Research Article

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DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ESTIMATION OF BLONANSERIN IN SYNTHETIC MIXTURE.

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Abstract:

This research manuscript describes simple, sensitive, accurate, precise and repeatable reverse phase high performance liquid chromatography method for the determination of Blonanserin in synthetic mixture. The sample was analyzed by reverse phase C_{18} column (Phenomenex C_{18}, 250 mm × 4.6 mm, 5µ) as stationary phase; acetonitrile : methanol : phosphate buffer, pH 3.0 (10 : 70 : 20 , v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. Quantification was achieved with Photo Diode Array detector at 238 nm. The retention time for Blonanserin was found to be 5.11 min. The linearity for the drug was obtained in the concentration range of 1-8 µg/ml with mean accuracies 99.89 ± 0.79. The method was successfully applied to synthetic mixture because no chromatographic interferences from formulation excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Blonanserin, Atypical antipsychotic, RP-HPLC, Synthetic mixture, Method validation.

Introduction

Blonanserin (BLS) is a relatively new atypical antipsychotic, having dopamine D_2 and serotonin 5-HT_{2A} receptor antagonist properties for the treatment of schizophrenia. Blonanserin is chemically, 2-(4-ethylpiperazin-1-yl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta[b]pyridine[1,2]. Literature survey reveals spectrophotometric[3], UPLC[4] and HPLC[5] methods for the estimation of Blonanserin in biological fluids and in pharmaceutical formulations.. Blonanserin is not yet official in IP, USP, BP, JP and EP, hence no official method is available for the estimation of Blonanserin in pharmaceutical dosage forms. The present communication describes simple, more sensitive, more accurate and more precise RP-HPLC method than reported method for estimation of drug in synthetic mixture.

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Shimadzu (Japan) RP-HPLC instrument (LC-2010C_{HT}) equipped with Photo Diode Array (PDA) detector and LC-solution software,

Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) were used in the study.

Reagents and materials

BLS bulk powder was kindly supplied as a gift samples from Astron research limited, Ahmedabad, Gujarat, India. The synthetic mixture containing 4 mg BLS was prepared in the laboratory using pharmaceutical excipients. Acetonitrile, Methanol, triple distilled water (S. D. Fine Chemicals Ltd., Mumbai, India) used were of HPLC grade. Potassium dihydrogen ortho-phosphate and Ortho-phosphoric acid (S.D Fine Chemicals Ltd., Mumbai, India) used were of AR grade. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Preparation of buffer solution

Phosphate buffer (0.02 M KH_2PO_4, pH 3.0) was prepared by dissolving accurately weighed 2.72 g of potassium dihydrogen phosphate in 1000 ml HPLC-grade water and the pH adjusted to 3.0 by diluted ortho-phosphoric acid.

Preparation of standard stock solutions

An accurately weighed quantity of BLS (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of BLS 100 µg/ml.

Chromatographic Condition

Stationary phase: C_{18} column (150 mm x 4.6 mm id., 5 µm).

Mobile phase: Acetonitrile: Methanol: Phosphate buffer pH 3.0

(10: 70: 20, v/v/v)

Flow rate: 1.0 ml/min

Injection volume: 20 µL

Temperature: 40 °C

Detection: At 238 nm using PDA detector.

Preparation of calibration curve

Accurately measured standard stock solutions of Blonanserin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 ml) were transferred to a series of 10 ml corning volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (20 µl) of each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated. Each response was average of three determinations.

Preparation of sample solution

The synthetic mixture containing 4 mg BLS was prepared in the laboratory. A quantity of powder equivalent to 4 mg of BLS was transferred to a 100 ml volumetric flask. Methanol (50 ml) was added and sonicated for 15 min. The flask was allowed to stand for 5 min at room temperature and the volume was adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with mobile phase to get a final concentration of 4 µg/ml of BLS. An aliquot (20 µl) of sample solution was injected under the operating chromatographic condition as described above and responses were recorded. The analysis procedure was repeated three times with synthetic mixture.

Method Validation

The method was validated in compliance with ICH guidelines[6].

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recovery of BLS by the standard addition method. Known amounts of standard solutions of BLS were added at 50, 100 and 150 % level to prequantified sample solutions of BLS (2 µg/ml). The amount of BLS was estimated by applying obtained values to the respective regression line equations.

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of BLS (5 µg/ml) without changing the parameters.
Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of BLS (2, 4 and 6 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[6].
$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Robustness

The robustness was studied by analyzing the same samples of BLS by deliberate variations in the method parameters. The change in the responses of BLS was noted. Robustness of the method was studied by changing the extraction time of BLS from synthetic mixture by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 2 ml/min and column oven temperature by± 2 ^{o}C. The parameters used in system suitability test were asymmetry of the chromatographic peak, tailing factor and theoretical plates, as RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for BLS was obtained with a mobile phase comprising of acetonitrile: methanol: phosphate buffer, pH 3.0 (10: 70: 20, v/v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 238 nm based on peak area. The peak with clear baseline was obtained (Figure 1). The retention time for BLS was found to be 5.11 min, respectively (Figure 1). Linear correlation was obtained between peak area versus concentrations of BLS in the concentration ranges of 1-8 µg/ml (Table 1) (Figure 2). The method was found to be specific as no significant changes in the responses of BLS was observed after 24 h. The mean recoveries obtained were 99.89 ± 0.79 % for BLS (Table 1 and 2), which indicates accuracy of the proposed method. The % RSD value for BLS was found to be <2 %, which indicates that the proposed method is repeatable. The low % RSD values of interday (0.94 – 1.17 %) and intraday (0.47 – 1.06 %) variations for BLS, reveal that the proposed method is precise. LOD value for BLS was found to be 0.1637 µg/ml and LOQ value for BLS was found to be 0.4963 µg/ml (Table 1). These data show that the proposed method is sensitive for the determination of BLS. The results of system suitability testing are given in Table 3. The amount of BLS present in the sample solutions were determined by fitting the responses into the regression equation of the calibration curve for BLS and the result obtained was comparable with the corresponding labeled claim (Table 4).

CONCLUSION

A simple, sensitive, repeatable and specific RP-HPLC method has been developed for the estimation of Blonanserin using a PDA detector. The method was validated for accuracy, precision, linearity, specificity, LOD & LOQ and robustness. In this proposed method the linearity is observed in the concentration range of 1-8 µg/ml with co-efficient of correlation, (r^2) = 0.9997 BLS at 238 nm. The result of the analysis of synthetic mixture by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the BLS in pharmaceutical dosage form without any interference of excipients.

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Table 1 Regression analysis data and summary of validation parameter for the proposed RP-HPLC method

Parameters	RP-HPLC method
Concentration range (µg/ml)	1-8
Slope	42463
Intercept	5840
Correlation coefficient	0.9997
LOD[a] (µg/ml)	0.1637
LOQ[b] (µg/ml)	0.4963
Accuracy (n[c] = 3)	99.89 ± 0.79
Repeatability (% RSD[d], n = 6)	0.83
Precision (%RSD)	
Interday (*n* = 3)	0.94-1.17 %
Intraday (*n* = 3)	0.47-1.06 %

a = Limit of detection b = Limit of quantification

c = number of determinations d = Relative standard deviation

Table 2 Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± % RSD (n=3)
BLS	I	2	50 %	99.71 ± 0.76
	II	2	100 %	100.19 ± 0.75
	III	2	150 %	99.79 ± 0.87

Table 3 System suitability test parameters for BLS for the proposed RP-HPLC method

Parameters	BLS ± % RSD (n = 6)
Retention time (min)	5.11 ± 0.25
Tailing factor	1.39 ± 0.31
Capacity factor	3.29 ± 0.65
Theoretical plates	2811.406 ± 1.27

Table 4 Analysis of synthetic mixture of BLS by proposed RP-HPLC method (n = 3)

Formulation	Label claim (mg)	Amount found (mg)	% Label claim ± % RSD (n=3)
Synthetic mixture	4	3.9936	99.84 ± 0.56

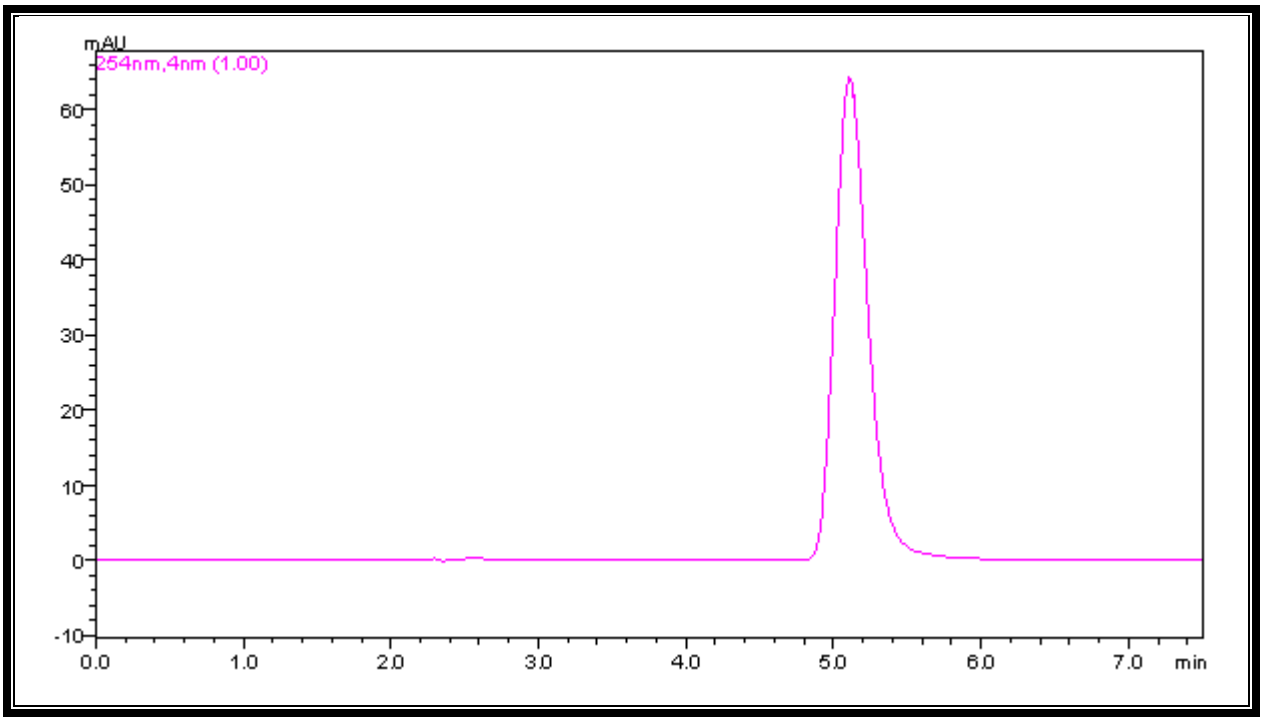

Figure 1. Chromatogram of BLS (5 µg/ml) at 238 nm

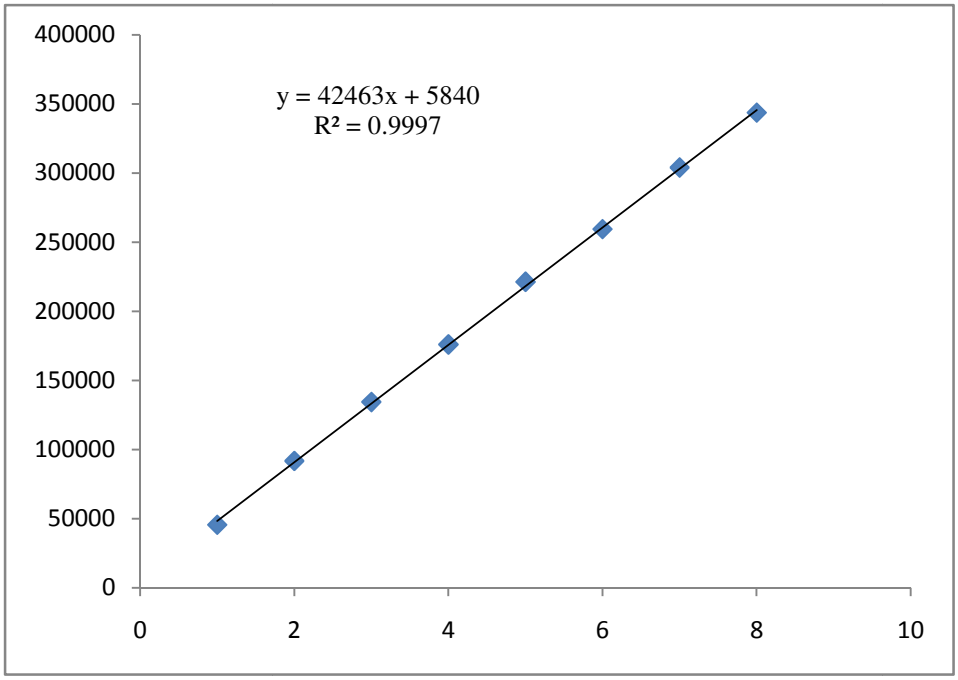

Figure 2. Linearity curve of Blonanserin (1-8 µg/ml)