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ORIGINAL ARTICLE

Development and ICH Q2(R2)-Compliant Validation of a Rapid and Cost-Effective RP-HPLC Method for Quantitative Determination of Ponesimod in Ponvory 20mg

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ABSTRACT

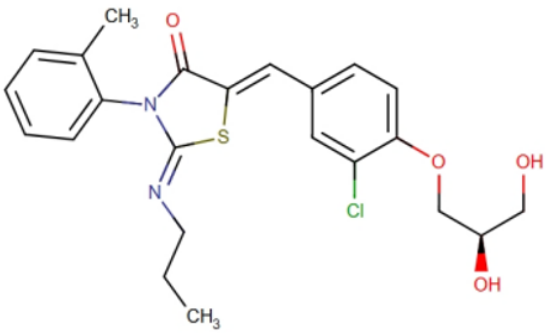
Ponesimod is a second-generation, orally active, highly selective sphingosine-1-phosphate receptor-1 ($S1P_1$) modulator. The quantitative detection of sphingosine-1-phosphate receptor modulators, especially in biological matrices, has been achieved by use of various chromatographic techniques. Following the latest ICH Q2(R2) guidelines, this study aimed to create and test an RP-HPLC method for the quantitative measurement of Ponesimod in both bulk medication and commercially available tablet dosage forms that was easy to use, fast, cheap, and reliable. Chromatographic separation was achieved by using a YMC Triart C18 column (250 × 4.6 mm, 5 µm) maintained at 25°C and an isocratic mobile phase containing 10 mM ammonium bicarbonate and acetonitrile (60:40, % volume by volume) supplied at a flow rate of 0.5 mL min^{-1}. Eluting ponesimod at 6.517 minutes resulted in column efficiency of 6540 theoretical plates and excellent peak asymmetry of 1.34. The method showed remarkable linearity over the concentration range of 5-150 µg mL^{-1}, as evidenced $R^2 = 0.9999$. Intermediate precision has a %RSD of 0.0234% and repeatability of 0.04%. Mean recoveries of 99.04% at the 50% level, 99.45% at the 100% level, and 101.15% at the 150% level proved the excellent analytical accuracy. The limits of detection (LOD) were 0.30 ng mL^{-1} and 1.00 ng mL^{-1}, respectively, with mean signal-to-noise ratios of 6.94 and 40.36. The fact that the chromatographic performance remained mostly unchanged after deliberately adjusting the flow rate and column temperature demonstrates outstanding robustness. When the process was applied to Ponvory® pills, an assay result of 101.43% was obtained. With great precision, accuracy, sensitivity, and durability, the RP-HPLC technology performed well. Its user-friendliness, fast analytical time, and cost-effective chromatographic conditions make it a great choice for quantitative evaluation of Ponesimod in Ponvory 20 mg and for periodic quality monitoring.

Keywords: Ponesimod; ICH Q2(R2); Pharmaceutical quality control; Analytical method validation; Sphingosine-1-phosphate receptor modulator

INTRODUCTION

Demyelination, axonal degradation, and increasing neurological impairment are hallmarks of multiple sclerosis (MS), an inflammatory, immune-mediated, chronic CNS illness. It is still one of the most common causes of non-traumatic neurological dysfunction globally, and it mostly strikes young adults. Significant therapeutic problems remain due to the varied character of multiple sclerosis (MS)

and its fluctuating clinical course, even though there have been notable advances in disease-modifying medicines. Therefore, a lot of research and development has gone into creating specific immunomodulatory drugs that can reduce disease activity while reducing systemic side effects[1, 2]. In the fight against autoimmune diseases, a watershed moment came with the discovery that sphingosine-1-phosphate (S1P) receptor signaling regulated lymphocyte trafficking. This approach to pharmacology resulted in the creation of Ponesimod, a second-generation orally active, extremely selective modulator of the sphingosine-1-phosphate receptor-1 ($S1P_1$)[3] (Fig. 1). A promising new treatment option for relapsing MS, ponesimod outperforms first-generation S1P receptor modulators in terms of receptor selectivity, pharmacodynamic reversibility, and safety profile. Consequently, once daily oral dose of ponesimod resulted in significant decreases in new gadolinium-enhancing lesions and favorable clinical outcomes in the randomized Phase II clinical study undertaken by Olsson *et al.*[4]. Relapsing MS patients benefited greatly from Ponesimod compared to teriflunomide in a number of ways, including a marked decrease in yearlyized relapse rates, fatigue symptoms, disease progression, and MRI lesion activity, all while maintaining an acceptable safety profile, according to the recent pivotal Phase III OPTIMUM trial[5]. These results have greatly improved the clinical use of Ponesimod and confirmed its efficacy as a disease-modifying medication. Analytical techniques designed for pharmaceutical applications must always be very ready, easy to operate, and cost-effective[6-11]. The quantitative detection of sphingosine-1-phosphate receptor modulators, especially in biological matrices, make it the method of choice for most of these procedures pharmacokinetic studies[12]. However, LC-MS/MS technologies aren't often used in pharmaceutical quality control labs because of the high maintenance costs, technically challenging sample preparation procedures, and analysts that are required to use them. Moreover, there is a severe lack of validated reverse phase methods to quantitatively determine Ponesimod in drug substances and drug products, according to a thorough literature review (see Table. 1) that found that analytical methods developed for Ponesimod are mostly bio-analytical in nature[13]. Systematic method development and rigorous validation are considered crucial components for guaranteeing long-term analytical reliability, according to recent breakthroughs in pharmaceutical analytical science that have focused on analytical life cycle management. The most recent standard from the International Council for Harmonization, ICH Q2(R2): Prior to implementing for regular pharmaceutical analysis, it is recommended to systematically evaluate the following analytical performance characteristics outlined in Validation of Analytical Procedures[14]. The current study aimed to create and verify a fast, selective, accurate, precise, robust, and cost-effective RP-HPLC method for routine pharmaceutical analysis due to the growing clinical importance of Ponesimod and the lack of available validated reverse phase methods. The evaluated parameters allowed the optimized method to be validated in line with the current ICH Q2(R2) guideline.





(a) **(b)**

Fig. 1: (a) Chemical structure of Ponesimod (b) Ponesimod as available in the market

Table 1: Comparison of the proposed RP-HPLC method with previously reported analytical methods for Ponesimod

Reference	Analytical technique	Application	Chromatographic conditions	Analytical performance	Limitations
Brossard *et al.*,[14]	LC–MS/MS	Pharmacokinetic evaluation in healthy subjects	-	Highly sensitive bio-analytical method for plasma quantification	Applicable only to biological matrices; requires costly LC-MS/MS instrumentation and extensive sample preparation

Reference	Analytical technique	Application	Chromatographic conditions	Analytical performance	Limitations
Olsson *et al.*,[3]	LC–MS/MS	Phase II clinical pharmacokinetic study	-	Quantification of Ponesimod during clinical studies	Developed exclusively for pharmacokinetic investigations and not suitable for routine pharmaceutical quality control
Kappos *et al.*,[4]	LC–MS/MS	Phase III OPTIMUM clinical study	-	Drug concentration monitoring during multicentre clinical trials	Sophisticated instrumentation and specialized laboratory facilities required
Dash *et al.*,[11]	Review of bioanalytical methods	S1P receptor modulators	-	Summarized highly sensitive bioanalytical approaches	Focused primarily on biological matrices rather than dosage-form analysis
Present study	RP-HPLC	Bulk drug and tablet dosage form	YMC Triart C18 (250 × 4.6 mm, 5 µm); 10 mM ammonium bicarbonate:acetonitrile (60:40, v/v); flow rate 0.5 mL min^{-1}	Retention time: 6.52 min; linearity: 5–150 µg mL^{-1} (R^2 = 0.9999); accuracy: 99.04–101.15%; precision (%RSD < 2%); robust and suitable for routine quality control	Simple, economical and readily transferable to conventional pharmaceutical quality control laboratories

MATERIALS AND METHODS

Chemicals and Reagents

From Hetero Drugs Pvt. Ltd. in Hyderabad, India, we received a gift featuring a sample of the Ponesimod reference standard. We shopped at a nearby pharmacy for Ponvory® 20 mg. We bought formic acid, methanol, and HPLC-grade acetonitrile from Rankem in India. The buffer was prepared using analytical reagent grade ammonium bicarbonate. Throughout the experiment, solutions were prepared and diluted using ultra-pure water that was created using a Millipore purification system.

Instrumentation

An Agilent G6125B system, which includes a UV detector, column oven, autosampler, and quaternary pump, was used to conduct the chromatographic analysis. The data from the chromatographic analysis were processed using the software that came with the equipment. Sample sonication was carried out using a LAB MAN Scientific ultrasonic cleaner (LMUC-12), while all weighing operations were performed using a Sartorius SECURA225D-1OIN analytical balance. Ultra-pure water was obtained using a LAB JAL LAB NE15UV purification system.

Chromatographic Conditions

Method development was performed by evaluating different chromatographic conditions to obtain acceptable retention behaviour, peak symmetry, and chromatographic efficiency. At 25°C, the YMC Triart C18 (250 mm × 4.6 mm, 5 µm) achieved the best separation. The mobile phase comprised of 10 mM ammonium bicarbonate in water and acetonitrile (60:40, % volume by volume) eluted in isocratic mode at a flown by 0.5 mL min^{-1}. The research was conducted with water as the diluent. Under the optimized chromatographic conditions, Ponesimod was eluted as a sharp and symmetrical peak suitable for quantitative analysis.

Getting Ready for the Mobile Phase (MP)

Stage A of Mobile phase (MP)

To make buffer solution (10 mM), 0.9609 g of ammonium bicarbonate (ABC) was dissolved in about 800 mL of distilled water. Once the substance had dissolved completely, it was blended with LC-grade water to a volume of 1000 mL.

Stage B of Mobile phase (MP)

The ideal mobile phase was a 60:40 (% volume by volume) mixture of acetonitrile and 10 mM ammonium bicarbonate buffer (ABC). Prior to chromatographic use, the mobile phase that had been prepared was sonicated for 15 minutes and filtered using a 0.45 µm membrane filter.

Standard Solution Preparation (SSP1)

A 50 mL volumetric flask was used to transfer 10 mg of Ponesimod reference standard, dissolve it in filtered water, and then dilute it to volume to create a stock solution with 200 µg mL^{-1}.To achieve concentrations of 6, 10, 50, 80, 100, 120, and 150 µg mL^{-1}, working standard solutions for linearity were made by transferring suitable portions of the standard solution preparation(SSP1)into calibrated 10 mL flasks later diluting by water.

Sample Solution Preparation (SSP2)

Twenty tablets of Ponvory® (label claim: 20 mg Ponesimod) were weighed, finely powdered, and an accurately weighed

quantity equivalent to the required amount of Ponesimod was transferring to a calibrated 100 mL flask. To make sure medicine got completely extracted, about 70 mL by volume of water (distilled) was added and the mixed for uniformity by sonication. After completely mixing the solution with purified water, it was filtered through Whatman filter paper to get it down to volume. To achieve the concentration needed for chromatographic analysis, suitable portions of the filtrate were further diluted with water.

Method Development

For optimal chromatographic performance, a variety of stationary phases, mobile phase compositions, solvent systems, and flow rates were investigated. At first, chromatographic separation was tried with a YMC Pack Pro C18 (100 × 4.6 mm, 5 µm) and a mobile phase of 0.1% formic acid-methanol (50:50, % volume by volume). Unfortunately, no identifiable Ponesimod peak was found. Analyte detection was also not sufficient in a second run that used a YMC Triart C18 (150mm in length maintaining the dimensions same),with 0.1% formic acid-acetonitrile (70:30, % volume by volume). Afterwards, YMC Triart C18 (250mm in length maintaining the dimensions same) was used in conjunction with 10 mM ammonium bicarbonate and acetonitrile (60:40, % volume by volume) flown by 0.5 mL min^{-1} to optimize the chromatographic conditions. This allowed for the production of a well-defined, symmetrical Ponesimod peak that was appropriate for quantitative estimation.

Method Validation

System appropriateness, specificity, linearity, precision, accuracy, robustness, detection capability, quantitation capability, and assay performance were evaluated to validate the optimized RP-HPLC technique in accordance with the criteria specified in the ICH Q2(R2) guideline.

System Suitability

Six separate injections of the reference solution were used to determine system appropriateness. We checked the reproducibility of the peak area, the retention period, the theoretical plate count(Tpc), and the tailing factor(Tf) before we analyzed the samples.

Specificity

Specificity was evaluated by injecting diluent and standard solutions to verify the absence of interfering peaks at the retention time of Ponesimod.

Linearity

The linearity was tested using seven different calibration levels over a concentration range of 6-150 µg mL^{-1}. The calibration curves were created by comparing the peak area to the analyte concentration and then analyzing the data using linear regression.

Precision

In order to assess the method's precision (repeatability), six separate injections of the standard solution were conducted under the same chromatographic circumstances. Two separate analysts, independently ascertained intermediate precision on separate days.

Accuracy

At 50%,100%, and 150% concentrations, the standard addition method was used to ascertain accuracy. We determined the percentage recovery by comparing the amount that was measured with the amount that was added.

Detection & Quantification Limit

By analyzing the signal-to-noise ratio that was achieved under optimal chromatographic conditions, the limits of detection (DL) and quantification (QL) were determined.

Robustness

Deliberately changing chromatographic parameters, such as flow rate (±0.1 mL min^{-1}) and column temperature (±5°C), was used to determine robustness, after which system suitability parameters were examined.

Ruggedness

To determine ruggedness, an other analyst used the same chromatographic technique to conduct the assay on a separate day. Chromatographic performance was consistent, which proved the approach could be reproduced under typical laboratory settings.

Evaluating Ponvory®

To quantify the amount of Ponesimod in the commercially available tablet (Ponvory®), the validated method was used. By comparing the sample solution's chromatographic response to that of the matching reference standard, the drug content might be determined.

RESULTS AND DISCUSSION

Method Development and Optimization

In order to develop an RP-HPLC method for Ponesimod that would produce satisfactory chromatographic performance, it was necessary to systematically optimize the stationery and mobile phases. During the initial trials, a YMC Pack Pro C18 (100 × 4.6 mm, 5 µm) column employing 0.1 ml by formic acid in 100 ml of water-methanol (50:50, v/v) failed to produce a detectable chromatographic response, indicating inadequate retention and poor interaction of

Ponesimod with the selected chromatographic system. A second trial performed on a YMC Triart C18 (similar dimensions as first trial) column replacing methanol with acetonitrile (70:30, v/v) similarly did not yield an analyte peak, suggesting that acidic mobile phase conditions were unsuitable for efficient chromatographic elution of Ponesimod. Subsequent optimization using a YMC Triart C18 column with 250 mm length, with 10 mM ammonium bicarbonate buffer and acetonitrile(ACN) (60:40, v/v) flown by 0.5 mL min^{-1} resulted in a sharp, symmetrical, and well-resolved chromatographic peak with a retention time of approximately 6.52 min. The improved chromatographic behaviour as displayed in Fig. 2, can be attributed to the mildly basic buffering environment created by ammonium bicarbonate, which enhanced analyte ionization control and improved interaction between Ponesimod and the stationary phase, leading to superior peak symmetry and chromatographic efficiency. Ammonium bicarbonate buffer was used because it provided a mildly basic chromatographic environment that controlled analyte ionization and reduced peak tailing. The optimized buffer–acetonitrile composition (60:40, v/v) had better peak symmetry, theoretical plate count, and reproducible retention than acidic mobile phase systems examined during preliminary optimization.

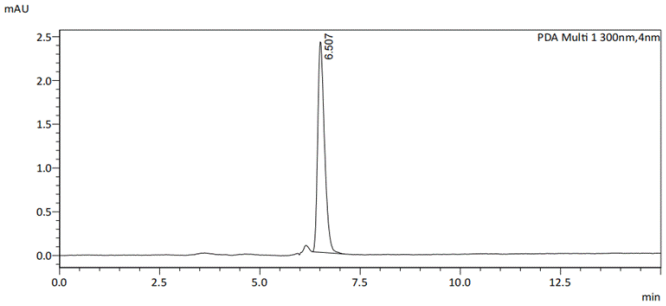


Fig. 2: Chromatogram of Ponesimod upon injection

Compared with previously reported RP-HPLC methods, the present method provides comparable chromatographic efficiency while employing a simple isocratic mobile phase without ion-pairing reagents or complex gradient elution. Sankar *et al*. reported an RP-HPLC method utilizing ammonium bicarbonate-based mobile phases with satisfactory chromatographic performance; however, the present method provides a shorter analytical procedure with excellent peak symmetry and reproducibility while maintaining comparable sensitivity. This RP-HPLC method is a good alternative to LC-MS/MS methods for pharmaceutical quality control. It originated in pharmacokinetics research. Standard HPLC equipment, little sample preparation, an isocratic mobile phase with less solvent, and superb analytical findings without expensive mass spectrometric detection are used in the approach. These properties make the recommended method suitable for quality assurance labs, batch release testing, and formulation analysis.

System Suitability

Table. 2 demonstrated satisfactory chromatographic performance before sample analysis. Six replicate injections produced a consistent retention time of 6.517 min with negligible variation (%RSD = 0.00%). The mean peak area was 1,106,396 with a %RSD of 0.234, indicating excellent injection repeatability. The average tailing factor of 1.34 confirmed symmetrical peak shape, while the theoretical plate count of 6540 exceeded the minimum acceptance criterion of 2000, indicating adequate column efficiency. The obtained system suitability parameters compare favorably with previously published methods, where tailing factors generally ranged between 1.2 and 1.6 and theoretical plate counts exceeded 4000. The present method demonstrated improved column efficiency while maintaining excellent chromatographic reproducibility.

Table 2: Depicting System Performance Metrics *(n=6)*

RT[a]	Peak area[b]	Tailing[f]	Plate count[g]
6.517	1106711	1.35	6535
6.517	1106575	1.31	6512
6.517	1106259	1.36	6588
6.517	1106376	1.3	6476
6.517	1105976	1.34	6580
6.517	1106479	1.35	6548
6.517[c]	1106396[c]	1.34[c]	6540[c]
0.00[d]	228.3[d]	0.02[d]	42.13[d]
0.00[e]	0.234[e]	1.82[e]	0.64[e]

[a] Peak elution time in minutes; [b] Total abundance of Ponesimod for each of the injections; [c] Mean *(n=6)*;[d] Standard Deviation; [e] % Relative standard Deviation

Specificity

Specificity was evaluated by injecting the diluent, Ponesimod reference standard, and tablet sample under the optimized chromatographic conditions. Chromatograms taken from the Ponesimod standard solution (Fig. 3**b**) and the diluent (Fig. 3**a**) are compared. Ponesimod exhibited full chromatographic selectivity since no interference peaks were detected at the retention time. Analyte peak remained well resolved without evidence of co-eluting impurities or excipient interference, demonstrating developed methodology is in fact particular for regular analysis. Absence of interference is consistent with previously published HPLC methods for Ponesimod and related sphingosine-1-phosphate receptor modulators, where chromatographic specificity was primarily achieved through optimized reversed-phase separation.

Precision

Table. 3 exhibited excellent repeatability. Repeatability studies performed on the same day produced a peak area %RSD of 0.04%, whereas intermediate precision performed on the succeeding day produced a %RSD of only 0.0234%. Retention times remained highly reproducible under both experimental conditions, confirming excellent method ruggedness. The observed precision values are substantially lower than the ICH acceptance limit of 2.0%, indicating excellent analytical reproducibility. Compared with earlier RP-HPLC methods for Ponesimod and other S1P receptor modulators, which generally reported precision values between 0.3 and 1.5%, the present method exhibited superior repeatability.

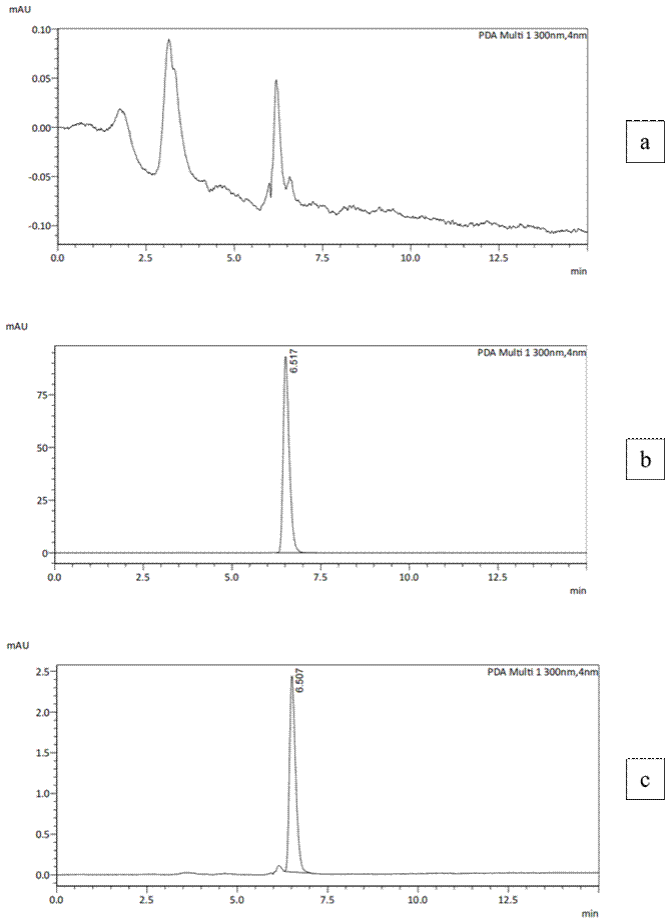


Fig. 3: Chromatogram of (a) Diluent, (b) Ponesimod, (c) Tablet sample

Linearity

The concentration range of 5-150 µg mL^{-1} was found to have excellent linearity, as shown in Table. 4 and Fig. 4. Linear regression analysis produced the equation y = 5552.84x + 2140.40 with a regression coefficient (R^2) of 0.9999, display a strong linear correlation between peak area and analyte concentration across the whole range under investigation, as depicted in Fig. 5. The calibration range adequately covers concentrations encountered during routine assay analysis. Compared with previously published RP-HPLC procedures, which commonly reported linearity over ranges of approximately 10–100 µg mL^{-1}, the present method offers a broader working range while maintaining excellent regression characteristics.

Table 3: Total abundance under Identical & Variable conditions

On the Day		Succeeding Day	
RT[a]	AREA[b]	RT[a]	AREA[b]
6.517	1106700	6.517	1106711
6.517	1106675	6.517	1106575
6.507	1107259	6.517	1106259
6.507	1106076	6.517	1106376
6.507	1105976	6.517	1105976
6.507	1106479	6.517	1106479
6.50[c]	1106528[c]	6.517[c]	1106396[c]
0.005[d]	468.4[d]	0.00[d]	258.350[d]
0.08[e]	0.04[e]	0.00[e]	0.0234[e]

[a] Peak elution time in minutes, [b] Total abundance of Ponesimod for each of the injections, [c] Mean *(n=6)*, [d] Standard Deviation, [e] % Relative standard Deviation

Table 4: Plot of Total abundance against Concentration

Preparation [a]	Peak Area [b]
5	28498
10	57232
50	280379
80	449734
100	556555
120	668927
150	833371

[a] µg/mL; [b] plentiful region for a specific concentration

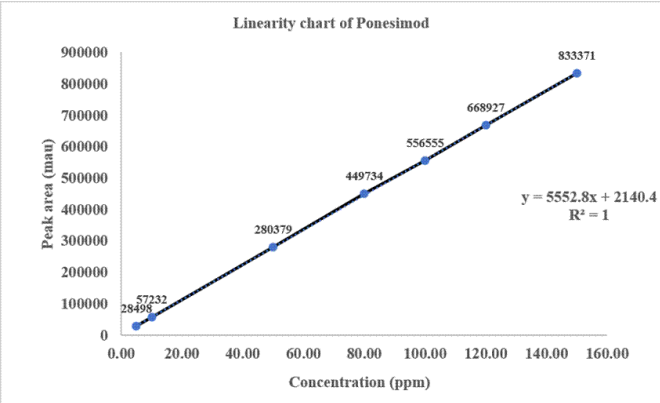


Fig. 4: A straightforward linear distribution for the dose range of 5-150 µg/mL

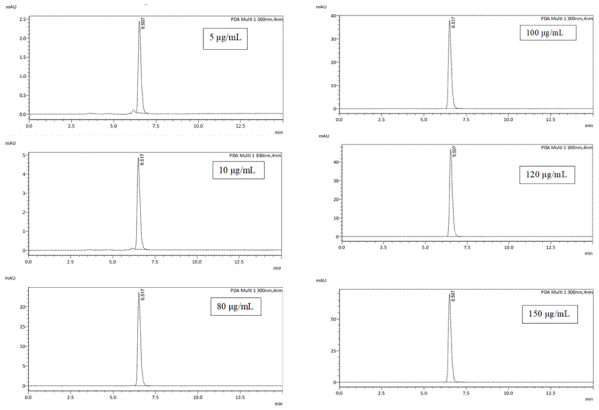


Fig. 5: Total abundance of Ponesimod [5,10,80,100,120,150µg/mL] in Chromatogram

Detection and Quantification Limits

Table. 5 demonstrated high analytical sensitivity with an LOD of 0.3 ng mL^{-1} and an LOQ of 1.0 ng mL^{-1}. Signal-to-noise ratios exceeded the acceptance criteria of 3 for LOD and 10 for LOQ, confirming reliable analyte detection and quantification at trace concentrations. Although LC-MS/MS methods generally achieve lower detection limits owing to superior detector sensitivity, the present RP-HPLC method provides sensitivity that is more than sufficient for routine pharmaceutical quality control analysis. For ICH Q2(R2) compliance, repeated injections verified the concentrations' chromatographic response and analytical precision.

Table 5: Precision at the Detection (DL) & Quantification Limit (QL)

Analytical parameter	DL (0.30 ng mL^{-1})	QL (1.00 ng mL^{-1})
Replicate injections (n)	6	6
Mean peak area	1334.50	5910.33
Area SD	186.73	42.75
Area %RSD	13.99	0.72
Mean signal-to-noise ratio	6.94	40.36
S/N SD	0.22	2.35
S/N %RSD	3.13	5.83
Acceptance criterion	S/N ≥ 3	S/N ≥ 10
Method suitability	Complies	Complies

SD: standard deviation; %RSD: percentage relative standard deviation

Accuracy

Table. 6 displays recovery trials conducted at 50%, 100%, and 150% concentration levels. The mean recoveries for these levels were 99.04%, 99.45%, and 101.15%, respectively. The %RSD values were found to be below 2%.These results confirmed excellent agreement between measured and theoretical concentrations, indicating negligible matrix interference and high analytical accuracy. The recovery values fall well within the ICH acceptance limits of 98–102% and compare favourably with previously published methods, which generally reported recoveries ranging from 98.5% to 101.8%.

Table 6: Evaluation of the recovery of Ponesimod

	DS [a]	DP [b]	Recovered amount	
			100.36	99.04 [c]
	25	50	98.58	1.15 [d]
50%			98.20	1.17 [e]
			99.14	99.45 [c]
	50	50	99.14	0.54 [d]
100%			100.07	0.54 [e]
			100.94	101.15 [c]
	75	50	101.01	0.31 [d]
150%			101.51	0.31 [e]

[a] concentration of pure substance spiked; [b] concentration of formulation; [c] Mean *(n=6);* [d] Standard Deviation; [e] % Relative standard Deviation

Table 7: Robustness by deliberate variation in COT (±5°C) and in FR (±0.1 mL min^{-1}) (n = 6)

Parameter	+5°C	–5°C	+0.1 mL min^{-1}	–0.1 mL min^{-1}
Retention time (min)	6.453 ± 0.00	6.560 ± 0.00	5.440 ± 0.00	8.13 ± 0.01
Peak area	1100493 ± 386.89	1099656 ± 122.71	918387 ± 52.03	1380316 ± 712.53
Tailing factor	0.98 ± 0.02	1.09 ± 0.05	0.95 ± 0.04	1.10 ± 0.04
Theoretical plates	9355 ± 99.55	9987 ± 138.25	9357 ± 43.57	9326 ± 104.28
%RSD (Peak area)	0.035	0.011	0.01	0.05
%RSD (Tailing factor)	0.193	1.210	0.243	0.254
%RSD (Plate count)	1.258	1.523	1.864	1.584
Result	**Complies with that of the required**			

Robustness

Chromatographic performance was unaffected by controlled changes in flow rate and column temperature, as shown in Table. 7. All parameters for system compatibility stayed within acceptable ranges. The robustness performance observed in the present investigation is comparable to previously reported RP-HPLC methods, confirming that the optimized chromatographic conditions provide reliable

routine analysis without requiring stringent operational control.

Ruggedness

To measure ruggedness, a designed experiment was carried out, with specified parameters purposefully altered within acceptable ranges as shown in Table. 8. The effects of these modifications on the method's performance are then assessed by comparing the results to those obtained under standard settings. Statistical Chromatograph analysis is used to establish the significance of these differences. It was judged that the criteria were met.

Table 8: Different lines with eluted Ponesimod (200 µg/mL) and its associated data

RT[a1]	AREA[a2]	RT[b1]	AREA[b2]
6.517	1106700	6.517	1106711
6.517	1106675	6.517	1106575
6.507	1107259	6.517	1106259
6.507	1106076	6.517	1106376
6.507	1105976	6.517	1105976
6.507	1106479	6.517	1106479
6.50[c]	1106528[c]	6.517[c]	1106396[c]
0.005[d]	468.4[d]	0.00[d]	258.350[d]
0.08[e]	0.04[e]	0.00[e]	0.0234[e]

[a1] Peak elution time in minutes obtained on Column I, Technician I; [a2] Total abundance of Ponesimod for each of the injections on Column I, Technician I; [b1] Peak elution time in minutes obtained by technician II on Column II; [b2] Total abundance of Ponesimod for each of the injections by Technician II; [d1] Mean peak elution time *(n=6);* [e1] Mean peak abundance *(n=6);*[f] Standard Deviation; [g] % Relative standard Deviation

Assay of Marketed Tablets

Application of the validated method to the commercial tablet formulation (Ponvory 20mg) demonstrated satisfactory assay performance, with the measured drug content complying with Pharmacopoeial acceptance criteria. Estimation was performed by injecting a sample (5 µg/mL) into an HPLC and applying the calculations listed below. The verified RP-HPLC method is ideal for routine pharmaceutical quality control due to its fast chromatographic analysis, ease of use, and low solvent consumption. Batch release testing, in-process quality monitoring, stability studies, and routine regulatory analysis can be done utilizing HPLC equipment found in most pharmaceutical labs.

sample area	wt of std	dilution of sample	purity	wt of tablet	100
standard area	dilution of std	wt of sample	100	label claim	
28485	99.9	100	99.8	19.3	
28498	10	94.81	100	20	

=1.0143*100 = 101.43 %

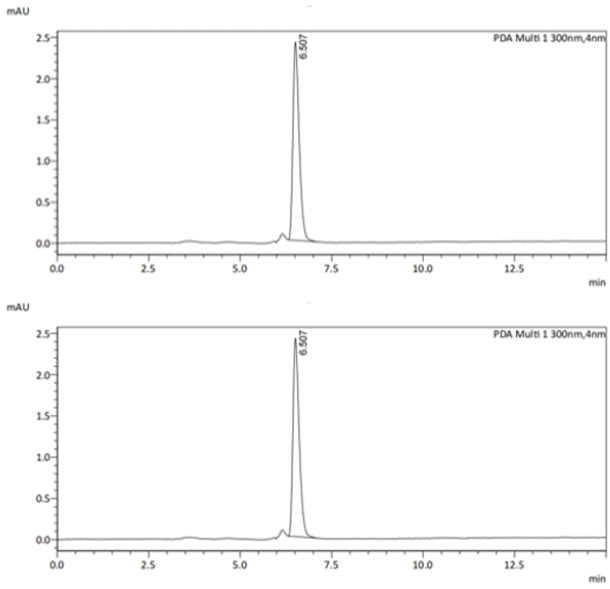


Fig. 6: Pictograph of height eluted time in minutes and total abundance for influx of (a) Ponesimod standard (b) Ponvory 20 mg

CONCLUSION

The quantitative determination of Ponesimod in bulk medication and tablets was accomplished by the development and validation of a reversed phase technique that is quick, selective, robust, and inexpensive. Using an isocratic mobile phase of 10 mM ammonium bicarbonate and acetonitrile (60:40, % volume by volume) flown by 0.5 mL min^{-1}, a well-resolved chromatographic peak with a retention time of 6.517 min was produced as the product was separated on a YMC Triart C18 (250 × 4.6 mm, 5 µm). A tailing factor of 1.34 and 6540 theoretical plates showed that the devised approach was quite suitable for the system. The good analytical performance was proven through validation that followed the current ICH Q2(R2) criteria. The method showed linearity with a regression coefficient (R^2 = 0.9999) over the concentration range of 5-150 µg mL^{-1}, for both repeatability and intermediate precision it produced %RSD values of 0.04% and 0.0234%, correspondingly. The analytical accuracy was shown to be excellent in recovery trials, with mean recoveries ranging from 99.04% to 101.15%. With acceptable signal-to-noise ratios, the detection limits (DL) were set at 0.30 ng mL^{-1} and 1.00 ng mL^{-1}, respectively. Experiments on robustness proved that chromatographic performance was unaffected by purposeful changes in flow rate and column temperature.

Without any interference from the formulation excipients, the quantitative evaluation of Ponvory® tablets was successfully carried out using the validated method, producing an assay result of 101.43%. The current RP-HPLC method provides an easy, affordable, and repeatable analytical alternative to previously documented LC-MS/MS techniques that were mainly used for pharmacokinetic studies. It is well-suited for regular pharmaceutical quality control, batch release testing, and formulation analysis.

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