IJPSR (2024), Volume 15, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 July 2024; received in revised form, 06 August 2024; accepted, 24 October 2024; published 01 December 2024

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF VIBEGRON IN PURE AND PHARMACEUTICAL DOSAGE FORM

S. Naazneen

St. Mary's College of Pharmacy, Secunderabad - 500025, Telangana, India.

Keywords:

Vibegron, Isocratic, RP-HPLC, Ammonium dihydrogen orthophosphate, Acetonitrile

Correspondence to Author:
Dr. S. Naazneen

Associate Professor,
St. Mary's College of Pharmacy,
Secunderabad - 500025, Telangana, India.

E-mail: umenaaz@gmail.com

ABSTRACT: An accurate, sensitive, precise, fast isocratic reverse phase HPLC technique has been developed and validated for the quantification of Vibegron in the pure and pharmaceutical dosage form. The best separation was achieved on a 250 mm x 4.6 mm ID., 5µ-particle size Inertsil®-Octadecyl-Silyl-3V-Reverse-Phase-C_{18}-column with 0.05M Ammonium dihydrogen orthophosphate in water: acetonitrile (60:40 v/ v) as mobile phase solvent at a speed of 1.0 ml/ min in the isocratic mode of elution. UV detection was observed at 238 nm. Retention time of Vibegron was found to be 4.0 minutes. With a correlation coefficient of about 0.9989, peak-response was obtained as function of concentration of Vibegron over the range of 80 to 240 µg/ ml. Vibegron had shown to have a percentage assay of 110.05 %. It had a limit of detection (LOD) and a limit of quantification (LOQ) of 0.1 µg/ ml and 0.3 µg/ ml, respectively. The presence of excipients in the formulation had no effect on the assay method. The procedure is swift and precise and can be employed appropriately for use in QC- laboratories.

INTRODUCTION: The IUPAC name of Vibegron is (6S)-N-[4-[[(2S, 5R)-5-[(R)-hydroxy (phenyl) methyl] pyrrolidin-2-yl] methyl] phenyl]-4-oxo-7, 8-dihydro-6H-pyrrolo[1,2-a]pyrimidine-6-carboxamide, it is having a molecular formula of $C_{26}H_{28}N_4O_3$ and molecular weight of 444.5g/mol [1]. It is a potent, selective beta-3 adrenergic receptor (β3) agonist used to treat overactive bladder which causes a frequent and sudden urge to urinate that is hard to control. It acts by relaxing the detrusor smooth muscle of the bladder while it is filling, thereby increasing bladder capacity [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(12).3617-22

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(12).3617-22

It was approved by the US Food and Drug Administration (FDA) in December 2020 and launched under the brand name Gemtesa® the following year 2021 [3]. Once Vibegron binds to the receptor, β3AR is stimulated and undergoes a conformational change and activates adenylyl cyclases (AC), which promotes the formation of cyclic adenosine monophosphate (cAMP).

Increased intracellular cAMP concentration leads to the activation of cAMP-dependent protein kinase A (PKA), which subsequently phosphorylates myosin light chains that are responsible for inhibiting the interaction of actin with myosin dependent on calcium – calmodulin complex [4]. Unlike Mirabegron, Vibegron is less likely to be associated with drug-drug interactions involving the CYP3A4, 2D6, or 2C9 enzymes [5]. Vibegron is sparingly soluble in water. Its solubility increases in acidic condition [6].

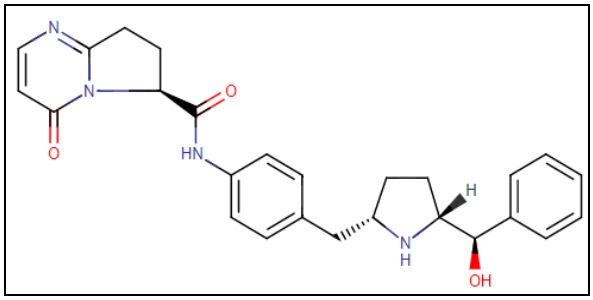

FIG. 1: CHEMICAL STRUCTURE OF VIBEGRON

A detailed literature survey revealed that only one method is reported previously to determine Vibegron simultaneously with Mirabegron by LCMS/MS method in biological fluids [7]. Nevertheless, no techniques for the determination of Vibegron in oral fixed dosage form have been published. Furthermore, no official or preliminary monograph on Vibegron has been published in any of the compendial pharmacopoeias. The goal of this study was to develop an accurate and efficient RP-HPLC method to estimate Vibegron in fixed dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards [8].

MATERIALS AND METHODS:

Chemicals and Reagents: The standard Vibegron (purity 99.7%) was obtained from SimSon Life Sciences Pvt. Ltd. Hyderabad, India. Marketted tablets with brand name Gemtesa® were obtained from the local market. HPLC-grade water and HPLC grade Acetonitrile were procured from Rankem Chemicals. Ammonium dihydrogen orthophosphate 85% (v/v) AR grade was purchased from Merck Company.

Chromatographic-Instrument: Quantitative HPLC was carried out on a Waters 2996 high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20 microliters injection volume and a quadra-pump. The column utilized was a Reverse Phase Inertsil Octa-Decyl-Silane-3V-C_{18} column (250mmx4.6 mm internal diameter with particle size 5µm). Empower Software was installed on the HPLC equipment. Ambient column temperature was maintained and eluted over 8.0 minutes at a mobile solvent speed of 1.0 ml/ min under isocratic condition. The mobile phase is 0.05M ammonium dihydrogen orthophosphate in water: acetonitrile (60:40 v/ v). It was degassed and filtered *via* 0.45µ Nylon membrane filters before use. An ultrasonic was used for the sonication of the mobile phase, standard solution and sample solution. For Vibegron, UV detection at 238 nm was used as the wavelength of detection with a PDA detector. Acetonitrile and water in the ratio of 40:60 (v/v) was used as diluent to make the standard dilutions. Vibegron was eluted at 4.0 min.

Preparation of the Primary Standard Drug Solutions: To make the primary standard stock solution, 200mg of Vibegron, was dissolved in a volumetric flask (100ml) with 20ml of diluent (40:60 v/v CH_3CN: H_2O), sonicated for 15 minutes, and then brought up to 100ml with diluent to get the primary standard stock solution containing 200µg/ ml of Vibegron.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50-ml volumetric flask, the flask was filled with 50 ml of diluent. This resultant solution, which includes 200 ug/ml of Vibegron is suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled at four degrees Celsius.

Sample Preparation: After measuring the weight of each individual tablet, we were able to calculate the average weight of twenty Gemtesa® pills. After weighing twenty tablets of Vibegron, they were crushed in a mortar and pestle and powdered. Tablet powder equivalent to 200.0 mg of Vibegron was weighed and transferred to 100 ml pre-calibrated volumetric flask and dissolved in a blend of acetonitrile and aqueous media with a volumetric ratio of 40:60 (v/v).

It was then sonicated for 15 minutes in the diluent and filtered via 0.45 µm PTFE filter, so as to get a primary working sample solution having concentration 2000 µg/ ml of Vibegron. After quantitatively transferring 5ml of the filtrate to a 50 ml precalibrated measuring flask, the diluent was added to bring the volume of the solution to 50.0 ml. This solution serves as a working testing solution having 200 µg/ ml of Vibegron. The stock solutions were kept in a dark place at 4 degrees centigrade.

DISCUSSION AND RESULTS: The purpose of this research was to create a chromatographic technique for the quantifiable determination of fixed-dose of Vibegron.

Optimized Chromatographic Conditions: The chromatographic conditions were optimized finally using 0.05M Ammonium dihydrogen orthophosphate in water: acetonitrile (60:40 v/ v) as the eluting solvents in isocratic mode at a flow rate of 1.0 ml/min with an injection volume of 20 µL.

Run time was 8 minutes, at an ambient column oven temp with acetonitrile and water in the ratio of 40:60 v/ v, sonicated and degassed, as the diluent in a Inertsil reverse phase C_{18} column (250mm x 4.6 mm internal diameter with particle size 5µm). The detection was done by Photo diode array (PDA) detector at a wavelength of 238nm. The retention time was found to be 4.0 min.

Linearity: Aliquots of Vibegron working stock solutions were placed in various 10ml volumetric flasks and made the volume up to the 10ml with the mobile phase, yielding in final strengths of 80- 240 µg/ ml **Table 2.**

The peak areas and retention times of the drug solution (loaded at 20µl) were measured thrice in the column. Using a PDA-detector set at 238 nm, a linearity-graph was generated by plotting peak areas-vs- Vibegron concentrations in µg/ ml.

Accuracy: The approach's accuracy was found by evaluating the drugs' recovery using the standard-spiking method. To assess if the analytes contained in the formulation caused positive or negative interventions, known amounts of each drug equivalent to 10 percent standard drug solution were added to 80 percent, 100 percent, and 120 percent of the target test concentrations a formulation mixture. Each set-of-addition was replicated thrice at each dilution level.

The results were compared to a competent reference standard after extraction of sample preparation. The percentage of analytes recovered by the assay was used to assess the accuracy. **Table 3** shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/ v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability is indicated by the low RSD value (1%) **Table 4.**

Limits of Detection and Quantification: The method's LOD was set at the lowest concentrations of active pharmaceutical components with a signal-to-noise (S/N) ratio of around 3. (LOD). The lowest active therapeutic medication concentrations that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ).

Method Applicability: In order to assess the newly developed approach, Vibegron was estimated using pharmaceutical tablets in this investigation.

Optimization of Chromatographic Conditions: An isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, reproducible, and quick-to-use method for the determination of Vibegron concentrations in pharmaceutical dosage forms. The impact of various HPLC technique variables were examined on the result of the study to optimize the chromatographic parameters. Various proportions of CH_3CN:H_2O, CH_3CN: *O*-H_3PO_3 and CH_3CN:KH_2PO_4, $NH_4H_2PO_4$:CH_3CN buffer were tested. After several early investigatory tests, 0.05M Ammonium dihydrogen orthophosphate in water: acetonitrile (60:40 v/ v) was chosen over other mobile phases because it resulted in improved peak shape of the active component.

This procedure gives the good chromatographic detection of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 238 nm. The analyte peak was highly defined and without any incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests: Method precision (RSD, percent), method accuracy (recovery percent & %RSD), linear range (r^2), and LOD & LOQ were explored as recommended method validation characteristics.

Linearity: With a correlation coefficient of 0.998, the graph of chromatographic-peak areas of the analyte versus respective concentrations was shown to be linear in the band of 80.0-240 µg/ ml for Vibegron **Table 2**. The least square fit data of linear regression analysis was derived from the measurements and is given in **Table 1**. The regression line for Vibegron is y = 86313x + 88516. **Table 1** presents the regression parameters for this technique that include slope, intercept and % RSD. These findings suggest that there was a significant correlation.

Accuracy: Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 94.60% to 102.4% for Vibegron, demonstrating the method's accuracy. The RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results **Table 3.**

Precision: Table 4 summarizes the intraday and interday fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs.

Limit-of-Detection & Limit-of-Quantifications: Vibegron has a limit of detection (LOD) and limit of quantification (LOQ) of 0.1 µg/ ml and 0.3 µg/ ml respectively. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify analytes over a wide concentration range.

Specificity: The retention time for Vibegron was determined to be 4.0 minutes, according to the representative chromatogram given in **Fig. 1**.

When the pharmaceutical tablets were evaluated, no indication of excipient interference signals was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed by any probable merging peaks. As a result, this technique can be employed with certainty.

TABLE 1: OPERATING-SYSTEM SUITABILITY RESULTS

Study-Parameter	Vibegron
Retention Time (min)	4.0
Peak areas	8864953
Percentage of peak areas	99.94
USP-Tailing	1.28
Theoretical Plates	5998.04
Resolution	3.27
Linear range (µg/ml)	80.0-240.0
Limit-of-Detection (µg/ml)	0.1
Limit of Quantification (µg/ml)	0.3
Correlation-Coefficient (r^2)	0.9989
Assay-in-Percentage (%)	110.05

TABLE 2: STANDARD CALIBRATION CURVE FOR LINEARITY EXPERIMENT

Calibration Standard Dilution Level	Concentration of Vibegron (µg/ml)	Peak Area
40 %	80	3494463
60 %	120	5367711
80%	160	6992239
100 %	200	8607390
120 %	240	10505905

TABLE 3: ACCURACY EVALUATION BY SPIKE-ANALYSIS METHOD

Accuracy study at 80% target level	Injection Number	Vibegron (Gemtesa-®) Area	
		Standard Soln.	Spiked Soln.
Gemtesa-® tablet dosage form solution at 80% level was spiked with 10% of standard solution of Vibegron	1	7029652	7893957
	2	7045251	7875675
	3	7055538	7863796
	Mean area	7043480.3	7877809.3
	Std. Dev	13033.9	15193.0
	% RSD	0.2	0.2
	%Recovery		94.60
80% of the target concentration is equivalent to Vibegron160 µg/ml in acetonitrile and water in the ratio of 40: 60 v/ v) as the diluent			
Accuracy study at 100% target level	Injection Number	Vibegron (Gemtesa-®) Area	
		Standard Soln.	Spiked Soln.

Gemtesa-® tablet dosage form solution at 100% level was spiked with 10% of standard solution of Vibegron	1	8717049	9683958
	2	8746621	9582060
	3	8748010	9608765
	Mean area	8737226.8	9624927.7
	Std. Dev	17488.0	52836.7
	% RSD	0.2	0.5
	% Recovery		101.6
100% of the target concentration is equivalent to Vibegron 200 µg/ml in acetonitrile and water in the ratio of 40: 60 v/ v) as the diluent			
Accuracy study at 120% target level	Injection Number	Vibegron (Gemtesa-®) Area	
		Standard Soln.	Spiked Soln.
Gemtesa-® tablet dosage form solution at 120% level was spiked with 10% of standard solution of Vibegron	1	10447002	11327423
	2	10463601	11338408
	3	10415940	11343795
	Mean area	10442180.9	11336541.7
	Std. Dev	24193.1	8344.0
	% RSD	0.2	0.1
	%Recovery		102.4
120% of the target concentration is equivalent to Vibegron240 µg/ml in acetonitrile and water in the ratio of 40: 60 v/ v) as the diluent			

TABLE 4: EVALUATION OF PRECISION ANALYSIS

Intra-Day Precision study of 100% standard dilution containing 200µg/ ml of Vibegron			**Inter-Day Precision study of 100% standard dilution containing 200µg/ ml of Vibegron**	
S. no.	**Vibegron**		**Vibegron**	
	Ret. time	**Peak area**	**Ret. time**	**Peak area**
1	4.04	8671982	4.04	8736646
2	4.03	864220	4.04	8736646
3	4.04	8656138	4.04	8738289
4	4.04	8651714	4.07	8723406
5	4.04	8649765	4.07	8683831
6	4.04	8646644	4.06	8733638
Average	4.04	8646644	4.1	8723410.4
Std. Dev	0.0	10384.8	0.0	20343.1
% RSD	0.1	0.1	0.4	0.2

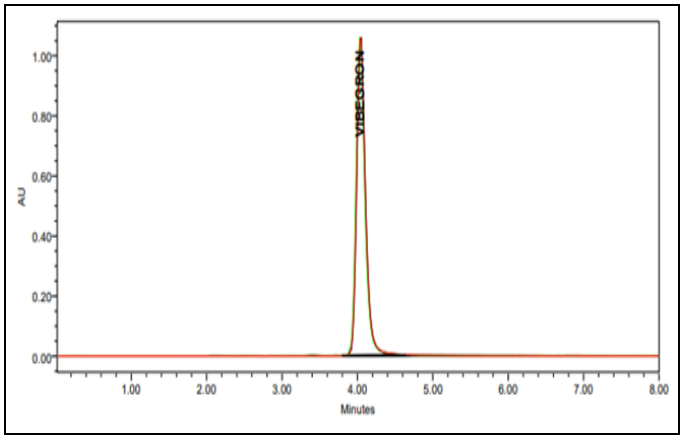

FIG. 2: STANDARD CHROMATOGRAM OF VIBEGRON

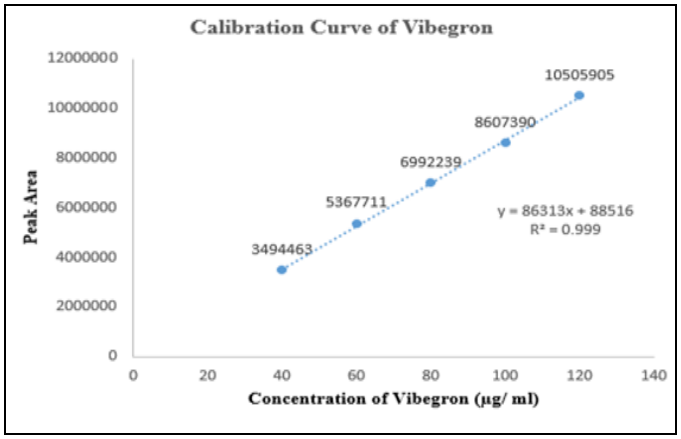

FIG. 3: LINEARITY GRAPH OF VIBEGRON OF STANDARD DILUTIONS

CONCLUSION: An effective and widely accessible HPLC approach was developed in this study to analyze Vibegron in unit dosage form. The main benefits of this approach are its considerably shorter run time, as well as its simplicity in usage and operation. While operating, each of these aspects is essential, particularly when examining a big number of samples. The validation trials showed that the procedural approach has a practical reliable sensitivity, a vast calibration concentration range, and appropriate precision & accuracy. The approach makes it possible to evaluate Vibegron in

a clear, precise, sensitive and routine manner for formulation QC-studies.

ACKNOWLEDGEMENT: Author is grateful to the management of St. Mary's College of Pharmacy, Secunderabad, India for providing laboratory facility for this work.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 44472635, Vibegron. Retrieved 16 May 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Vibegron.
2. Frankel J, Staskin D, Varano S, Kennelly MJ, Jankowich RA and Haag-Molkenteller C: An evaluation of the efficacy and safety of vibegron in the treatment of overactive bladder. Ther Clin Risk Manag 2022; 18: 171–182.
3. Clinical Trials Arena. Projects July 21 2023. GEMTESA for the treatment of overactive bladder, US. Retrieved 16 May 2024 from https://www.clinicaltrialsarena.com/projects/gemtesa-vibegron-for-the-treatment-of-overactive-bladder-oab.
4. Drug Bank Online. DB14895. Vibegron. Mechanism of Action. Retrieved 02 June 2024 from https://go.drugbank.com/drugs/DB14895.
5. Schena G and Caplan MJ: Everything you always wanted to know about β3-AR. But Were Afraid to Ask. Cells 2019; 8(4): 357.
6. Center for Drug Evaluation and Research Application Number: 213006Orig1s000 Product Quality Review(S). NDA213006 Gemtesa (Vibegron) tablets Assessment #1. Executive Summary. Quality Assessment Overview. Retrieved on 5 May 2024 from https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/213006Orig1s000ChemR.pdf.
7. Lou Y, Cheng M, Cao Q, Li K, Qin H, Bao M, Zhang Y, Lin S and Zhang Y: Simultaneous quantification of Mirabegron and Vibegron in human plasma by HPLC-MS/MS and its application in the clinical determination in patients with tumors associated with overactive bladder. J Pharm Biomed Anal 2024; 240: 115937.
8. ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use, Geneva 2005; 1-13.

How to cite this article:
Naazneen S: RP-HPLC method development and validation for the estimation of vibegron in pure and pharmaceutical dosage form. Int J Pharm Sci & Res 2024; 15(12): 3617-22. doi: 10.13040/IJPSR.0975-8232.15(12).3617-22.