RESEARCH ARTICLE

Reverse Phase High Performance Liquid Chromatography Method Development and Validation for Estimation of Flibanserin in Bulk and Dosage Form

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Received: 25th July, 2020; Revised: 27th September, 2020; Accepted: 26th October, 2020; Available Online: 25th Decemeber, 2020

ABSTRACT

The Present study described new, simple, accurate precise, development for estimation of Flibanserin by reverse phase high performance liquid chromatography (RP-HPLC) method.

The chromatographic method standardized using C18 column (Inertsil ODS 3, 250 x 4.6mm x 5 µ) and mobile phase containing 0.01M Ammonium acetate buffer pH 5(pH adjusted with GAA): acetonitrile (30:70 v/v) at flow rate of 1mL/min the eluents were detected by DAD detector at 257 nm. The retention time was found to be 5.9. The system suitability parameters for Flibanserin such as theoretical plates and tailing factor, were found 15724 &1.03 respectively. The linearity study of Flibanserin was found in a concentration range of 1–3 µg/mL and correlation coefficient (r2) was found to be 0.9995, % recovery was found to be at each level was 99.76–100.73%

% RSD for interday and intraday precision was found 0.74–0.48%. The analytical method was validated and applied on a marketed formulation.

Keywords: Flibanserin, RP-HPLC, Acetonitrile: Ammonium acetate buffer (70:30).

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.4.8

How to cite this article: Manjaramkar DS, Hamrapurkar PD, Kaple SR. Reverse Phase High Performance Liquid Chromatography Method Development and Validation for Estimation of Flibanserin in Bulk and Dosage Form. International Journal of Pharmaceutical Quality Assurance. 2020;11(4):501-504.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The Project entitled new simple method development and validation of assay method by RP-HPLC for determination of Flibanserin. The literature survey reveals the HPLC method is available for the estimation of Flibanserin from bulk drugs and dosage form. Objective of this work for the development of new, simple, rapid, accurate, efficient and reproducible RP-HPLC method by optimizing chromatographic conditions for the analysis of Flibanserin. The developed method was validated according to International Council for Harmonisation (ICH) guidelinesQ2 (R1) (Figure 1).[1]

Flibanserin is used to treat premenopausal women with developed, generalized hypoactive sexual desire disorder (HSDD). It is categorized by low sexual desire that causes interpersonal difficulty or marked distress. It is a serotonin 1A receptor agonist and a serotonin 2A receptor antagonist, but the mechanism by which the drug improves sexual desire and related distress is unknown.[2]

MATERIALS AND METHODS

Pure API was procured as gift samples from Symed Labs Limited, Hyderabad. HPLC grade Acetonitrile, Methanol and Water of Merck make was provided by Haffkine institute for training, research and testing, Mumbai. Buffer materials and all other chemicals were of analytical reagent grade used.

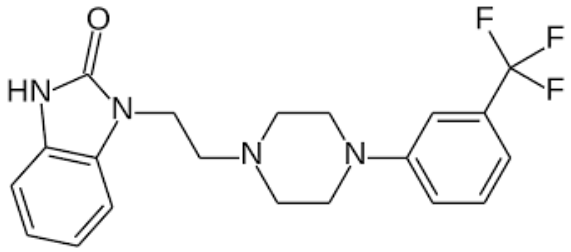

Figure 1: Structure of Flibanserin

IUPAC NAME: 1-(2-{4-[3-(trifluoromethyl) phenyl] piperazin-1-yl} ethyl)-2,3-dihydro-1H-1,3-benzodiazol-2-one. Its molecular formula is $C_{20}H_{21}F_3N_4O$ and its molecular weight is 390.4021 g/mol. [(3)]

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Equipment

Thermo Fisher HPLC equipped with dionex ultimate 3000 quaternary gradient HPLC Pump and Integrator Chromeleon 6.8 software. The chromatographic separations were performed using Inertsil ODS-3 C18 Column (250 × 4.6mm, 5 µ) column, Analytical Balance (AKERN), pH meter (SCIENTIFIC SALES), and a sonicator (GRANT).

Method Development[4]

Various trials were conducted for the selection of mobile phase for the method development. The summary of these Trials is stated in Table 1.

PREPARATION OF SOLUTION

A) Mobile Phase Preparation: (Solution A: Solution B; 30:70)
i) Solution A: 0.77 g of Ammonium acetate was dissolved in 1000 mL water and pH was adjusted to 5 using glacial acetic acid. The resulting solution was sonicated and filtered using 0.45 µm membrane filter.
ii) Solution B: Acetonitrile HPLC grade.

Preparation of Mobile Phase

Mix a mixture of above solution A 300 mL (30%) and 700 mL of solution B (70%) and degassed in an ultrasonic water bath for 15 minutes.
B) Diluent: A mobile phase used as diluent.
C) Preparation of Standard Solution
10 mg of Flibanserin was accurately weighed and transferred to 10 mL of volumetric flask, add about 4 mL of methanol, sonicated to dissolve at room temperature and volume was makeup with methanol to give stock solution of 1000 µg/mL (solution A). A 1 mL of this solution A was further diluted to 10 mL with diluent to give a concentration of 100 µg/mL (solution B). A total of 1-mL of this solution B was further diluted to 10 mL of diluent to give a concentration of 10µg/mL (solution C). 2 mL of solution C was diluted to 10 mL of diluent to give final concentration of 2µg/mL (solution D).

Preparation of Sample Solution (Marketed formulation)

10 tablets of Flibanserin 100 mg each were accurately weighed and the average weight of a tablet was calculated. Then 10 tablets were finely powdered, and powder equivalent to 10 mg of Flibanserin was taken and transferred into a 10 mL volumetric flask. 5 mL of methanol was added and sonicated with occasional shaking for few minutes, and the volume was made upto the mark with methanol (solution A). A 1-mL of this solution A was further diluted to 10 mL with diluent to give a concentration of 100 µg/mL (solution B). A 1-mL of this solution B was further diluted with 10 mL diluent to give a concentration of 10 µg/mL (solution C). 2.0 mL of solution C was diluted with 10 mL of diluent to give a final concentration of 2.0 µg/mL (solution D).

METHOD VALIDATION[1]

The developed method was validated on the parameters such as system suitability, specificity, linearity, precision, accuracy and limit of detection (LoD) and limit of quantification (LoQ) in accordance with the Specifications of ICH guidelines.

A) System Suitability

System suitability was performed by using a working concentration of Standard, i.e., 2 µg/mL concentration was prepared and six replicates were injected on HPLC. Here in this test %RSD of Peak area, retention time and theoretical plates were evaluated.

B) Specificity

The method's specificity was determined by recording the chromatogram of the working level of the of Flibanserin solution (2.0 µg/mL) and Blank chromatogram (only diluent). Specificity signifies the identification of analyte, interference from other peaks.

C) Linearity and Range

The method's linearity was evaluated in the range of 1.0µg/mL–3.0µg/mL for Flibanserin. Drug levels of these concentrations were prepared and each linearity level was injected into HPLC, chromatograms and peak area was recorded for all the peaks. The calibration curve was plotted as the mean peak area of the analyte against the concentration of the drug in µg/mL.

D) Precision

The standard solution was injected of 3 concentration 1, 2, 3µg/mL, i.e., 50, 100, 150%, respectively, and measured the area for all injections in HPLC. The same procedure followed and a new sample was prepared at different time interval injected in HPLC for intraday precision and by repetition on the next day (i.e., Interday precision). The %RSD for the area of each concentration injection was calculated.

E) Accuracy

The % recovery study was performed by using minimum three concentration levels, each in triplicate determinations. It was carried out by adding 50, 100, and 150% of nominal concentration to blank diluent of Flibanserin standard in triplicates.

F) Limit of Detection and Limit of Quantification

The LoD and LoQ of the developed method were determined by injecting progressively low concentrations of the Standard

Table 1: Experimental Trials for choice of Mobile Phase.

Mobile Phase composition	*Observation*	*Inference*
Water: Methanol	No precision in retention time, and broad peak	Use of acetonitrile required for improving Peak shape
Water: Acetonitrile	Improvement in peak shape, but no precision in retention time.	Use of buffer required to improve precision in retention time.
Buffer: Acetonitrile	Precision in retention time with good peak shape and resolution.	Chosen for optimization of the method.

Table 2: Optimized chromatographic conditions

Parameter	*Specification*
HPLC Instrumentation	Thermo Fisher HPLC equipped with Dionex Ultimate 3000 Quaternary Gradient HPLC Pump, with DAD Detector and Chromeleon 6.8 software
Column	Inertsil ODS-3 C18 Column (250x4.6mm, 5 µ)
Wavelength	257 nm
Injection loop	10 µL
Mob Phase	0.01M Ammonium Acetate Buffer (pH 5): Acetonitrile (30:70 v/v)
Flow Rate	1mL /min
Diluent	Mobile phase
Elution pattern	Isocratic
Column temperature:	28*C
Run time:	18 min

Table 3: System suitability data for Flibanserin

Sr. No.	*System suitability parameters*	*Observations*	*Acceptance criteria*
1	Flibanserin Standard Solution	2.00 µg/mL	--
2	Area % RSD	0.75%	NMT 2%
3	Retention Time	5.9	--
4	NTP	15724	NTP > 2000
5	Symmetry Factor	1.03	0.8-2.0

Table 4: Accuracy data of Flibanserin.

Sr. No	*Concentration (µg/mL)*	*% Recovery*
1	1	100.73
2	2	100.46
3	3	99.76

solution of Flibanserin using the developed HPLC method. This was done until a signal to noise ratio of NLT 3:1 and NLT 10:1 is maintained for LoD and LoQ, respectively.

RESULTS AND DISCUSSION

The summary of optimized chromatographic conditions is stated in Table 2

A) System Suitability

The Flibanserin Standard Solution of 2.0µg/mL was injected in six replicates. The mean of System Suitability parameters were obtained and are summarized in Table 3.

B) Specificity

The method was quite selective for Flibanserin as there was no other interfering peak seen around the retention time of Flibanserin (Rt-5.9 minutes). Also, the baseline did not show any significant peak. Thus, the method was found to be highly specific for Flibanserin Representative chromatogram for a blank in Figure 2 and Flibanserin standard is in Figure 3.

C) Linearity and Range:

The linearity was confirmed within the range 1.00µg/mL – 3.00µg/mL. The Correlation Coefficient (r^2) was found to be 0.9995 and the equation of the line was found to be y = 0.4436x–0.0183 as evident from the calibration curve. Thus, the data showed that the response to be linear. This clearly indicates that an excellent correlation existed between the peak

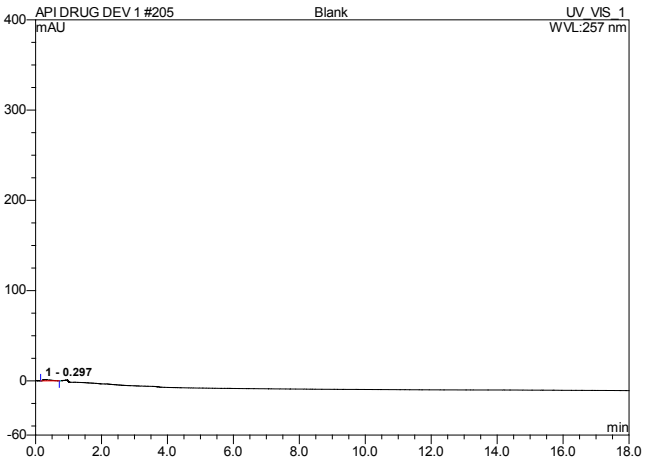

Figure 2: Chromatogram for blank.

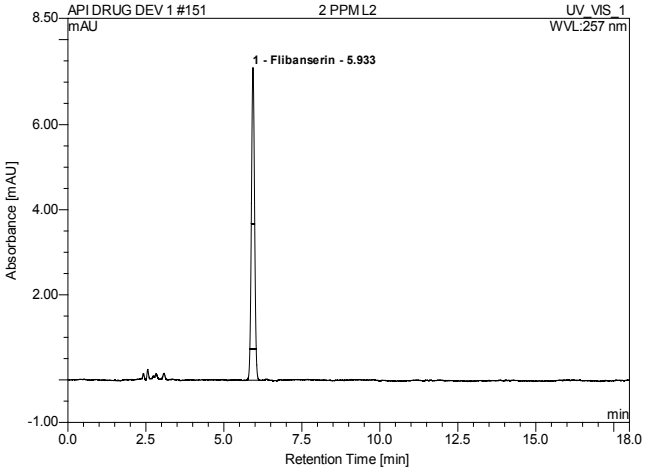

Figure 3: Chromatogram of Flibanserin standard (2.0µg/mL)

Table 5: Assay of the marketed formulation.

Sample	*% Assay*
Marketed formulation	98.36

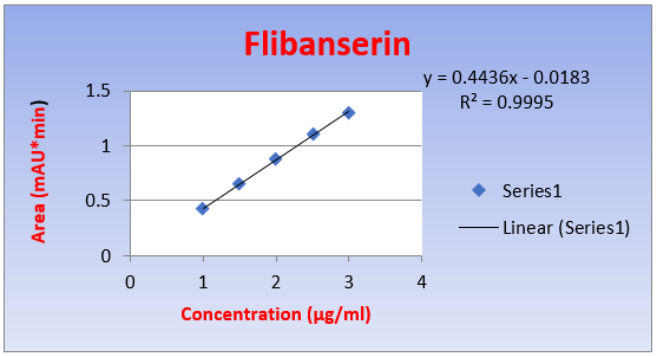

Figure 4: Calibration curve for Flibanserin

area and concentration of the analyte. The calibration curve is shown in Figure 4.

D) Precision

Intraday Precision (Repeatability) of Flibanserin was determined by taking six replicates of 50, 100 and 150% of working level i.e. 1.0, 2.0 and 3.0 µg/mL concentration at different time intervals and Interday Precision (Intermediate Precision) by six replicates of same 3 concentration on two consecutive days. The % RSD values for Intraday and Interday Precision was found 0.48% and 0.74%.

E) Accuracy

% Recovery study was performed using a minimum of 3 concentration levels, each in triplicate determinations. It was carried out by spiking 50, 100 and 150% of nominal level concentration to blank diluent of Flibanserin in triplicates. Results for % Recovery are summarized below.

F) Limit of Quantification and Limit of detection

The LoD value for Flibanserin was found to be 0.10 µg/mL and the LoQ value 0.50 µg/mL, respectively. This proved that the method developed to be sensitive

APPLICATION OF DEVELOPED AND VALIDATED METHOD ON MARKETED FORMULATION

The validated method was successfully applied to estimate Flibanserin from the marketed formulation. The representative chromatogram for the Marketed formulation of Flibanserin is shown in Figure 5 and the assay result is stated in Table 5.

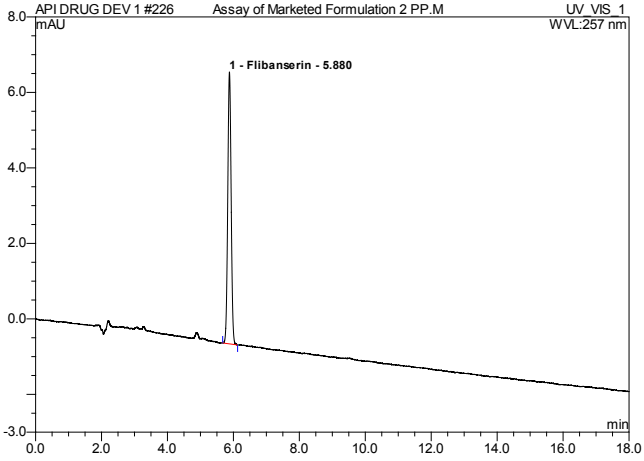

Figure 5: Chromatogram of Marketed Formulation of Flibanserin

CONCLUSION

The RP-HPLC assay method developed for the determination of Flibanserin is linear, accurate, precise, rapid and specific, as evident from the validation results.

ACKNOWLEDGEMENT

The authors are grateful to Symed Labs Hyderabad for providing Pure Flibanserin API as a gift sample, and authors are grateful to the staff members of the chemotherapy department of Haffkine Institute for training, research and testing for providing facilities for the completion of the project.

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