method is employed for determination of Blonanserin in formulation. Hence, the method is validated.

Keywords: Blonanserin, Analytical Method Validation, Reversed Phase HPLC.

Abbrevations
BLS- Blonanserin, HPLC – High Performance Liquid Chromatography, LOD – Limit of Detection , LOQ – Limit of Quantification , ICH – International Conference on Harmonization , USP – United States Pharmacopeia

I. INTRODUCTION

Blonanserin is a novel drug in the category of atypical antipsychotics [1] that belongs to a class of 4-phenyl-2-(1-piperazinyl) pyridines. Blonanserin acts as an antagonist at dopamine D2, D3, and serotonin 5-HT 2A receptors [2]. Safety and efficacy studies of Blonanserin's have been studied in schizophrenic and delirium patients, and it is found to be effective and well-tolerated in both conditions [3, 4, 5]. Blonanserin has been known to improve some types of cognitive associated with preprontal cortical function in patients with first-episode and chronic schizophrenia. It has now emerged as a potential candidate to treat 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 a stable, authentic, quick, and established analytical approach is critical.

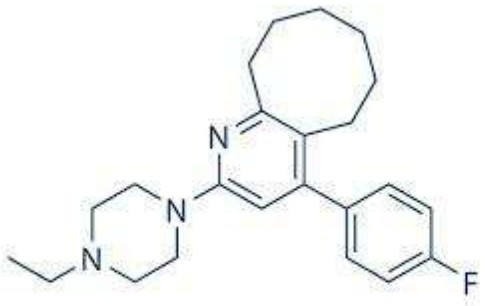

Fig. Chemical structure of Blonanserin

Blonanserin is not yet officially recognized by the I.P., B.P., USP, or any other pharmacopeia, 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]. All the methods of analysis

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have their own set of constraints, such as detection limits, quantification and analysis times, and a low level of linearity[13]. The current research task aimed to eliminate all drawbacks and develop a fast, stability-indicating RP-HPLC with UV Visible Detector method for estimating Blonanserin in bulk drugs and tablet dosage forms, as well as a validation study (R1), in order to comply with the International Conference on Harmonization (ICH) Guidelines Q2 [14].

1.0 Materials & Methods

1.1 Apparatus

Shimadzu Liquid chromatography (LC 2010CHT HPLC System) having UV- Visible detector and Lab Solution Software, octadecylsilane (C18)250 x 4.0 mm, 5 micron shimpack shimadzu stainless steel Column As stationary Phase, Shimadzu Analytical Balance AP Series. Sonicator (Precioustech), Digital pH Meter (Hanna instruments), Glass wares like Pipette, Beaker, Volumetric Flask, etc From Supertek. Syringe, nylon filter 0.45micron size, Milli Q Water System (Labjal) (HPLC Grade Water System), Dissolution apparatus (Electrolab)

1.2 Chemicals and Materials

Blonanserin Working reference Standard was gift sample from Consern Pharma Ltd. Potassium di- hydrogen orthophosphate AR, Acetonitrile HPLC grade, Methanol HPLC grade, Milli Q Water, Ortho-phosphoric Acid, Blonanserin 4 mg tablet (Blonanserin Tablet From Consern Pharma Ltd).

1.3 Chromatographic Conditions

Column – 250 x 4.0 mm 5 µ, C18 (Octadecylsilane)
Wavelength – 237nm
Column Oven temperature – Ambient
Injection volume – 20 µl
Flow rate – 1 ml per min
Dissolution Medium : 0.1 M HCl 900 ml, rotating paddle at 50 revolutions per minute for 30 minutes.

1.4 Preparation of Mobile Phase

Mobile phase is Phosphate Buffer: Acetonitrile: Methanol In ratio of 40:40:20 having pH 4.
Phosphate Buffer is prepared by dissolving 2.72 gm of potassium di hydrogen orthophosphate in 400 ml Milli Q water (HPLC Grade Water) in a one liters beaker. Then beaker is placed in ultra sonicator for mixing of solute particles, after mixing of solute particle, 400ml Acetonitrile and 200 ml Methanol is added in beaker. Then beaker is placed in ultra sonicator for up to 15 to 20 minutes for complete degassing of mixture. After degassing, Mixture is placed on pH meter with continuous stirring with magnetic stirrer. Glass probe of pH meter is dipped in mixture and then pH is maintained to 4 with drop wise addition of ortho-phosphoric acid in mixture. After maintaining the pH, the mixture is filtered by using vacuum filtration assembly with filter of 0.4µsize.

1.5 Preparation of Standard solution

20 mg of Blonanserin WRS is weighed in 50 ml volumetric flask using highly sensitive Analytical Balance (Shimadzu AP series). The flask was pre-rinsed with mobile phase as Mobile Phase is used as solvent for standard preparation. Then volumetric flask is placed in ultra sonicator for complete mixing of Blonanserin WRS. After mixing of standard in mobile phase, volumetric flask is placed in cold water for 4-5 minutes. Then volume is made to mark with mobile phase. 1 ml of sample was pipette out from it and transferred to 10 ml volumetric flask and again volume was made to mark with mobile phase. So, Standard Solution was prepared with concentration of 40 ppm.

1.6 Preparation of Sample Solution

20 tablets of Blonanserin were weighed and crushed in a pestle mortar to get a fine powder of tablets, then weight of powder equivalent to 20 mg of BLS was transferred in 50 ml volumetric flask which was prerinsed with mobile phase. Then volumetric flask was placed in ultra sonicatior for complete mixing of powdered tablets in volumetric flask. After mixing of sample powder in Mobile Phase the volumetric flask was placed in cold water for cooling of sample solution. After cooling, volumetric flask was filled to mark. After that, 1 ml was pipette out from it and transferred to 10 ml volumetric flask and again volume was made to mark with mobile phase. So, Sample Solution was also prepared with concentration of 40 ppm.

2.0 Analytical Method Validation

The parameter for performing method validation are linearity, accuracy, recovery, specificity, limit of detection, limit of detection, limit of quantification and robustness are studied.

II. RESULT & DISCUSSION

2.1 Linearity

Different concentration of samples like 32ppm, 36ppm, 40ppm, 44ppm, 48ppm were prepared, which are 80%, 90%,100%,110%,120%

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of the main concentration of sample. The plot found to be linear from the concentration vs area, and correlation coefficient r^2 was found to be **0.9998**. Triplicate injection of different concentrations was runed to determine average area of different triplicate injections. The area under curve obtained of different injections was mentioned below in table.

Concentration	1st injection area	2nd injection area	3rd injection area	Average area	Std deviation	%RSD
32ppm(80%)	1634092	1633083	1633523	1633566	505.8725136	0.030967375
36ppm(90%)	1839533	1829178	1839978	1836236.333	6116.388504	0.333093752
40ppm(100%)	2036881	2039622	2047354	2041286	5431.093107	0.266062374
44ppm(110%)	2246439	2245184	2242677	2244766.667	1915.407615	0.085327693
48ppm(120%)	2454791	2454551	2462975	2457439	4795.818178	0.195155126
						0.182121264

Table 1 shows triplicate injection of different concentration of sample and area under curve observed of different injections. Average area, standard deviation and relative standard deviation of area under curve

Retention time of different concentration injections are mentioned below

Concentration	Injections	Area	Retention Time	%RSD	Avg retention time
32ppm	1st	1634092	9.839	0.018	9.840
	2nd	1633083	9.840		
	3rd	1633523	9.842		
36ppm	1st	1839533	9.836	0.009	9.836
	2nd	1829178	9.835		
	3rd	1839978	9.836		
40ppm	1st	2036881	9.832	0.032	9.836
	2nd	2039622	9.838		
	3rd	2041286	9.837		
44ppm	1st	2246439	9.833	0.031	9.835
	2nd	2245184	9.834		
	3rd	2242677	9.838		
48ppm	1st	2454791	9.832	0.016	9.834
	2nd	2454551	9.833		
	3rd	2462975	9.835		

Table 2 shows the retention time of different peaks of sample, relative standard deviation of retention time and average retention time.

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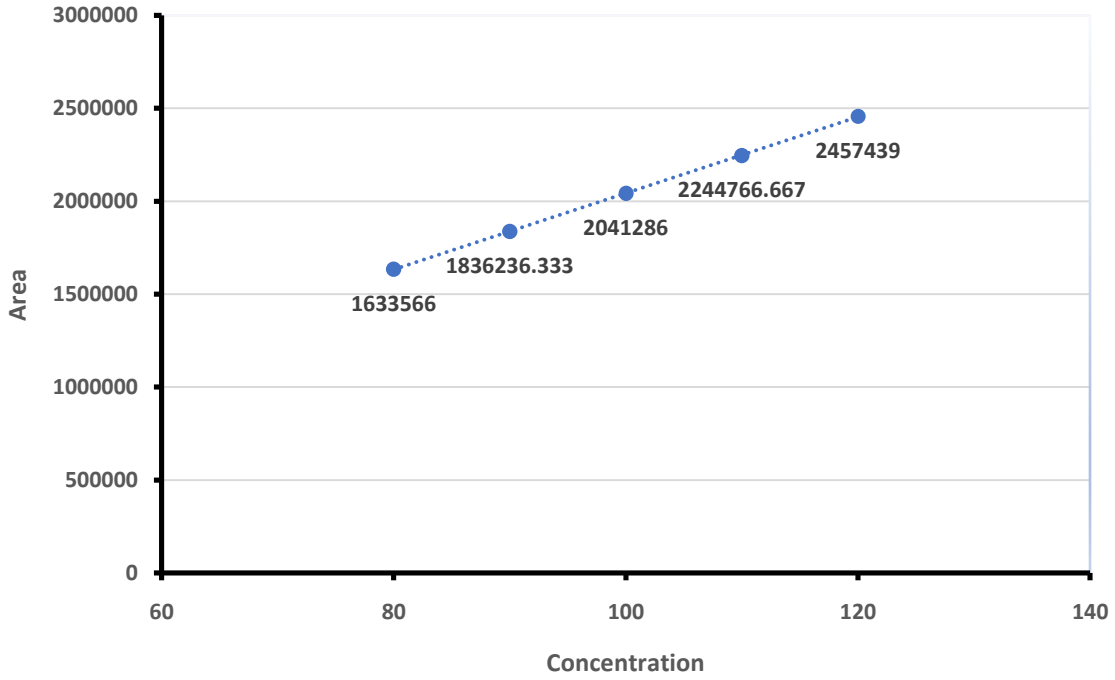
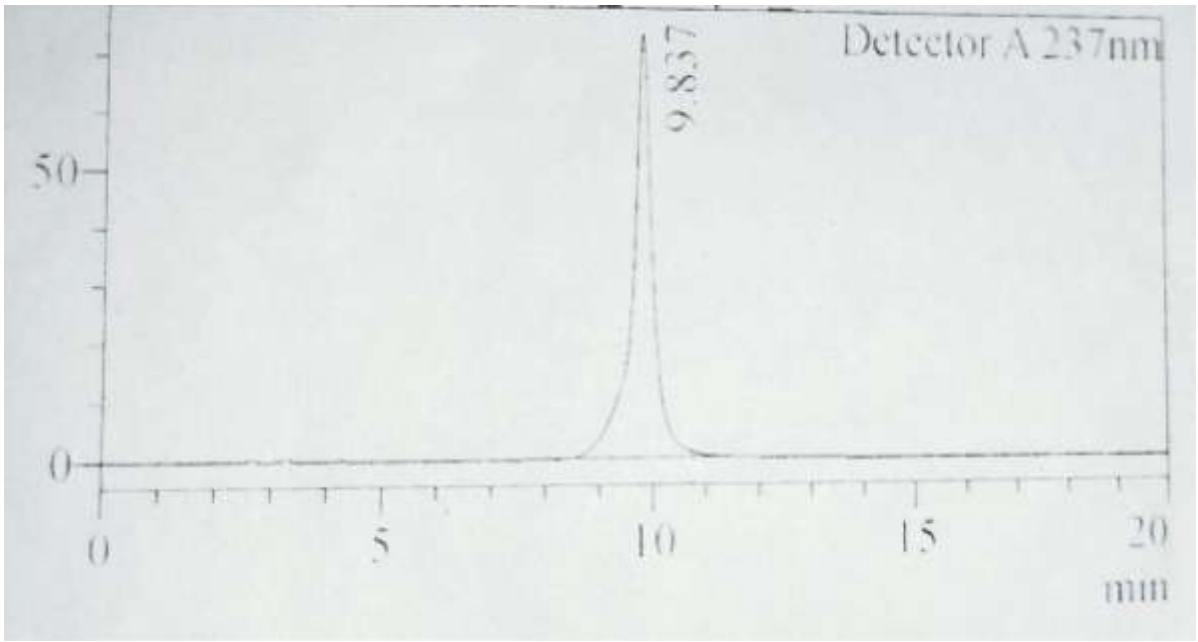
Area v/s Concentration



Plot -1



1 Chromatogram - BNS sample (40ppm) at 237 nm

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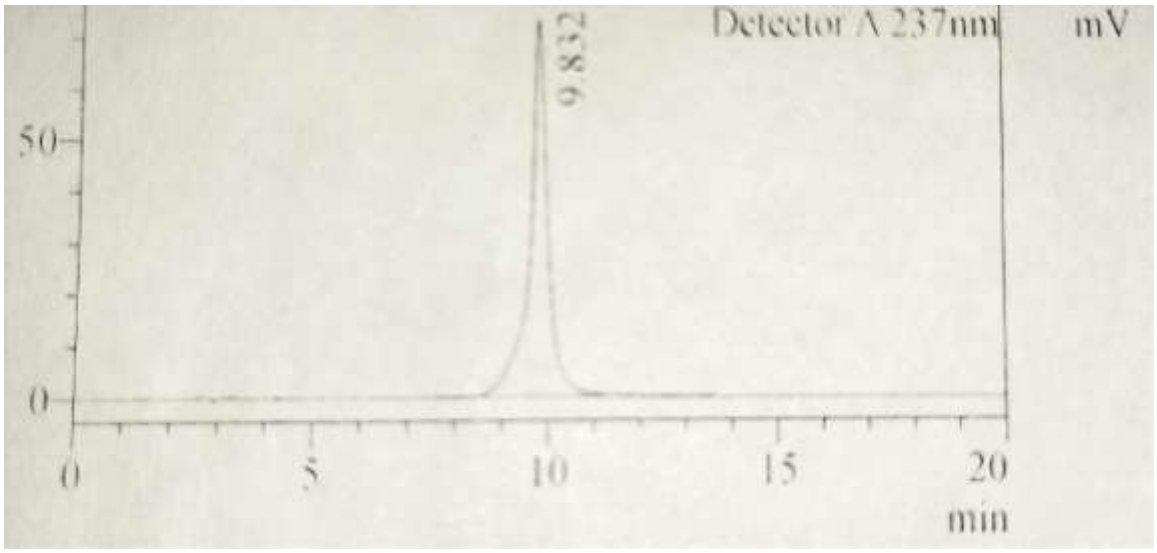

2 Chromatogram - BNS Standard (40ppm) at 237 nm

2.2 Accuracy / Recovery

Average recoveries of BNS triplicates 80% , 90% , 100%, 110%, 120% was in acceptable criteria (as mentioned in Table 2). Excellent recovery with low relative standard deviation was observed .which means that the method can be succesfully applied for the assay determination in potent BLS Tablet.

2.3 Specificity

Specificity is a ability to access unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004) . the placebo of the formulation was also injected , 1 chromatrogram indicates that there was no interference of excepients with the peak of BLS , hence the method is specific for the determination of BLS.

2.4 Method Precision

Six replicate injections of sample solution are injected. %RSD was found below 2%. this indicates that the method is precised with multiple injections . and retention time is 9.83 ± 0.02.

2.4 Stability of sample and standard solution

After succesfull running of batch on day 1, the sample and standard are preserved at room temperature to get reinjected on day 2, and after 24 hour same sample set was runned after completion of batch we observed that RSD is below 2 % , this indicated that sample and standard was stable on day 2.

2.5 Limit of Detection & Limit of Quantification

LOD and LOQ were calculated by using equations designated by ICH. LOD and LOQ values were calculated as signal-to noise ratio of 3:1 and 10:1 respectively.

$LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Value of slope in Plot 1 is 51407 and standard deviation is 10431.78521

So , after applying values in formula , we can able to LOD and LOQ.

The LOD is found to be 1.49 µg/ml and LOQ is found to be 4.53µg/ml.

2.6 Robustness

Robustness of the analytical method is checked by performing minor variation in method parameters and mobile phase preparation. in method parameters the columnoven temperature is varied ±2 Cand in mobile phase preparation the pH is varied ±0.5 .

Hence , No major difference in the results is observed with minor changes in Retention time of sample and standard . So the method is validated for robustness.

2.7 Dissolution

The dissolution is performed in electrolab dissolution apparatus, the dissolution medium i.e 0.1 M HCL is prepared and transferred to vessels of apparatus , each vessel filled with 900 ml 0.1 M HCL, Temperature of vessel is maintained at 37.5 °C. Six tablets are selected randomly and placed in each vessel. Paddles are attached to apparatus and then paddles are rotated in vessel with 75

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revolution per minuet for 30 minutes. After completion of time, samples are collected in testtubes.

No.of Injection of standard	Area of standard	No. of Injection of six tablets	Area of six tablets
1st	2117442	1st	2112666
2nd	2117239	2nd	2112605
3rd	2113972	3rd	2116242
4th	2117142	4th	1966980
5th	2116069	5th	2116445
		6 th	2107434
Averge	2116373		2088729

Table 3

The area of standard and samples are compared. After comparing areas we are able to find that all the six tablets have dissolution values in between 95 to100 %.

III. CONCLUSION

A simple , senstive isocratic method is developed on RP-HPLC with UV Visible Detector . The method is validated for Linearity, Accuracy, Recovery, Specificity, Precision, Stability, LOD, LOQ and Robustness.

BLS Showing excellent detection at 237 nm in 25x 4.0,5µ C18 Column.sample is runned at flow rate of 1ml/min. with injection volume of 20µl. Uv-visible Detector's detection found efficient for BLS sample at 237nm. The calibration curves found to be linear upto 32 ppm to 48 ppm concentration with r^2 value of 0.9998 . Method is found to be specific without interference of excepients with peak of BLS . Method gets precised by injecting multiple replicate injections with RSD below 2%. Sample is also tested for stability upto 24 hours . LOD and LOQ values were calculated as signal-to noise ratio of 3:1 and 10:1 respectively. LOD and LOQ values are 1.49 µg/ml and 4.53 µg/ml. The result of analysis states that the method is highly Reproducible and cab be employed for routine testing of BLS in dosage forms.

REFERENCES

[1]. Garcia E, Robert M, Peris F, Nakamura H, Sato N, Terazawa Y, 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: 615-625.

[2]. Tenjin T, Miyamoto S, Ninomiya Y, Kitajima R, Ogino S, Miyake N, Yamaguchi N, Profile of blonanserin for the treatment of schizophrenia. Neuropsychiatr Dis Treat. 2013;9:587-94.

[3]. Ghosh R, Bhatia MS, Bhattacharya SK, Blonanserin in management of Schizophrenia. Delhi Psychiatry J. 2012; 15(1):406- 411.

[4]. Xia C, Hongyun W, Jiang J, Rui C, Ying Z, Wen Z, Hongzhong L, Pei H, The Pharmacokinetic and Safety Profiles of Blonanserin in Healthy Chinese Volunteers

[5]. After Single Fasting Doses and Single and Multiple Postprandial Doses. Clin Drug Investig. 2014;34(3):213-222

[6]. Patil AD, Efficacy and safety of blonanserin versus other antipsychotics: a review. Int. J. Basic. Clin. Pharmacol, 2013;2(6):689- 692.

[7]. Modi G, Chandrul K, A simple and validated reverse phase HPLC method for the determination of Blonanserin in pharmaceutical dosage forms. J. Chem. Pharm. Res, 2011; 3(2) 274-279.

[8]. Mondal P, Shobharani S, Raparla R. A New Stability Indicating Quantitative RP-HPLC Method For Blonanserin-A Novel Antipsychotic Agent. Indo Am J Pharm Res. 2014;4(4):1968-1976.

[9]. Modi G, Chandrul K, Padia M, Spectrophotometric estimation of Blonan-serin in pure drug and pharmaceutical formulation. J. Chem. Pharm. Res. 2011;3: 670-675.

[10]. Mondal P, Shobharani S, Raparla R, Quantification of Blonanserin in Human Plasma Using Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrophotometry-Application to

Pharma-cokinetic Study, J Young Pharm. 2016; 8(4):406-414

[11]. Ying Z, Ming L, Ji Jiang, Hongyun W, Hu P, Simultaneous determination ofblonanserin and its four metabolites in human plasma using ultra-performance liquid chromatography–tandem mass spectrometry, J. Chromatogr. B. 2013;939: 59-66.

[12]. Yu-Guan W, Xiao J, N-Ming Z, Xia L, De-wei S, Simultaneous determination of blonanserin and its metabolite in human plasma and urine by liquid chromatography – tandem mass spectrometry: Application to a pharmacokinetic study. J. Chromatogr. B. 2012;903:46-52.

[13]. Stability testing of new drug substances and products Q1 A (R2).International conference on harmonization, IFPMA, 2003, Geneva.

[14]. Acharyya S, Si A, Kumar HK, S. A Stability Indicating Novel UPLC-PDA Method for the Estimation in Bulk and Capsule Dosage form of Isavuconazole Effective for Mucormycosis Caused by COVID-19 Pandemic. J Pharm Res International. 2021;33(43A):505-513.

[15]. Mondal P, Shobharani S, Ramakrishna, R. Novel stability indicating validated RPHPLC method for simultaneous quantification of Artemether and Lume-fantrine in Bulk and Tablet. Curr Pharm Anal. 2014;10(4):271-278.