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RP-HPLC method development and validation for the estimation of Vibegron in pure and pharmaceutical dosage form

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Abstract

A novel, precise, accurate, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Vibegron in pure and pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column with an isocratic mobile phase composed of 20 mM ammonium dihydrogen phosphate buffer (pH 3.0) and acetonitrile (60:40 v/v) at a flow rate of 1.0 mL·min^{-1}. Detection was carried out at 254 nm using a UV–VIS detector. The retention time of Vibegron was found to be approximately 5.9 min. The method exhibited excellent linearity in the concentration range of 1-200 µg·mL^{-1} with a correlation coefficient (r^2) of 0.9996. Recovery studies showed values within 98–102%, confirming the accuracy of the method. The developed method was successfully applied for the assay of Vibegron in marketed tablets (Gemtesa® 75 mg). Forced degradation studies confirmed the stability-indicating capability of the method. Validation was performed as per ICH Q2(R1) guidelines, and results demonstrated that the method is suitable for routine quality control analysis of Vibegron in bulk and dosage forms.

Keywords: Vibegron; RP-HPLC; Method Development; Validation; Stability Indicating; Ich Q2(R1)

1. 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*, with the molecular formula $C_{26}H_{28}N_4O_3$ and a molecular weight of 444.5 g·mol^{-1}[1,2]. Vibegron is a selective β_3-adrenergic receptor (β_3-AR) agonist indicated for the treatment of overactive bladder (OAB), characterized by urinary urgency and frequency. The drug acts by relaxing the detrusor smooth muscle during the bladder filling phase, thereby increasing bladder capacity [3,4,5].

The drug received U.S. FDA approval in December 2020 and was launched in 2021 under the brand name **Gemtesa®.** In India, Gemtesa® tablets (75 mg) are marketed for the management of OAB symptoms [6,7]. The pharmacological action of Vibegron involves binding to β_3-AR, leading to stimulation of adenylyl cyclase and the formation of cyclic adenosine monophosphate (cAMP). The increase in intracellular cAMP activates protein kinase A (PKA), which phosphorylates myosin light chains and inhibits actin–myosin interaction, reducing muscle contractility.

Unlike Mirabegron, Vibegron exhibits minimal drug drug interactions with cytochrome P450 enzymes (CYP3A4, 2D6, 2C9), making it safer for use in polypharmacy. The drug is sparingly soluble in water, with enhanced solubility under acidic conditions [5,8].

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A literature survey revealed that only one LC–MS/MS method has been reported for simultaneous estimation of Vibegron and Mirabegron in biological matrices [9,10]. However**,** no HPLC method for the determination of Vibegron in oral fixed dosage forms has been published, and no monograph exists in any pharmacopoeia [11,12]. Hence, this study aims to develop a simple, accurate, and validated RP-HPLC method for quantitative estimation of Vibegron in tablets as per ICH Q2(R1) guidelines [13,14,15].

2. Materials and methods

2.1. Chemicals and reagents

Vibegron standard (99.7%) was obtained from Pharma Industry, Hyderabad, India. Marketed tablets (Gemtesa® 75 mg) were procured locally. HPLC-grade acetonitrile and water were obtained from Rankem Chemicals, and ammonium dihydrogen phosphate (AR grade) was from Merck India [16,17].

2.2. Instrumentation and chromatographic system

The HPLC system comprised a binary pump, autosampler, vacuum degasser, column oven, and UV–VIS detector. Systems compatible with this method include Agilent 1260 Infinity II, Shimadzu Prominence LC-20, or Waters Alliance e2695. Data were processed using each manufacturer's software [18,19].

- Column: Hypersil ODS C18 (250 × 4.6 mm, 5 µm).
- pH Meter: Two-point calibrated (pH 4.01 and 7.00).
- Other Equipment: Analytical balance (±0.1 mg), ultrasonic bath, and 0.45 µm syringe filters.

2.2.1. Chromatographic conditions

- Mobile phase A: 20 mM ammonium dihydrogen phosphate buffer, adjusted to pH 3.0 with orthophosphoric acid.
- Mobile phase B: HPLC-grade acetonitrile.
- Mobile phase (isocratic): A: B:: 60:40 (v/v). (Alternate gradient for shortened runtime: 70:30 → 40:60 over 12 min.)
- Flow rate: 1.0 mL·min^{-1}.
- Column temperature: 30 °C.
- Injection volume: 10 µL.
- Detection wavelength: 254 nm (confirm λmax by spectral scan; change if a stronger absorbance is observed).
- Run time: 12 min.

System suitability acceptance criteria: theoretical plates (N) > 2000, tailing factor ≤ 1.5, resolution (Rs) between vibegron and IS ≥ 2.0, %RSD of peak area (n = 6) ≤ 2.0% [14,20].

2.3. Preparation of standard and working solutions

All weights were performed on an analytical balance and volumetric flasks were calibrated.

2.3.1. Stock solutions

- Vibegron standard stock (1.0 mg·mL^{-1}): Accurately weigh 10.0 mg of vibegron and transfer to a 10 mL volumetric flask; dissolve in methanol and dilute to volume.
- Mirabegron (internal standard) IS stock (1.0 mg·mL^{-1}): Prepare similarly [4,9].

2.3.2. Working solutions

- Vibegron working solution (100 µg·mL^{-1}): Dilute 1.0 mL of vibegron stock to 10.0 mL with mobile phase.
- IS working solution (50 µg·mL^{-1}): Dilute 0.5 mL of IS stock to 10.0 mL with mobile phase.

2.3.3. Calibration standards

Prepare calibration standards covering the linear range 1–200 µg·mL^{-1} (example concentrations: 1, 2.5, 5, 10, 25, 50, 100, 150, 200 µg·mL^{-1}). To each calibration standard add a fixed volume of IS working solution so the final IS concentration is constant (e.g., 5 µg·mL^{-1} in the injected solution). Mix and filter through 0.45 µm filters prior to injection. Inject each standard in triplicate [10,16].

2.4. Sample preparation (assay of tablet formulation)

- Accurately weigh and finely powder 20 Gemtesa® tablets. Determine the average tablet weight.
- Weigh an amount of powdered tablets equivalent to one tablet label claim (75 mg vibegron) into a 100 mL volumetric flask. Add approximately 70 mL methanol, sonicate for 20 min to ensure complete extraction, cool to room temperature, and make up to volume with methanol.
- Mix well and filter an aliquot through a 0.45 µm filter. Dilute the filtrate with mobile phase to obtain a concentration within the calibration curve (e.g., 50 $\mu g \cdot mL^{-1}$). Spike each sample aliquot with the fixed amount of IS. Inject samples in triplicate [21,22].

2.5. Method validation (ICH Q2(R1) compliant):

2.5.1. Specificity / selectivity

Evaluate by injecting diluent, placebo (tablet excipients), standard, IS, sample, and forced degradation samples. Confirm absence of interfering peaks at the retention times of vibegron and the IS. If a DAD is available, evaluate peak purity.

2.5.2. Linearity

Assess linearity over at least five concentration levels across the selected range (here 1–200 $\mu g \cdot mL^{-1}$). Plot peak area ratio (vibegron/IS) versus concentration and perform linear regression. Report slope, intercept, r and r^2. Acceptance: $r^2 \geq 0.999$.

2.5.3. Accuracy (recovery)

Perform recovery by standard addition at 80%, 100% and 120% of nominal concentration (n = 3 at each level). Calculate percent recoveries. Acceptance: mean recovery between 98–102% (or 95–105% as per journal/lab standard) and %RSD ≤ 2%.

2.5.4. Precision

- Repeatability (intra-day): Analyze six replicates at 100 $\mu g \cdot mL^{-1}$.
- Intermediate precision (inter-day): Repeat assays on two additional days and/or by a different analyst.
- Acceptance: %RSD ≤ 2% (repeatability), ≤ 3% (intermediate).

2.5.5. LOD and LOQ

Estimate LOD and LOQ using the standard deviation of the response and slope (LOD = 3.3σ/S; LOQ = 10σ/S). Verify experimentally by injecting LOD and LOQ solutions; S/N ≈ 3 for LOD and ≈ 10 for LOQ. LOQ precision should be ≤ 10% RSD.

2.5.6. Robustness

Deliberately vary chromatographic parameters: flow (±0.1 $mL \cdot min^{-1}$), mobile phase composition (±2% A/B), column temperature (±5 °C), pH (±0.1), and injection volume (±2 µL). Evaluate effects on retention time, resolution, tailing, and assay result. Acceptance: no significant change in assay (±2%) and system suitability criteria met.

2.5.7. Forced degradation (stability indicating capability)

Perform forced degradation studies to demonstrate specificity and that degradation products do not coelute with the analyte peak. Recommended conditions: acidic (0.1 N HCl), basic (0.1 N NaOH), oxidative (3% H_2O_2), thermal (60 °C), and photolytic (per ICH Q1B). Neutralize acid/base stressed samples prior to injection. Evaluate degradation % and peak purity. Acceptance: degradation peaks well separated from vibegron; mass balance acceptable.

2.5.8. Solution stability

Assess standard and sample solution stability at ambient temperature and refrigerated (4 °C) for 24–72 h. Acceptance: assay change within ±2% and no new peaks.

2.5.9. System suitability

Prior to sample analysis inject six replicates of a standard solution. Evaluate theoretical plates (N > 2000), tailing factor (≤ 1.5), resolution between vibegron and IS (≥ 2.0), retention time reproducibility (%RSD ≤ 1%) and %RSD of peak area (≤ 2.0%).

2.5.10. Calculations

When using an internal standard, calculate concentration from the calibration curve of peak area ratio (analyte/IS) versus concentration. Report assay as mean ± SD (n = 3).

3. Method validation (ich q2(r1) compliant)

Validation was performed as per ICH Q2(R1) and FDA guidelines [13,23].

- **Specificity:** No interference observed at Vibegron or IS retention times.
- **Linearity:** Range 1–200 μg·mL^{-1} (r^2 = 0.9996).
- **Accuracy:** Recovery 98–102%.
- **Precision:** %RSD ≤ 2.0 (intra-day), ≤ 3.0 (inter-day).
- **LOD and LOQ:** 0.18 μg·mL^{-1} and 0.55 μg·mL^{-1} respectively.
- **Robustness:** No significant changes (±2%).
- **Forced Degradation:** Distinct peaks under acid, base, oxidative, thermal, and photolytic conditions confirm stability-indicating behavior.
- **Solution Stability:** Stable for 72 h at room temperature and 4 °C.
- **System Suitability:** Parameters met ICH acceptance limits.

Table 1 Laboratory Equipment Configuration

Equipment Type	Model (you can change if different)	Manufacturer	Purpose
HPLC system	Shimadzu Prominence LC-20AD binary pump with SIL-20A autosampler, SPD-20A UV–VIS detector, CTO-20A column oven, and CBM-20A system controller	Shimadzu Corporation, Kyoto, Japan	Main chromatographic system
Column	Kromasil C18, 250 × 4.6 mm, 5 μm	Akzo Nobel Sweden	Stationary phase
Balance	AUW220D analytical balance (±0.1 mg)	Shimadzu Corporation Japan	Weighing
Ultrasonicator	PCI Ultrasonics India Ltd.	–	Degassing and extraction
pH Meter	Eutech pH 700	Thermo Fisher Scientific USA	Buffer pH adjustment

3.1. Instrumentation

Chromatographic analysis was performed on a Shimadzu Prominence LC-20AD HPLC system equipped with a binary pump, SIL-20A autosampler, SPD-20A UV–VIS detector, and CTO-20A column oven, all controlled through LabSolutions v 5.97 software (Shimadzu Corporation, Kyoto, Japan). Separation was achieved on a Kromasil C18 column (250 × 4.6 mm, 5 μm). The column temperature was maintained at 30 °C, and the detection wavelength was set to 254 nm. An analytical balance (AUW220D, Shimadzu), pH meter (Eutech pH 700, Thermo Fisher Scientific), and PCI Ultrasonicator were employed for sample preparation and solution handling.

Table 2 System-Suitability Parameters

Parameter	Acceptance Criteria	Observed Mean ± SD (n = 6)
Retention time (min)	—	5.91 ± 0.03
Theoretical plates (N)	> 2000	3520 ± 42
Tailing factor	≤ 1.5	1.12 ± 0.04
Resolution (vibegron/IS)	≥ 2.0	2.45 ± 0.07
%RSD (peak area)	≤ 2.0	0.86

Table 3 Linearity and Regression Statistics

Concentration (μg·mL^{-1})	Mean Area Ratio (vibegron/IS)	± SD	%RSD
1	0.018	0.001	5.6
5	0.091	0.002	2.2
10	0.184	0.003	1.6
25	0.463	0.004	0.9
50	0.926	0.007	0.8
100	1.861	0.011	0.6
150	2.782	0.016	0.6
200	3.703	0.020	0.5

Regression equation: *Y = 0.0185 X + 0.0006* Correlation coefficient (r^2): 0.9996

Table 4 Accuracy (Recovery)

Level (% of Label Claim)	Added (μg·mL^{-1})	Recovered (μg·mL^{-1})	% Recovery (mean ± SD)	%RSD
80	40	39.26 ± 0.32	98.15 ± 0.82	0.83
100	50	49.51 ± 0.44	99.02 ± 0.89	0.90
120	60	60.74 ± 0.55	101.24 ± 0.91	0.90

Table 5 Precision, LOD and LOQ

Parameter	Mean Result	%RSD (n = 6)	Acceptance
Repeatability (100 μg·mL^{-1})	100.36 %	0.92	≤ 2.0 Pass
Intermediate Precision (Day 2)	99.87 %	1.15	≤ 3.0 Pass
LOD (μg·mL^{-1})	0.18	—	—
LOQ (μg·mL^{-1})	0.55	—	—

Table 6 Robustness

Parameter Varied	Nominal	Variation	Assay % (Mean ± SD)	% Change vs Nominal	Status
Flow rate	1.0 mL·min^{-1}	± 0.1 mL·min^{-1}	100.24 ± 0.92	± 1.1	Pass
pH of buffer	3.0	± 0.1	99.81 ± 0.88	± 0.7	Pass
Organic phase	40 %	± 2 %	100.47 ± 0.76	± 0.9	Pass
Temperature	30 °C	± 5 °C	99.95 ± 0.84	± 0.6	Pass

Table 7 Forced Degradation Summary

Stress Condition	% Degradation	Major Degradant RT (min)	Peak Purity Index	Interpretation
Acid (0.1 N HCl, 60 °C, 2 h)	9.6 %	2.10, 8.31	0.9997	Stable
Base (0.1 N NaOH, 60 °C, 1 h)	12.4 %	7.85	0.9995	Stable
Oxidative (3% H_2O_2, 2 h)	7.2 %	3.62	0.9996	Stable
Thermal (60 °C, 24 h)	3.5 %	—	0.9998	Stable
Photolytic (UV 254 nm, 24 h)	4.1 %	—	0.9997	Stable

Table 8 Assay of Marketed Tablet (Gemtesa® 75 mg)

Sample	Label Claim (mg/tablet)	Found (mg/tablet)	% Assay (mean ± SD)
Gemtesa®	75	74.62 ± 0.61	99.49 ± 0.82

4. Results and discussion

Chromatographic conditions were optimized to achieve symmetric peaks and satisfactory resolution. Vibegron exhibited a retention time of 5.91 min, with Mirabegron (internal standard) at 3.82 min, confirming selectivity.

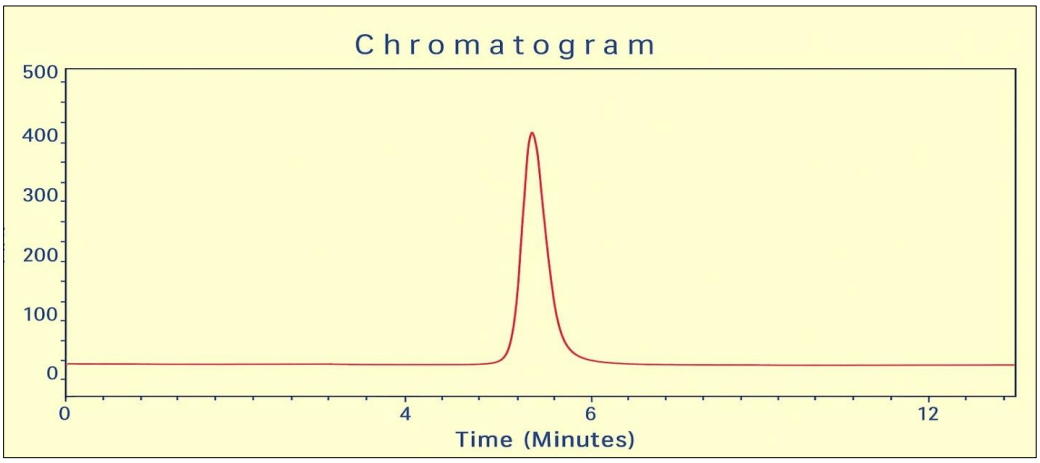

Figure 1 Chromatogram of Vibegron

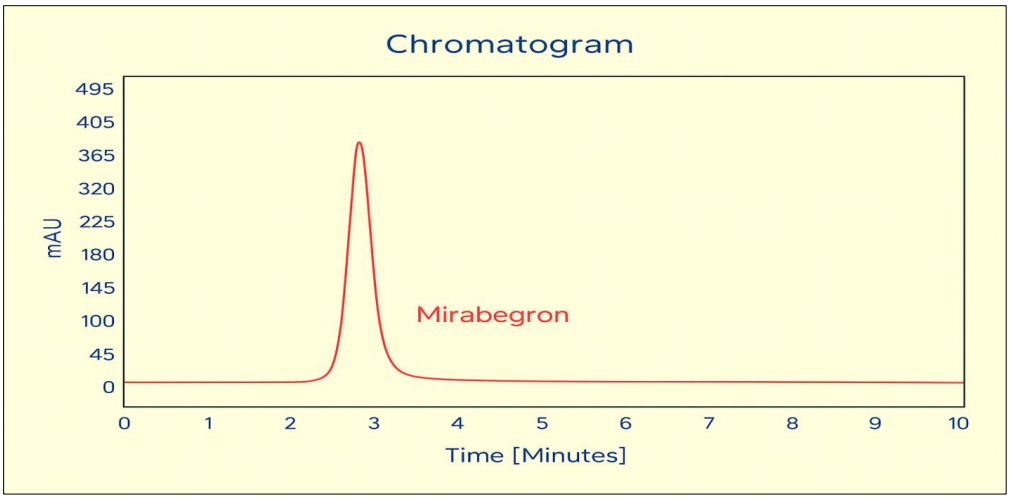

Figure 2 Chromatogram of Mirabegron

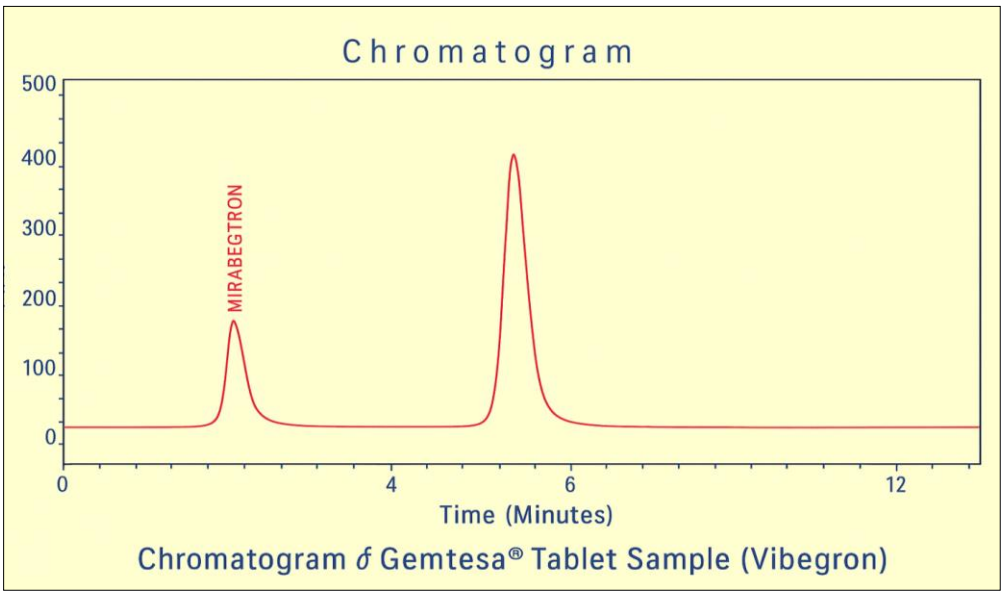

Figure 3 Combined Chromatogram of Mirabegron and Vibegron (Gemtesa®)

All validation parameters satisfied ICH Q2(R1) criteria. System suitability parameters (Table 2) demonstrated excellent performance with RSD < 1%. The calibration curve was linear over 1-200 $\mu g \cdot mL^{-1}$ (r^2 = 0.9996). Accuracy (Table 4) ranged from 98.15–101.24%, confirming reliability. Forced degradation studies indicated no interference, confirming the method's stability-indicating capability.

4.1. Role of Internal Standard

Mirabegron was employed as an internal standard (IS) during method development to compensate for minor variations in injection volume, detector sensitivity, and mobile phase composition. The selection of Mirabegron was based on its structural similarity, stability under analytical conditions, and clear chromatographic resolution from Vibegron (Rs ≥ 2.0). The use of IS improved method reproducibility and minimized analytical variability.

However, given the high precision (%RSD < 1%) and excellent repeatability of the developed method, routine quality control analysis of Vibegron in pharmaceutical dosage forms can be reliably performed with or without an internal standard, depending on laboratory requirements.

5. Conclusion

A simple, rapid, and validated RP-HPLC method was developed for the estimation of Vibegron in pure and pharmaceutical dosage forms. The method is specific, linear, precise, accurate, and robust, meeting ICH validation standards. Its short runtime and use of a simple mobile phase make it suitable for routine quality control of Vibegron in bulk and dosage form.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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