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ORIGINAL ARTICLE

Establishment and Validation of a Stability-Indicating RP-HPLC Protocol for Mavacamten in Oral Capsule Preparations

Pratibha Atul DAROI[1]*, Pranjali PATIL[1], Vijay MUNIPALLI[2], Bhushan SONAWANE[2]

[1]LSHGCT's Gahlot Institute of Pharmacy, Department of Pharmaceutical Quality Assurance, Koparkhairne, Navi Mumbai, Maharashtra, India
[2]Central Drugs Testing Laboratory, Zonal FDA Bhavan, Department of Analytical Research and Development Mumbai, Maharashtra, India

ABSTRACT

Objectives: Mavacamten (MYK-461), approved for hypertrophic cardiomyopathy, is a cardiac myosin inhibitor. At present, no pharmacopoeial method for its quantification has been reported. Our study was designed to establish a validated stability-indicating reverse-phase high-performance liquid chromatography method to quantify MYK-461 in its capsule formulation.

Materials and Methods: For chromatographic separation, an Inertsil ODS-3V C18 column (250 × 4.6 mm, 5 µm) with the mobile phase of 0.1% trifluoroacetic acid and acetonitrile (50:50 v/v) was used. The flow rate (1.0 mL/min), column temperature (35 ˚C), injection volume (10 µL), total run time (10 minutes), and detection wavelength (269 nm) were applied.

Results: The validated method showed precision, accuracy, and robustness, with relative standard deviation < 2% and recovery of 100.5%. Linearity was observed over the range 5–75 µg/mL (r = 0.9994). We achieved a clear, well-resolved separation of MYK-461 from impurities, with estimated limit of detection (1.58 µg/mL) and limit of quantification (4.8 µg/mL). In forced degradation studies, we observed the highest degradation under acidic conditions (10.37%), while minimal degradation under photolytic (0.93%), thermal (1.66 %), oxidative (2.56%), and alkaline (4.19%) conditions.

Conclusion: The developed method is precise and reliable, and offers a practical tool for routine quality control and future research, while emphasising the need for controlled storage conditions to ensure long-term drug integrity.

Keywords: Forced degradation, mavacamten, cardiomyopathy, RP-HPLC, validation

INTRODUCTION

Cardiomyopathies represent a diverse array of myocardial anomalies denoted by structural and functional impairments without having overt coronary artery disease, hypertension, valve dysfunction, or structural malformation at birth.[1-3] Advances in genetic and molecular diagnostics have revealed that many forms of cardiomyopathy, including hypertrophic, dilated, and arrhythmogenic types, are frequently associated with pathogenic gene variants affecting sarcomere or desmosomal proteins.[4] Recent studies have also emphasised the role of inflammation, autoimmunity, and metabolic deregulation in the pathogenesis and progression of these conditions, offering novel targets for precision therapy.[5-8] International Union of Pure and Applied Chemistry of mavacamten is 6-{[(1S)-1-phenylethyl] amino}-3-propane-2-yl-1H-pyrimidine-2,4-dione, as depicted in Figure 1.

Mavacamten got FDA approval in April 2022 for managing symptomatic obstructive hypertrophic cardiomyopathy (HCM), thus offering a unique treatment option for adults with obstructive HCM under New York Heart Association (NYHA) class II-III.[9,10]

***Correspondence:** pratibhadaroi@gmail.com, **ORCID-ID:** orcid.org/0000-0002-2481-4960

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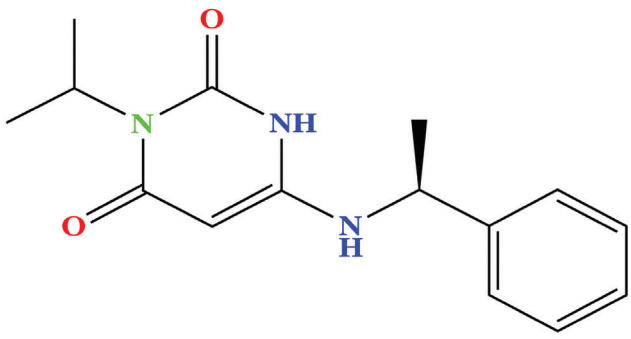

Figure 1. Mavacamten structure.

HCM is marked by abnormal thickening of the myocardial wall, which increases the propensity for severe arrhythmias and transient cardiac arrest. This condition typically stems from excessive thickening of the wall of the left ventricle, which compromises cardiac output. In cases of symptomatic obstructive HCM, mavacamten has become a game-changing treatment.[11] Mavacamten binds reversibly to cardiac β-myosin heavy chain, a motor protein involved in cardiac muscle contraction. It inhibits myosin ATPase activity, reducing excessive ATP-driven cross-bridge formation between actin and myosin. In HCM, hypercontractility results in left ventricular outflow tract (LVOT) obstruction.[12] Mavacamten inhibits cross-bridging between actin and myosin filaments, thus normalising contractile force without overly compromising systolic function. By reducing myocardial tension, it promotes ventricular relaxation and lowers the LVOT gradient, resulting in improved diastolic filling. Eventually, all these mechanisms result in decreased cardiac hypertrophy, improved exercise capacity, and reduced risk of symptoms associated with obstructive HCM.[13] The most common side effects reported with mavacamten during the clinical studies are dizziness and fainting.[14] The MOA of mavacamten is unique, and it differs from that of drugs like β-blockers as well as calcium channel blockers, commonly employed to treat HCM cases.[15] Mavacamten has demonstrated significant clinical benefits in reducing symptoms, improving exercise tolerance, and reversing heart muscle thickening in patients with obstructive HCM. However, it requires close monitoring, as it may temporarily reduce cardiac function. Precise and reliable analytical methods are essential to ensure proper dosing, effectiveness, and safety in clinical use.

Being a pioneering cardiac myosin inhibitor of its kind, mavacamten is approved on five continents for the treatment of HCM in individuals classified under NYHA class II or III, aiming to modulate cardiac efficiency and alleviate symptoms.[16] To the best of our knowledge, no reverse-phase chromatographic techniques for mavacamten have been reported in the literature, nor has there been an analytical method documented in any pharmacopoeia. To address this need, we developed a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method that can be reliably used to quantify mavacamten in capsules. This method was subsequently validated under the ICH Q2 (R1) standards.

It has been increasingly emphasized that ensuring the intrinsic stability and degradation behaviour of pharmaceutical compounds is important. Forced degradation (FD) studies were undertaken to serve this objective, as they are critical to pharmacological efficacy and safety of the product under investigation. Stress testing and FD investigation underscored the stability-indicating nature of the developed method, focusing on the predominant degradation pathways, notably acid-induced hydrolysis. Our study provides a rapid chromatographic technique that caters to both advanced clinical studies and routine quality assurance testing.

MATERIALS AND METHODS

Reagents and chemicals

A certified reference standard of mavacamten (purity: 99.74%) and a bulk drug sample were obtained from the Central Drug Testing Laboratory, Mumbai, India. Commercial capsule formulations containing mavacamten (CAMZYOS®, in strengths equivalent to 2.5, 5, and 10 mg), manufactured by Bristol-Myers Squibb, were used in method development and validation studies.

Chromatographic analyses were performed using acetonitrile of HPLC grade (purity ≥ 99.9%). Trifluoroacetic acid, triethylamine, and potassium dihydrogen phosphate of analytical reagent grade (purity ≥ 99.0%) were sourced from Rankem, India. High-purity water generated through a Milli-Q® water purification system (18.2 MΩ·cm resistivity) was employed for the preparation of mobile phase components, standard solutions, sample solutions, and all subsequent dilutions. All reagents and solvents were used as received without further purification.

Instruments

For chromatographic analysis of mavacamten, a Dionex Ultimate 3000 HPLC system from Thermo Scientific was used. Because the UV/VIS detector was compatible with the chromophoric groups of mavacamten, it was used with an adjustable wavelength selection, allowing sensitive detection at 269 nm.

Methodology

Preparation of a mobile phase

A mobile phase comprising acetonitrile and 0.1% (v/v) trifluoroacetic acid (50:50, v/v) was prepared. The 0.1% trifluoroacetic acid solution was obtained by diluting 1.0 mL of trifluoroacetic acid to 1000 mL with HPLC-grade purified water. The resulting mobile phase was filtered through a 0.45 µm membrane filter, sonicated, and degassed before chromatographic analysis.

Preparation of the sample solution from formulated capsules

To obtain a 50 µg/mL concentration, 5 mg of mavacamten was dissolved in the same diluent, and the solution was diluted to 100 mL to prepare the sample solution. The solution was then passed through 0.45 µm membranes and sonicated for 10 minutes to ensure complete solubility and to avoid particle interference.

Preparation for the standard and calibration curve solution

The intermediate stock solution of 100 μg/mL was prepared. For this purpose, a 10-mg mavacamten reference standard was accurately weighed and dissolved in a diluent in a volumetric flask to obtain a final volume of 100 mL. By utilising a 70:30% v/v ratio of acetonitrile-water as the diluent, 5 mL of a 100 μg/mL intermediate solution was diluted to 10 mL to produce the standard solution with a concentration of 50 μg/mL. A 100 μg/mL stock solution was used for calibration and subsequent dilutions. Mavacamten standard solutions at concentrations between 5 and 75 μg/mL were prepared and added to the system. A calibration curve established the relationship between the analyte content and the detector response, enabling evaluation of the method's linearity.

Chromatographic conditions

The UV spectrophotometer was used to perform spectral scanning between 200 and 400 nm of a standard solution of mavacamten (50 μg/mL). UV detection revealed an absorption peak (λ_{max}) at 269 nm. Separation was achieved on an Inertsil ODS-3V C18 column (250 × 4.6 mm, 5 μm) at 35 °C, employing an isocratic mobile phase with a 50:50 v/v composition of 0.1% trifluoroacetic acid added to acetonitrile. To reduce carryover, ensure mobile phase integrity, and maintain constant chromatographic performance, an autosampler with a fixed-volume loop and integrated needle rinse was used to add a 10 μL injection. Flow uniformity and baseline stability of the detector were achieved using degassing devices. A Millipore filtration system with 0.45 μm membrane filters was used to remove particulate matter.

Validation

System suitability

Key chromatographic variables such as peak area, number of theoretical plates, tailing factor, and retention time were assessed by 6 consequetive injections of standard mavacamten solution (50 μg/mL).

Specificity

The chromatograms of the blank, the mavacamten sample, and the standard were recorded and evaluated for the presence of interfering or impurity peaks.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Standard solutions of mavacamten in the concentration range of 5–75 μg/mL were prepared and injected into the HPLC system. A calibration curve was constructed by plotting peak area against analyte concentration to evaluate the linearity of the developed method.

Linearity was assessed using least-squares linear regression analysis. The slope, intercept, and correlation coefficient (r) were obtained from the regression equation. The limits of detection and quantification were estimated using the standard deviation (SD) of the response (σ) and the slope of the calibration curve (S), according to the following equations: LOD = 3.3σ/S, LOQ = 10σ/S.

Accuracy

Using the standard addition method, sample solutions were spiked at 4 levels, viz., 100%, 110%, 120%, and 130%, and the mean recovery (%) was assessed. Solutions at concentrations of 50 μg/mL, 55 μg/mL, 60 μg/mL, and 65 μg/mL were prepared. Accuracy was evaluated using a recovery study, and the results were expressed as percent recovery.

Precision

The precision of the developed RP-HPLC method was evaluated in terms of repeatability (intraday precision) and intermediate precision (interday precision).

Intraday precision was assessed by analysing six replicate preparations of mavacamten at a concentration of 50 μg/mL across three time points on the same day: morning (10:00 AM), afternoon (1:00 PM), and evening (4:00 PM). The assay values obtained at each time point were used to determine the mean, SD, and percentage relative SD (RSD %).

Intermediate precision was evaluated by analysing six replicate preparations of mavacamten (50 μg/mL) on two different days by different analysts under the same chromatographic conditions. The assay results obtained on each day were statistically evaluated in terms of mean assay, SD, and RSD %.

The precision of the method was expressed as the percentage relative SD (RSD %), and values not exceeding 2.0% were considered acceptable.

Robustness

Robustness was evaluated to assess the influence of small, deliberate changes in chromatographic conditions. Variations were introduced in the detection wavelength (± 2 nm), flow rate (± 0.2 mL/min), column temperature (± 2 °C), and mobile phase composition (± 2%, v/v). The influence of these changes on the assay results was evaluated by calculating the assay percentage and RSD %.

Content uniformity analysis of mavacamten capsules

The developed method was applied to determine the content uniformity of mavacamten capsules available in 2.5 mg, 5 mg, and 10 mg dosage strengths to assess the consistency of drug distribution among individual dosage units. Sample solutions (50 μg/mL) were prepared using a diluent of acetonitrile: water (70:30 v/v) by precisely diluting each dosage strength to final volumes of 50 mL (2.5 mg), 100 mL (5 mg), and 200 mL (10 mg).

FD studies

FD studies were performed under acidic, alkaline, oxidative, thermal, and photolytic stress conditions to evaluate the stability-indicating capability of the developed RP-HPLC method. For acidic degradation, 1.0 mL of the standard stock solution was mixed with 1.0 mL of 0.1 M hydrochloric acid, and the mixture was maintained under the specified stress conditions. Following the degradation period, the solution was neutralised with an equivalent volume of 0.1 M sodium hydroxide and then diluted to 10 mL with a diluent consisting of acetonitrile and water (70:30 v/v).

For alkaline degradation, 1.0 mL of the standard stock solution was treated with 1.0 mL of 0.1 M sodium hydroxide. After completion of the stress exposure period, the solution was neutralised using an equivalent volume of 0.1 M hydrochloric acid and diluted to 10 mL with the diluent.

Oxidative degradation was carried out by treating the standard stock solution with 3% (v/v) hydrogen peroxide and maintaining the mixture under prescribed conditions before dilution. Thermal degradation studies were performed by exposing the standard solution to 60 °C in a hot air oven for 24 h. For photolytic degradation, the standard solution was exposed to ultraviolet radiation at 254 nm in a UV chamber for 24 h.

After completion of the respective stress treatments, all samples were appropriately diluted with the diluent, filtered through a 0.45 µm membrane filter, and analysed using the developed RP-HPLC method.

Statistical analysis

Data acquisition and chromatographic processing were performed using the Chromeleon™ Chromatography Data System (version 7.2.6, Thermo Fisher Scientific, USA). Statistical calculations were performed using Microsoft Excel (version 2021; Microsoft Corporation, Redmond, WA, USA).

Ethical approval and informed consent

Ethics committee approval and informed consent were not required for this study, as the research involved only the development and validation of an analytical method for pharmaceutical drug samples and did not involve human participants, animals, human-derived biological samples, or identifiable personal data.

RESULTS

Method development

Chromatographic separation

Accurate detection of the analyte at 269 nm was facilitated by the use of an Inertsil ODS-3V C18 column (4.6 mm × 250 mm, particle size 5 µm), acetonitrile and a 0.1% trifluoroacetic acid solution (50:50 v/v) as the mobile phase, a runtime of 10 minutes, and a flow rate of 1.0 mL/min. A symmetric peak tailing factor of 1.12 and a retention period of 5.06 minutes indicated separation.

Method validation

Linearity

The developed RP-HPLC method exhibited a satisfactory linear relationship within the validated concentration range of 5–75 µg/mL, yielding a correlation coefficient (r) of 0.9994. The corresponding peak area responses at different concentration levels are summarised in Table 1, whereas the calibration profile is illustrated in Figure 2. Regression analysis yielded the equation y = 744.38x + 3061, where y denotes the chromatographic peak area and x represents the analyte concentration (µg/mL). The sensitivity of the method was evaluated by determining the limits of detection and quantification, which were found to be 1.58 µg/mL and 4.8 µg/mL, respectively. Low limits of detection and quantification indicate that the proposed method has adequate sensitivity for the routine estimation of mavacamten in capsule dosage forms.

Table 1. Linearity data of mavacamten.

Concentration (µg/mL)	Peak area 1	Peak area 2	Peak area 3	Average area
5	5819	5818	5817	5820
10	10571	10574	10573	10574
20	18202	18201	18200	18201
30	25877	25876	25878	25878
40	33203	33204	33203	33204
50	40561	43560	40562	40562
60	48215	48216	48217	48216
75	57907	57906	57908	57907

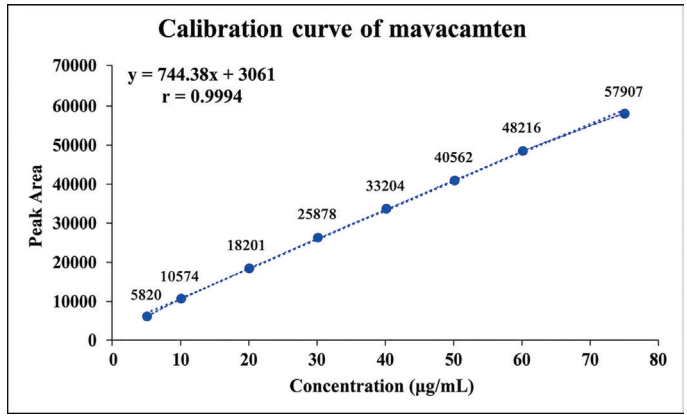

Figure 2. Linear calibration curve depicting the concentration-response relationship of mavacamten.

Table 2. Intraday precision data of mavacamten.

Sr. no	Morning (10:00 AM)	Afternoon (1:00 PM)	Evening (4:00 PM)
	Assay %	Assay %	Assay %
1	100.64	99.81	99.78
2	100.23	100.0	99.41
3	100.16	99.76	100.52
4	100.66	100.53	101.33
5	100.48	99.84	100.59
6	100.09	100.81	100.10
Mean	100.38	100.13	100.29
SD	0.226	0.438	0.678
RSD %	0.225	0.437	0.676
Limit for RSD %	NMT 2%		

SD, standard deviation; RSD %, percent relative standard deviation; NMT, not more than.

Precision

The intraday precision study showed mean assay values of 100.38%, 100.13%, and 100.29% for the morning, afternoon, and evening measurements, respectively, as shown in Table 2. The corresponding % RSD values were 0.225%, 0.437%, and 0.676%, demonstrating good intraday precision of the developed method. Interday precision was assessed by analysing mavacamten samples on two separate days, and the results are summarised in Table 3. The percentage assay values ranged from 99.70 ± 0.171% to 100.43 ± 0.245%, with corresponding RSD % values between 0.120% and 0.712%. All RSD % values were below the acceptance limit of 2.0%, indicating good intermediate precision and reproducibility of the developed RP-HPLC method for routine analysis of mavacamten capsules.

Accuracy

The accuracy of the developed RP-HPLC method was assessed by the standard addition method at four concentration levels (100–130%).[17] Percentage recoveries ranged from 100.40% to 100.90%, with RSDs % between 0.34% and 0.62% (Table 4). All RSD % values were below the acceptance limit of 2.0%, indicating satisfactory accuracy and reproducibility of the method. The results confirm the absence of interference from formulation excipients and demonstrate the suitability of the method for the accurate quantification of mavacamten in capsule dosage forms.

System suitability

During validation, the system's suitability characteristics remained unaltered. The average peak area was 40,745; the average theoretical plate count was 15,224; the mean retention time was 5.06 minutes; and the tailing factor was 1.12. Table 5 presents system suitability results, indicating satisfactory performance.

Content uniformity

The assay results met the predetermined limits (98–102%), indicating consistent active pharmaceutical ingredient concentration in mavacamten capsules. The content uniformity results are shown in Table 6.

Robustness

The technique performed robustly under these conditions, as indicated by RSD % readings consistently below 2%, supporting the conclusion that the procedure was resilient to small operational variations. The findings from robustness evaluations are shown in Table 7.

Validation summary

A summary of the validation results for the evaluated RP-HPLC method is presented in Table 8.

FD studies

The specificity of the developed RP-HPLC method was evaluated by analysing blank, standard, sample, and FD preparations.[18] The blank chromatogram did not exhibit any peak at the retention time corresponding to mavacamten, indicating the

Table 3. Interday precision data of mavacamten.

Assay	Day 1		Day 2	
	*Assay % ± SD	RSD %	*Assay % ± SD	RSD %
1	99.70 ± 0.171	0.712	100.39 ± 0.121	0.120
2	100.43 ± 0.245	0.244	99.72 ± 0.242	0.242
Limit for RSD %	NMT 2%			

*Average of 6 determinations; SD, standard deviation; RSD %, percent relative standard deviation; NMT, not more than.

Table 4. Accuracy data by the standard addition method.

Accuracy % level	Amount spiked (µg/mL)	% Mean* recovery	SD	RSD %
100	0	100.9	0.35	0.34
110	5	100.40	0.37	0.37
120	10	100.7	0.5	0.50
130	15	100.6	0.6	0.62
Limit for RSD %	NMT 2%			

*Average of 3 determinations; SD, standard deviations; RSD %, percent relative standard deviation; NMT, not more than.

Table 5. System suitability data of mavacamten.

Sr. no	Area	Retention time	Theoretical plates	Tailing factor
1	40547.81	5.06	15376	1.13
2	40582.48	5.04	15325	1.12
3	40886.32	5.05	15207	1.11
4	40761.51	5.08	15191	1.12
5	40859.36	5.06	15148	1.13
6	40532.95	5.04	15101	1.12
Average	40745.07	5.06	15224	1.12
SD	145.85	0.02	105.44	0.01
RSD %	0.36	0.30	0.69	0.67
Limit	NMT 2%	NMT 2%	NLT 2000	NMT 2%

SD, standard deviation; % RSD, percent relative standard deviation; NMT, not more than; NLT, not less than.

Table 6. Content uniformity data of mavacamten.

Sr. no	Label claim (mg/capsule)	Estimated amount (mg)	Purity %
1	2 mg	1.97	99.7
2	5 mg	5.01	100.28
3	10 mg	10.11	101.27
Limit for peak purity	98-102%		

Table 7. Robustness data of mavacamten.

Parameter	Deliberate variation	Percent estimation	Mean % estimation	SD	RSD %
Wavelength (nm)	267	99.72	100.42	0.52	0.5
	269	100.58			
	271	100.97			
Flow (ml/min)	0.8	99.80	100.13	0.328	0.327
	1.0	100.58			
	1.2	100.02			
Temperature (°C)	33	100.29	100.5	0.141	0.14
	35	100.60			
	37	100.58			
Mobile phase composition (v/v)	48:52	99.88	100.34	0.375	0.374
	50:50	100.33			
	52:48	100.80			
Limit for RSD %	NMT 2%				

SD, standard deviation; RSD %, percent relative standard deviation; NMT, not more than.

Table 8. Summary of validation results of mavacamten.

Sr. no	Parameter	Result	Acceptance criteria	Remark
1	Linearity-correlation coefficient (r)	0.9994	r ≥ 0.99	Passed
2	Limit of detection (µg/mL)	1.58	S/N = 3:1	Passed
3	Limit of quantification (µg/mL)	4.8	S/N = 3:1	Passed
4	Accuracy (mean % recovery)	100.50	Recovery % = 98–102%	Passed
5	Precision (mean RSD %)	< 2	RSD % ≤ 2	Passed
6	Robustness (mean RSD %)	< 2	RSD % ≤ 2	Passed
7	Content uniformity (%)	100.2	98–102%	Passed
8	System suitability RSD %	< 2	RSD % ≤ 2	Passed
	Theoretical plates	15,224	≥ 2000	Passed
	Tailing factor	1.12	≤ 2	Passed

RSD %, percent relative standard deviation.

absence of interference from diluent components. Similarly, no interfering peaks arising from formulation excipients were observed in the sample chromatograms. Under various stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic, additional degradation peaks were generated; however, these peaks were well resolved from the Mavacamten peak, as shown in Figure 3. The retention time of mavacamten remained consistent, and no interference was observed at the analyte elution position. These findings demonstrate that the developed method is capable of selectively quantifying mavacamten in the presence of its degradation products and formulation-related components. Table 9 displays the computed results.

DISCUSSION

The current project was designed to develop and validate a rapid and reliable RP-HPLC method for quantifying mavacamten in capsule dosage forms. The increasing clinical value of mavacamten as a pioneering cardiac myosin inhibitor necessitates stringent quality control and the availability of a validated analytical method suitable for routine analysis.

Chromatographic conditions were selected to achieve acceptable retention, adequate peak symmetry, and satisfactory column efficiency within a short analysis time. The Inertsil ODS-3V C18 column, combined with an isocratic mobile phase consisting of acetonitrile and a trifluoroacetic acid solution, produced a sharp, symmetrical peak for mavacamten with a retention

time of approximately 5 min. The relatively short retention time reduces solvent consumption and improves analytical throughput, thereby making the method suitable for routine quality control. The system suitability parameters, including tailing factor and theoretical plate count, indicated efficient chromatographic performance and adequate interaction between the drug and the stationary phase.

Method validation, performed according to ICH recommendations, demonstrated excellent linearity over the studied concentration range, indicating a proportional relationship between analyte concentration and detector response, thereby confirming the method's suitability for quantitative applications such as assay and content-uniformity determinations. The calculated limits of detection and quantification further indicate that the method possesses adequate sensitivity for the analysis of finished pharmaceutical products.

The precision studies revealed low intra-day and inter-day variability, underscoring satisfactory repeatability and intermediate precision. The small variation observed among replicate injections and analyses performed on different days confirms the reproducibility of the developed procedure under

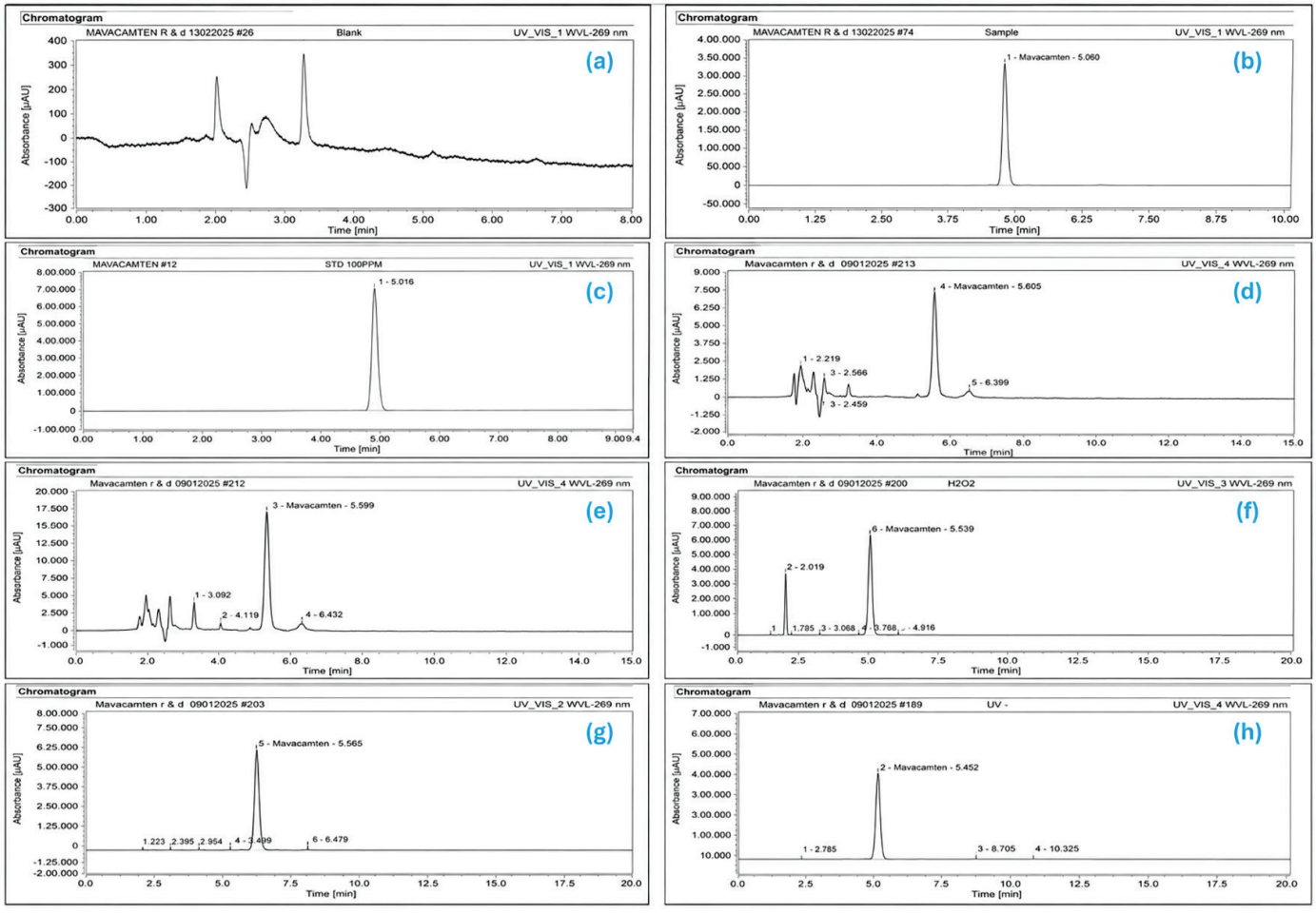

Figure 3. Representative RP-HPLC chromatograms of mavacamten under various conditions: (a) blank; (b) test sample; (c) reference standard; (d) acid-induced degradation; (e) base-induced degradation; (f) oxidative stress condition; (g) thermal degradation; (h) photolytic degradation.

Table 9. Forced degradation data of mavacamten.

Test	Forced degradation conditions	Duration	Percent (%) degradation	Percent (%) residual drug
1	Acidic (0.1 M HCL)	24 hours at room temperature	10.97	89.63
2	Alkali (0.1M NAOH)	24 hours at room temperature	4.19	95.81
3	Oxidative (3% H_2O_2)	24 hours at room temperature	2.56	97.44
4	Thermal (60 °C)	24 hours at 60 °C	1.66	98.34
5	Photolytic (at 254 nm)	24 hours at room temperature	0.93	99.07

normal laboratory operating conditions. Such reproducibility is particularly important for analytical methods intended for routine quality control and stability testing.

Accuracy evaluation using recovery experiments produced values close to 100%, indicating that the excipients in the capsule formulation did not interfere with the quantification of mavacamten. The low variability observed during recovery studies further confirms the reliability of the method for determining the drug in pharmaceutical matrices. These findings demonstrate that the method possesses adequate selectivity toward the analyte in the presence of formulation components.

System suitability results remained within the prescribed acceptance criteria throughout validation, demonstrating the consistency and reliability of the chromatographic system. Stable retention time, acceptable peak symmetry, and satisfactory column efficiency indicate that the analytical system can generate reproducible results during prolonged use; this is particularly important for methods intended for routine stability assessment.

The developed method was successfully applied to the analysis of marketed capsule formulations, yielding assay values within the pharmacopeial acceptance limits. Content uniformity studies demonstrated minimal variability among individual dosage units, indicating homogeneous distribution of mavacamten within the capsule formulations. These results support the practical applicability of the method for routine quality control testing of different capsule strengths.

Robustness evaluation demonstrated that deliberate changes in chromatographic conditions, including flow rate, mobile phase composition, and detection wavelength, did not significantly affect analytical performance. The absence of substantial variation in assay results and system suitability parameters confirms the rugged nature of the method and indicates that minor operational changes are unlikely to compromise analytical performance during routine laboratory use.

FD studies constitute an essential component of method development because they provide information on the intrinsic stability characteristics of drug substances and demonstrate the ability of the analytical method to separate degradation products from the intact drug. In the present investigation, mavacamten exhibited differential susceptibility to various stress conditions. The drug remained relatively stable under thermal, photolytic, and oxidative conditions, whereas acidic conditions produced the greatest extent of degradation.

The pronounced degradation observed under acidic stress may be attributed to proton-mediated chemical transformations within the mavacamten molecule. The presence of amide functionality can facilitate acid-catalysed hydrolysis through protonation of the carbonyl group, thereby increasing the susceptibility of the molecule toward nucleophilic attack. Additionally, protonation of nitrogen-containing heterocyclic moieties may alter the electronic distribution within the molecule and promote subsequent degradation pathways. The greater extent of degradation under acidic conditions, therefore, suggests that acid-catalysed reactions represent the predominant degradation mechanism of mavacamten under the investigated stress conditions.

Importantly, the developed chromatographic method achieved adequate resolution between the parent drug and degradation products without interference at the retention time of mavacamten. The ability to selectively quantify the intact drug in the presence of degradation products makes the method suitable for stability studies, shelf-life evaluation, and quality control applications.

The findings of the present study have practical significance for pharmaceutical industries and quality control laboratories. The method combines short analysis time, satisfactory validation characteristics, and stability-indicating capability, thereby offering an economical and efficient analytical approach for routine analysis of mavacamten capsule formulations. Furthermore, the degradation behaviour observed during stress studies may provide useful information for formulation development, packaging selection, and optimization of storage conditions.

Overall, the developed RP-HPLC method demonstrated satisfactory validation characteristics, adequate specificity, and effective separation of degradation products from the parent drug.

Study limitations

Although the method is suitable for routine quality control, we acknowledge certain limitations. The moderate sensitivity and reliance on isocratic elution may restrict its applicability to highly complex impurity profiling. Additionally, the use of trifluoroacetic acid, while beneficial for improved peak shape and resolution, may have implications for long-term system maintenance. Table 10 summarises the limitations of the proposed method. Nevertheless, within the context of routine assay, content uniformity, and stability evaluation of mavacamten capsules, these limitations do not compromise the method's overall suitability.

CONCLUSION

The present investigation resulted in the successful development of a selective, stability-indicating RP-HPLC method for the determination of mavacamten in capsule dosage forms. The optimised chromatographic conditions enabled rapid analysis with satisfactory peak characteristics and effective separation of the drug from its degradation products, demonstrating the suitability of the method for stability-related applications.

Comprehensive validation, performed in accordance with ICH recommendations, established the reliability of the method with respect to linearity, accuracy, precision, robustness, sensitivity, and specificity. The consistent analytical performance observed during validation and the successful analysis of commercial capsule formulations confirm the applicability of the method for routine pharmaceutical quality assessment.

Table 10. Limitations of the developed RP-HPLC method for mavacamten.

Limitation	Impact
Moderate LOD/LOQ	Not suitable for eluting low-level analytes
Isocratic system	Not as appropriate for complex impurity profiles
Trifluoroacetic acid usage	Causes issues with advanced detectors
Limited characterization of degradants	There can be an oversight in the regulatory filings

RP-HPLC, reverse-phase high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification.

Stress-degradation studies provided important insights into the chemical stability profile of mavacamten. The drug exhibited the highest susceptibility to acidic degradation, whereas limited degradation was observed under thermal, oxidative, and photolytic conditions. The clear resolution of degradation products from the parent drug peak demonstrates the method's ability to accurately quantify mavacamten in the presence of these products.

The developed analytical procedure offers practical advantages, including simple sample preparation, short chromatographic run time, reproducible performance, and suitability for routine laboratory operation. In addition to supporting assay and content- uniformity testing, the method may be effectively employed for stability studies, quality- control investigations, and the evaluation of pharmaceutical products during storage.

The study provides a validated, reliable analytical approach for determining mavacamten in capsule formulations and valuable information on its degradation behaviour. The proposed method may serve as a useful tool for pharmaceutical industries and quality control laboratories involved in the development, evaluation, and stability monitoring of mavacamten-containing products.

Ethics

Ethics Committee Approval: Ethics committee approval was not required for this study, as the research involved only the development and validation of an analytical method for pharmaceutical drug samples and did not involve human participants, animals, human-derived biological samples, or identifiable personal data.

Informed Consent: Informed consent approval was not required for this study, as the research involved only the development and validation of an analytical method for pharmaceutical drug samples and did not involve human participants, animals, human-derived biological samples, or identifiable personal data.

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Footnotes

Authorship Contributions

Concept: P.A.D., V.M., Design: P.A.D., V.M., B.S., Data Collection or Processing: P.P., Analysis or Interpretation: P.A.D., V.M., Literature Search: P.A.D., B.S., Writing: P.P., B.S.

Conflict of Interest: The authors declare no conflicts of interest.

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