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Research Article

Analytical Method Development and Validation of a Cardiovascular Drug By HPLC

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ABSTRACT

A simple, accurate, precise and economical reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Vericiguat, a cardiovascular drug used in the treatment of chronic heart failure [19-20]. The chromatographic separation was achieved on an Agilent C18 column (100 mm × 4.6 mm, 2.5 µm particle size) using a mobile phase of methanol and water containing 0.1% acetic acid in the ratio 50:50 (v/v), pH adjusted to 3.2. The mobile phase was pumped isocratically at a flow rate of 0.8 mL/min, with detection carried out at 258 nm using a diode array detector. Under these optimised conditions, Vericiguat eluted at a retention time of 5.268 min with sharp, symmetrical peaks and a theoretical plate count exceeding 12,000. The method demonstrated linearity across the concentration range of 10–50 µg/mL, yielding a correlation coefficient of 0.999 and the regression equation y = 31.33x + 46.50. Mean recovery at three spiking levels fell within 98–102%, confirming accuracy. Intra-day and inter-day precision studies returned %RSD values well below 2%. The limit of detection and limit of quantitation were determined to be 0.1320 µg/mL and 0.3949 µg/mL respectively. Robustness and specificity studies gave satisfactory results. The proposed method is simple, accurate, precise, economical and reproducible, and is suitable for the routine quality-control analysis of Vericiguat [21-24].

INTRODUCTION

Analytical chemistry concerns itself with the analysis of material samples to gain a thorough understanding of their chemical composition and structural architecture. It is, at its core, a measurement science — a collection of powerful ideas and methods that find use across every field of science and medicine. The discipline seeks ever-improved means of measuring the chemical

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composition of both natural and artificial materials [1,2].

In recent decades, the centre of gravity in analytical chemistry has shifted decisively toward instrumental methods. The speed and sensitivity of these physical and physicochemical approaches have far surpassed what classical gravimetric and volumetric analysis can deliver. Currently, most instrumental analysis rests on comparing a signal from the sample against that from a standard of known composition [3,4].

Analytical chemistry divides into qualitative analysis, which identifies the elements, ions or compounds present in a sample, and quantitative analysis, which determines how much of one or more constituents is present [29]. Classical methods rely on precipitation, extraction or distillation to separate components. Instrumental methods exploit the interaction of matter with energy to extract analytical information. The principal instrumental families include spectroscopy, electrochemistry, chromatography, thermal analysis and microscopy [5–8].

Chromatography is unique in the history of analytical methodology and is probably the most powerful and versatile separation technique available [9]. In a single procedure it can separate a mixture into its individual components and simultaneously provide quantitative data on the amount of each component present [10]. HPLC stands for high-resolution, high-pressure, high-speed liquid chromatography. It has several times the resolving power of open-column liquid chromatography and is used for speedy resolution of complex mixtures [11-12]. It provides a specific, sensitive and precise method for analysis, with ease of sample preparation and rapid turnaround [13–15]. Validation is defined as documented evidence that provides a high degree of confidence that a process will consistently produce a product meeting its predetermined specifications. The ICH Q2(R1) guideline identifies the following parameters: accuracy, linearity, precision (repeatability and intermediate precision), detection limit, quantitation limit, specificity, range and robustness [16–18].

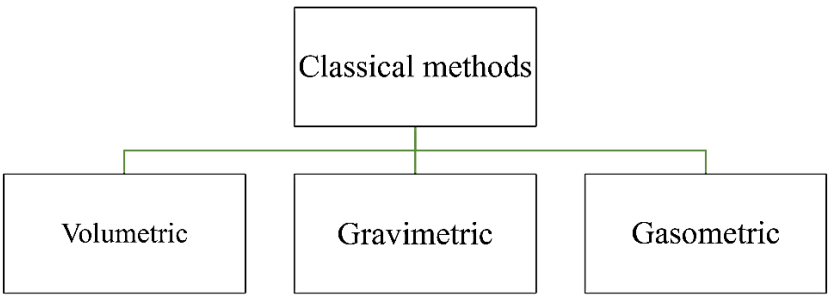

Fig. 1: Classical Analytical Methods

2. DRUG PROFILE

Vericiguat was approved by the United States FDA on December 22, 2015 for the treatment of pulmonary arterial hypertension (PAH) to delay disease progression and reduce risk of hospitalization. PAH is a relatively rare disease with usually a poor prognosis requiring more treatment options to prolong long-term outcomes. Marketed by Actelion Pharmaceuticals under brand name Uptravi, Vericiguat and its active metabolite, ACT-333679 (MRE-269), act as agonists of the prostacyclin receptor to increase vasodilation in the pulmonary circulation and

decrease elevated pressure in the blood vessels supplying blood to the lungs [19,20].

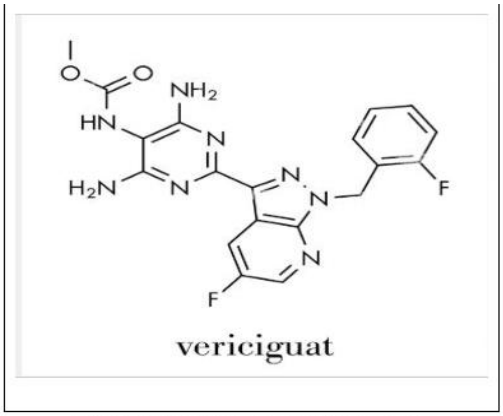

Fig. No.5 Structure of Vericiguat

Fig. 2: Structure of Vericiguat

Table 1: Profile of drug

Parameter	Details
Molecular formula	$C_{19}H_{16}F_2N_8O_2$
Molecular weight	426.388 g/mol
Chemical name	Methyl N-[4,6-diamino-2-[5-fluoro-1-[(2-fluorophenyl) methyl]pyrazolo[3,4-b]pyridin-3-yl]pyrimidin-5-yl]carbamate
Description	White crystalline powder
Category	Cardiovascular drug

Heart failure involves the impaired synthesis of nitric oxide (NO) and decreased activity of soluble guanylate cyclase (sGC). Vericiguat directly stimulates sGC by binding to a target site on its beta-subunit, bypassing the need for NO-mediated activation, and causes an increase in the production of intracellular cGMP, resulting in vascular smooth muscle relaxation and vasodilation.

3. LITERATURE SURVEY

Mandhare et al. (2024) developed a QbD-based RP-HPLC method using a C18 column with methanol and 0.1% OPA (76:24) at 331 nm. LOQ was 0.7209 µg/mL, LOD was 0.2379 µg/mL [21].

Mustafa et al. (2023) used an Inertsil ODS-C18 column with water:acetonitrile (70:30) at 332 nm. Retention time was 4.500 min, linearity 2–20 µg/mL, $r^2 = 0.9996$ [22].

Madhavi et al. (2024) used a Kromacil C18 column with KH_2PO_4:acetonitrile (50:50) at 252

nm. RT was 4.42 min, linearity 30–70 µg/mL, recovery 99.92–100.61% [23].

Bodke et al. (2023) used a Hemochrom C18 column with 0.1% TFA:acetonitrile (65:35) at 327 nm. RT was 8.3 min, linearity 5–150 µg/mL [24].

Patel et al. (2023) used a Zorbax Eclipse Plus C18 column with KH_2PO_4:methanol (60:40) at 256 nm. RT was 6.9 min, linearity 50–150 µg/mL, r^2 = 0.9995 [25].

Gadapa N. LC-Based Analytical And Assay Methodologies For Multiclass Drug Quantification. Authors Click Publishing; 2026 Jan 23 [27].

Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An overview of experimental designs in HPLC method development and validation. Journal of pharmaceutical and biomedical analysis. 2018 Jan 5;147:590-611 [28].

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Manasa K, Shiva S, Gowtham C, Akanksha S, Sreeja K, Sandhya N. A Review On Method Development And Validation Ofselected Anticancer And Cardiovascular Drugs Using Different Analytical Techniques. Journal For Innovative Development in Pharmaceutical and Technical Science (JIDPTS). 2026 Jan;9(1) [50].

Prakash L, Himaja M, Subbaiah BV, Vasudev R, Srinivasulu C, Haribabu R. Isolation, identification and characterization of degradant impurities in Tolterodine tartrate formulation. Journal of Pharmaceutical and Biomedical Analysis. 2014 Mar 5;90:215-21 [51].

4. MATERIALS AND METHODS

4.1 Drug, Chemicals and Reagents

Table 2: Drug and Drug Supplier

Name of Drug	Drug Supplier
Vericiguat	Swapnroop Drug and Pharmaceutical

Table 3: List of Reagents and Chemicals used

Sr. No.	Chemical	Manufacturer
1	Acetonitrile (HPLC grade)	Merck Ltd., India
2	Methanol (HPLC grade)	Merck Ltd., India
3	Water (HPLC grade)	Merck Ltd., India
4	0.1% OPA (HPLC grade)	Merck Ltd., India
5	0.1% Acetic acid (HPLC grade)	Merck Ltd., India

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4.2 Instrumentation

Table 4: Instrument (HPLC) Details used during Method Development

Sr. No.	Instrument	Company
1	HPLC system	Agilent with autosampler (DAD, Chemstation)

2	UV Spectrophotometer	Analytical Technologies Limited
3	Column (C18)	Agilent C18 (100mm × 4.6mm, 2.5µm)
4	pH meter	VSI pH meter (VSI 1-B)
5	Balance	Wensar™ High Resolution Balance
6	Sonicator	Ultrasonic electronic instrument

4.3 UV Spectrum

A 10 µg/mL solution of Vericiguat in methanol was scanned over 200–400 nm. The drug showed maximum absorbance at 258 nm

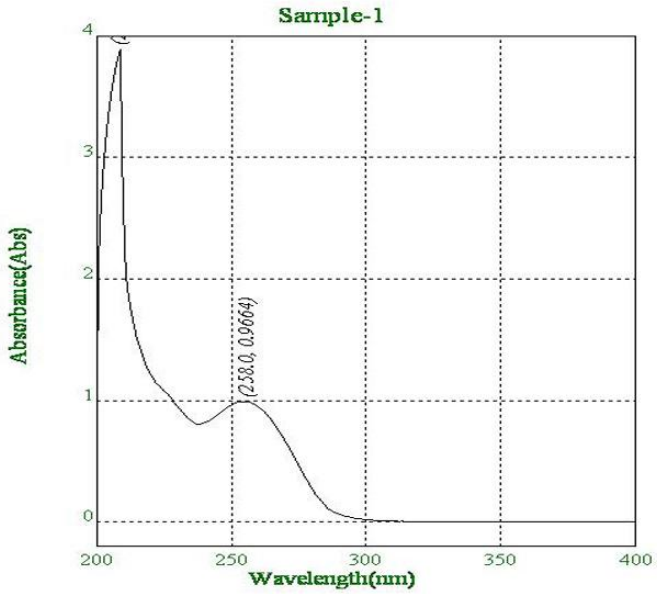


Fig. 2: UV spectrum of Vericiguat (λmax 258 nm)

4.4 Method Validation

The developed method was validated as per ICH Q2(R1) guidelines for linearity, accuracy, precision, robustness, specificity, LOD and LOQ [31,34,35].

5. RESULTS AND DISCUSSION

5.1 Method Development

Several mobile phase combinations were screened [25-26,30]. The trials and outcomes are summarised below.

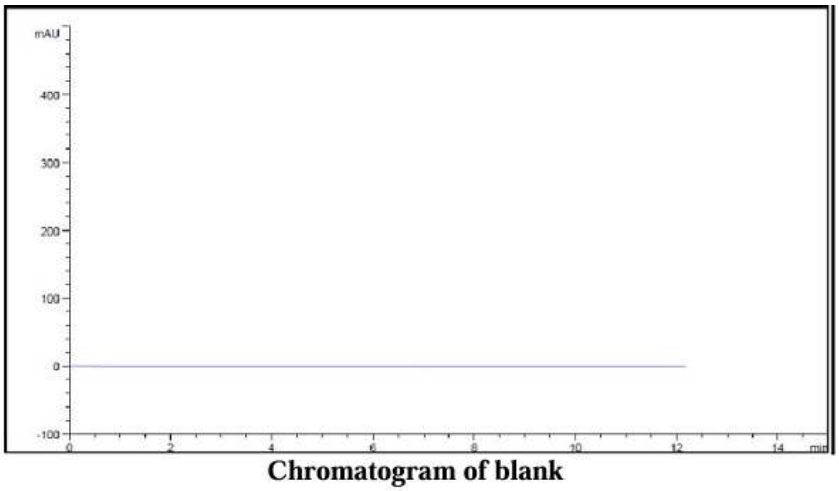
Table 5: Different Trials of Chromatographic Condition

Trial	Mobile phase, flow rate, wavelength	Observation	Conclusion
1	0.1% OPA water + MeOH (10:90), 0.7 mL, 258 nm	Peak splitting	Rejected
2	0.1% acetic acid water + MeOH (20:80), 0.7 mL, 258 nm	RT too long	Rejected

3	0.1% OPA water + MeOH (30:70), 0.7 mL, 258 nm	RT too long, low plates	Rejected
4	0.1% OPA water + MeOH (35:65), 1.0 mL, 258 nm	RT long	Rejected
5	MeOH + 0.1% acetic acid water (90:10), 0.7 mL, 258 nm	RT too long	Rejected
6	MeOH + 0.1% acetic acid water (75:25), 0.8 mL, 258 nm	RT long	Rejected
7	MeOH + 0.1% acetic acid water (50:50), 0.8 mL, 258 nm	Sharp peak obtained	Selected

The selected mobile phase (50:50 v/v), pH 3.2, flow rate 0.8 mL/min gave retention at 5.268 min with TP = 12,255 and TF = 0.85.

Blank chromatogram:



Chromatogram of blank

Standard chromatogram of Vericiguat:

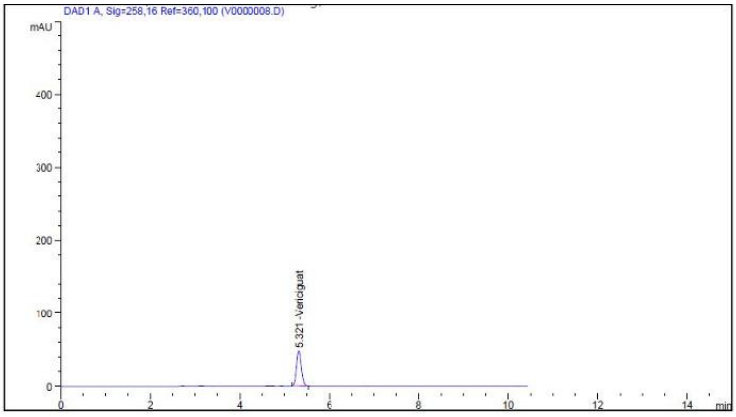


Fig. 3: Chromatogram of standard Vericiguat

Table 6: Result for standard Chromatogram of Vericiguat

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.321	358.03867	12504	0.86	-

5.2 Linearity

Working standard solutions at five concentration levels (10–50 µg/mL) were injected in duplicate and the peak areas recorded.

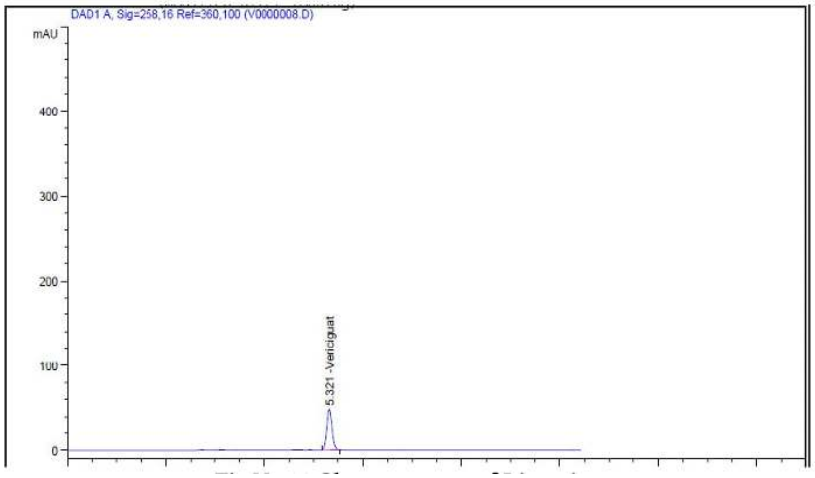


Fig. 4: Chromatogram of Linearity

Table 7: Result for standard Chromatogram of Vericiguat

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.321	358.0386	12504	0.86	-

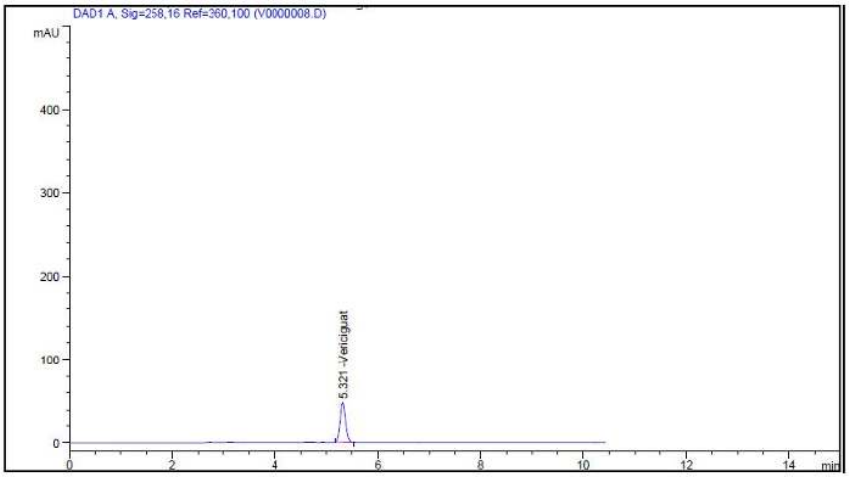


Fig. 5: Chromatogram of Linearity 10mcg-01

Table 8: Result for Chromatogram of linearity 10mcg-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.321	358.03867	12504	0.86	-

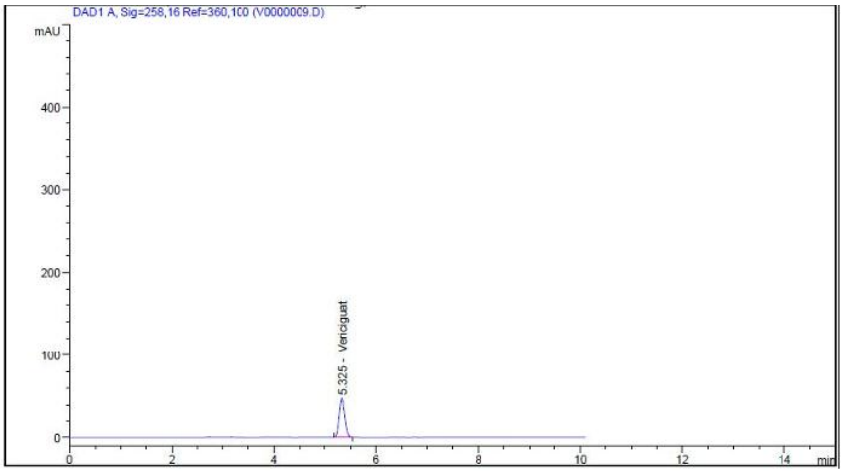


Fig. 6: Chromatogram of Linearity 10mcg-02

Table 9: Result for Chromatogram of linearity 10mcg-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.325	359.6866	12523	0.87	-

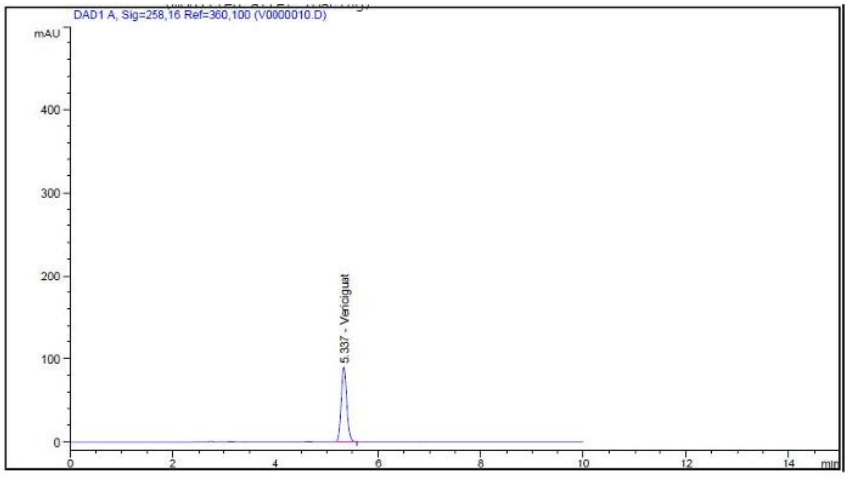


Fig. 7: Chromatogram of Linearity 20mcg-01

Table 10: Result for Chromatogram of Linearity 20mcg-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.337	664.86511	12287	0.86	-

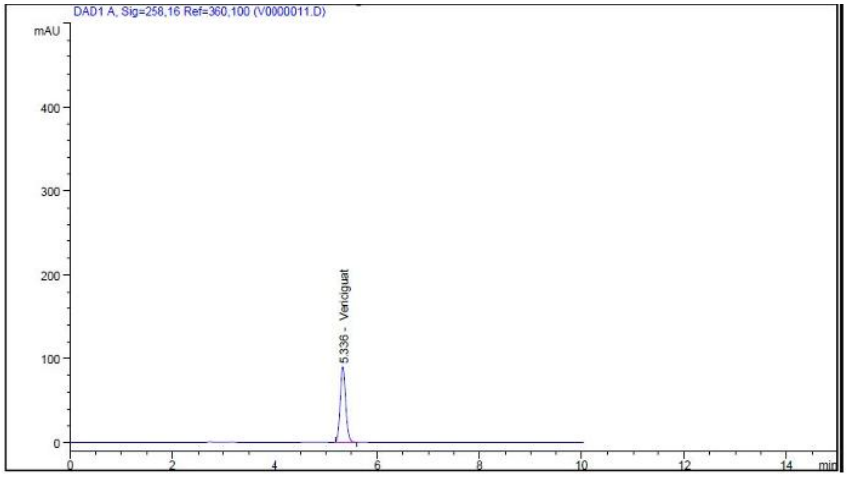


Fig. 8: Chromatogram of Linearity 20mcg-02

Table 11: Result for Chromatogram of linearity 20mcg-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.336	669.19025	12878	0.85	-

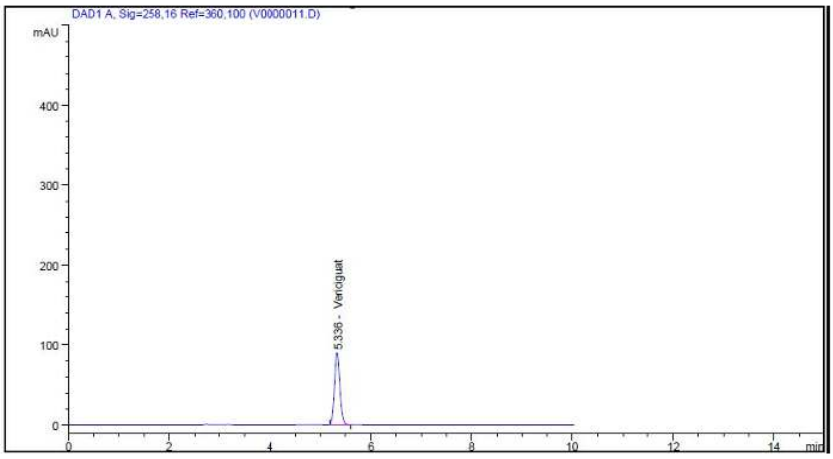


Fig. 9: Chromatogram of Linearity 30mcg-01

Table 12: Result for Chromatogram of linearity 30mcg-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.336	994.97418	12475	0.85	-

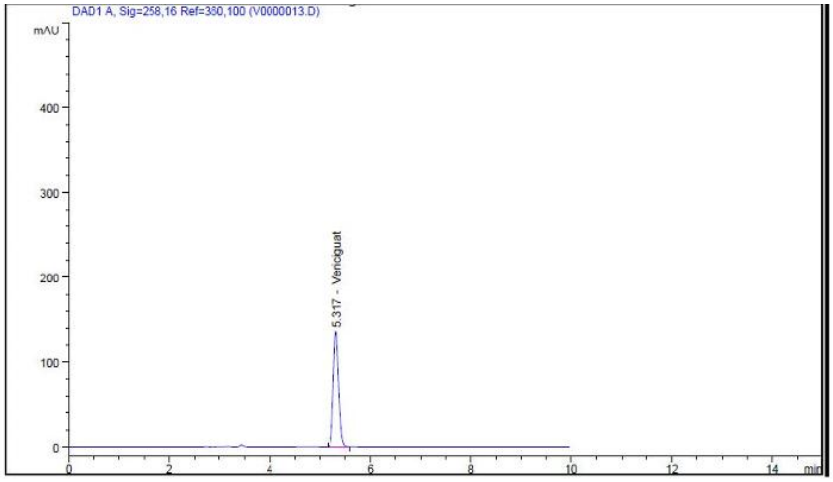


Fig. 10: Chromatogram of Linearity 30mcg-02

Table 13: Result for Chromatogram of linearity 30mcg-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.317	995.19849	12487	0.85	-

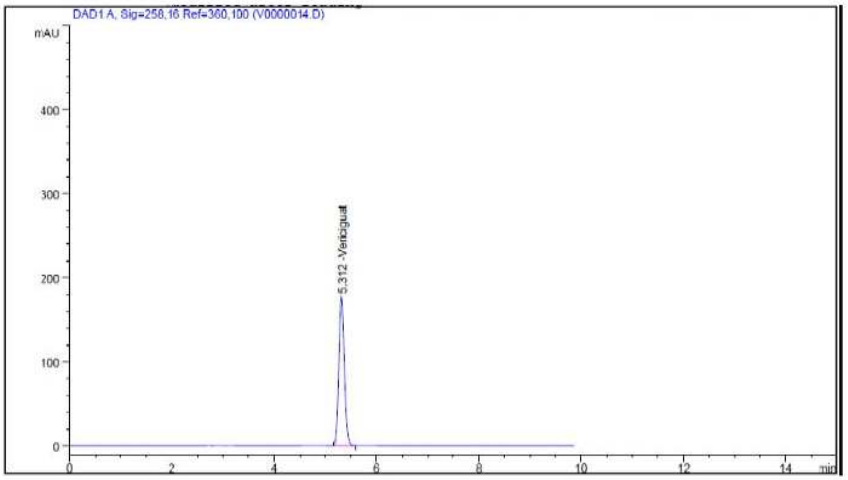


Fig. 11: Chromatogram of Linearity 40mcg-01

Table 14: Result for Chromatogram of Linearity 40mcg-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.312	1305.08679	12460	0.85	-

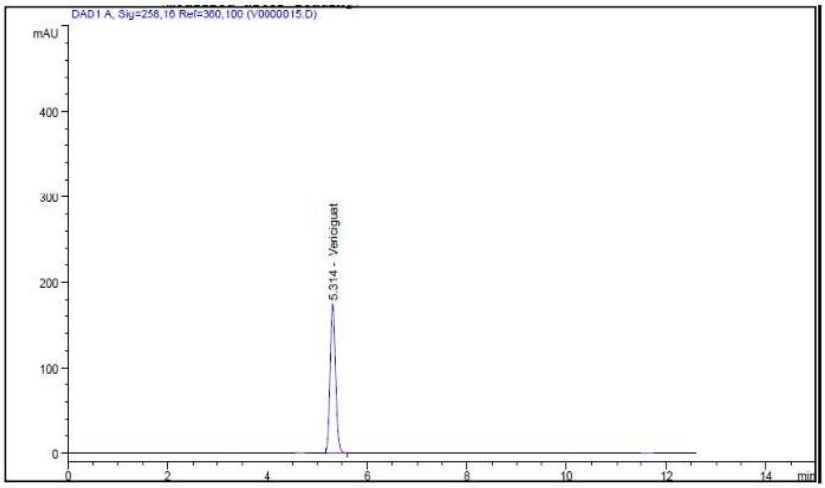


Fig. 12: Chromatogram of Linearity 40mcg-02

Table 15: Result for Chromatogram of Linearity 40mcg-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.314	1304.59705	12178	0.85	-

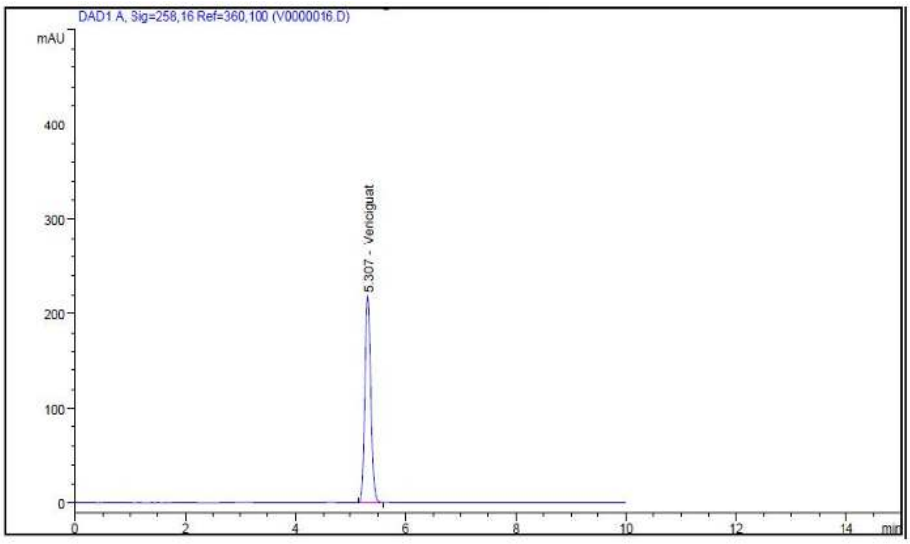


Fig. 13: Chromatogram of Linearity 50mcg-01

Table 16: Result for Chromatogram of linearity 50mcg-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.307	1607.66833	12440	0.86	-

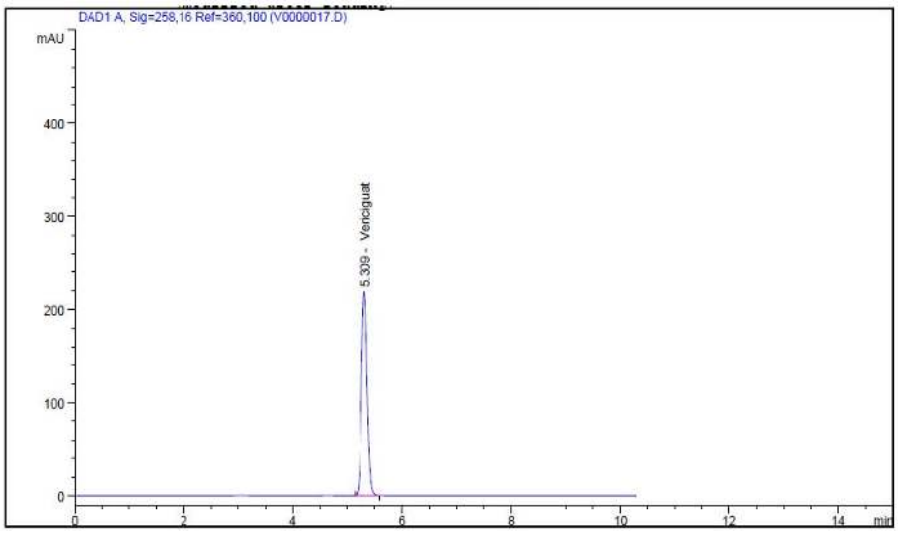


Fig. 14: Chromatogram of Linearity 50mcg-02

Table 17: Result for Chromatogram of Linearity 50mcg-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.309	1605.47900	12450	0.86	-

Table 18: Linearity of Vericiguat (HPLC)

Sr. No.	Concentration (µg/mL)	Area Vericiguat
1	10	358.86
2	20	667.03
3	30	995.09
4	40	1304.84
5	50	1606.57

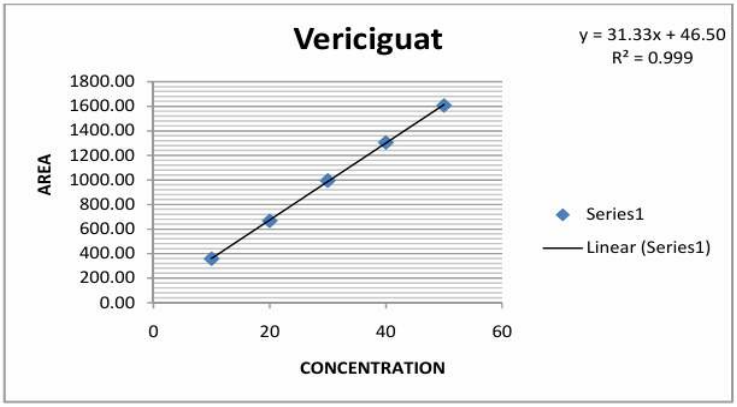


Fig. 15: Calibration curve of Vericiguat (HPLC)

Table 19: Regression equation data for Vericiguat

Regression parameter (y = mx + c)	Value
Slope (m)	31.33
Intercept (c)	46.50
Correlation coefficient (r^2)	0.999

5.3 Accuracy (Recovery)

Recovery studies were performed at 80%, 100% and 120% levels to validate accuracy.

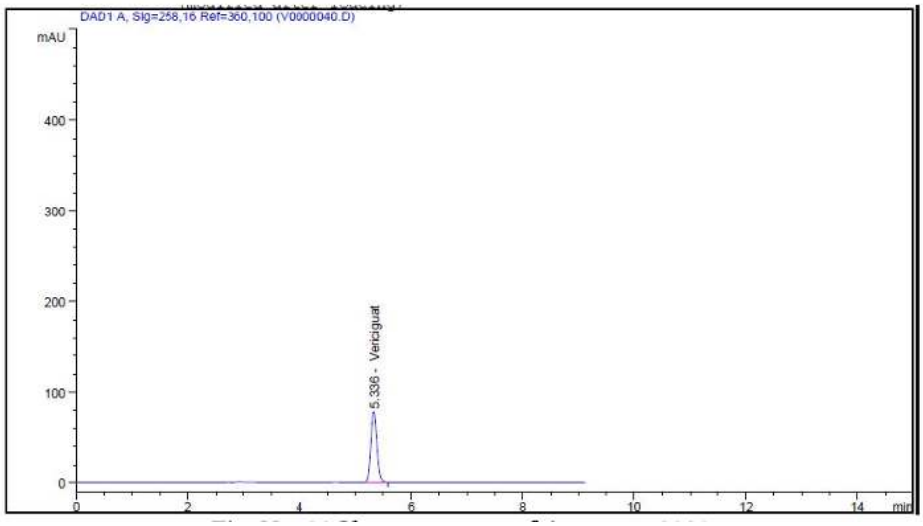


Fig. 16: Chromatogram of Accuracy 80%

Table 20: Result for Chromatogram of Accuracy 80%

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.336	608.92470	12281	0.85	-

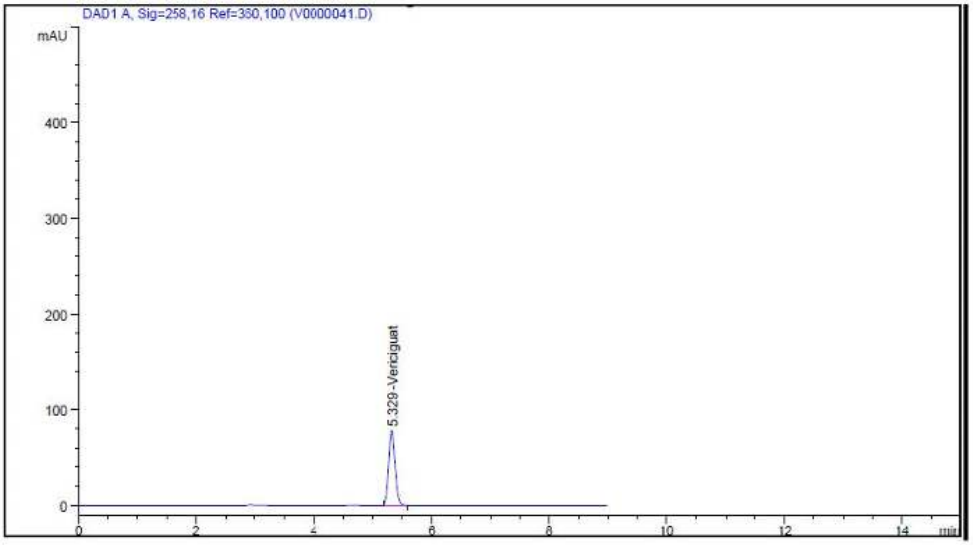


Fig. 17: Chromatogram of Accuracy 80%-02

Table 21: Result for Chromatogram of Accuracy 80%-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.329	609.76007	12249	0.86	-

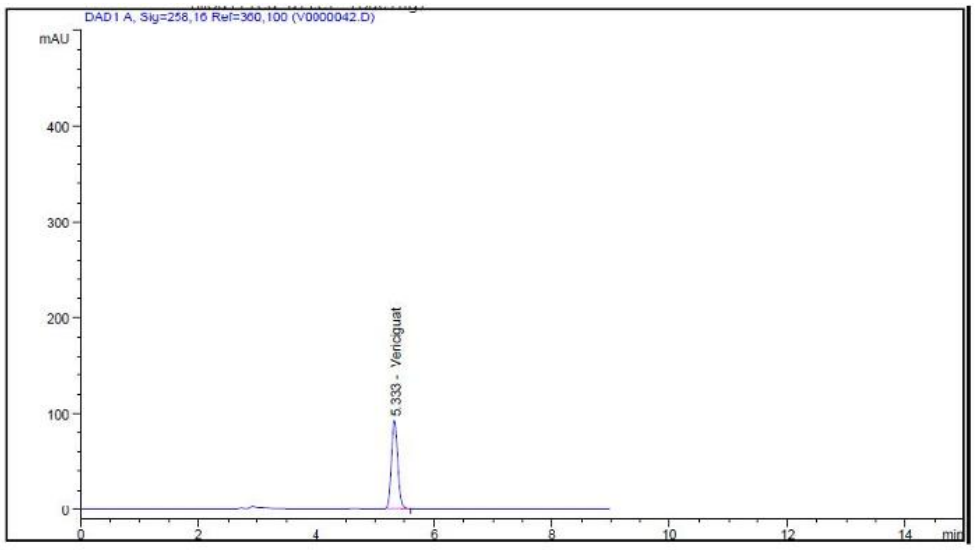


Fig. 18: Chromatogram of Accuracy 100%

Table 22: Result for Chromatogram of Accuracy 100%

No.	RT[min]	Area[mV*s]	TP	TF	Resolution

1	5.333	672.89670	12561	0.86	-

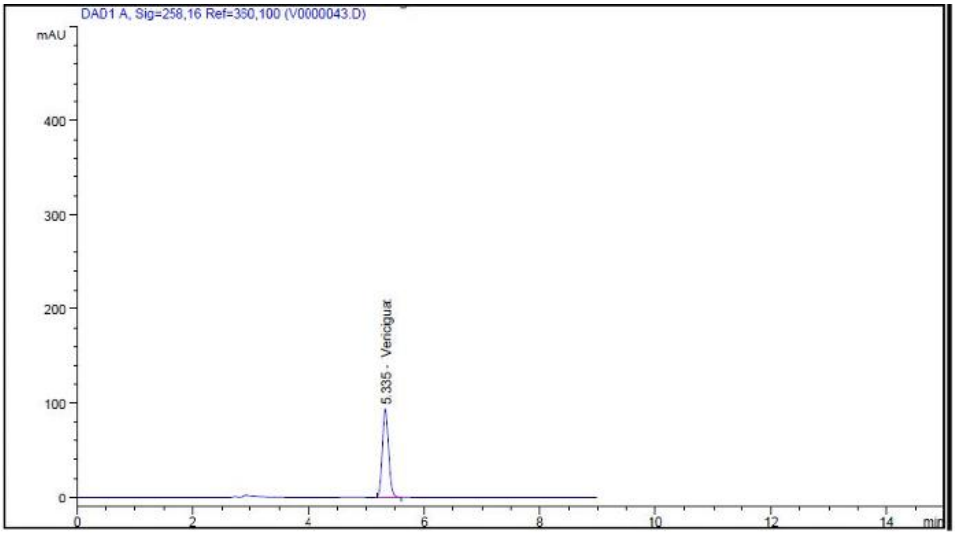


Fig. 19: Chromatogram of Accuracy 100%-02

Table 23: Result for Chromatogram of Accuracy 100%-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.335	675.71906	12568	0.85	-

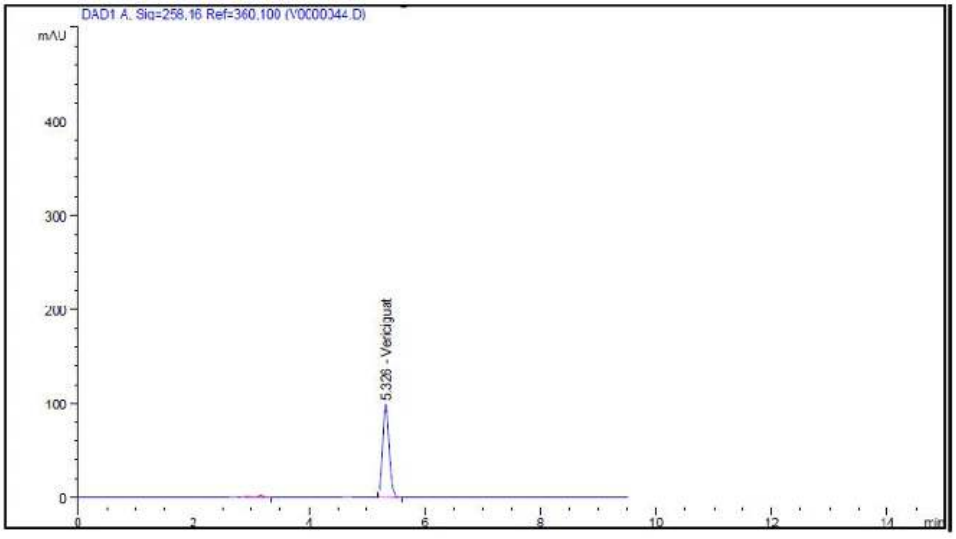


Fig. 20: Chromatogram of Accuracy 120%

Table 24: Result for Chromatogram of Accuracy 120%

No.	RT[min]	Area[mV*s]	TP	TF	Resolution

1	5.326	731.43109	12236	0.85	-

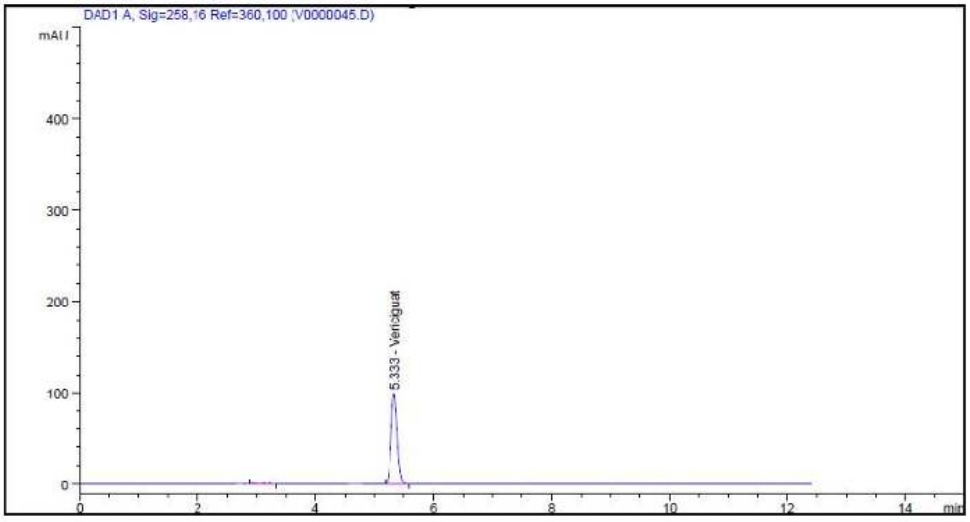


Fig. 21: Chromatogram of Accuracy 120%-02

Table 25: Result for Chromatogram of Accuracy 120%-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.333	730.09045	12560	0.86	-

Table 26: Result of Recovery data for Vericiguat

Level (%)	Amt. taken	Amt. added	Area Mean ± SD	Amt. recovered Mean ± SD	% Recovery Mean ± SD
80	10	8	17.95±0.01	7.96±0.01	99.56±0.24
100	10	10	20.04±0.06	10.0±0.06	100.39±0.64
120	10	12	21.84±0.03	11.84±0.03	98.67±0.25

Table 27: Statistical Validation of Recovery Studies Vericiguat

Level (%)	Mean % Recovery	SD	% RSD
80	99.56	0.24	0.24
100	100.39	0.64	0.63
120	98.67	0.25	0.26

5.4 System Suitability (Repeatability)

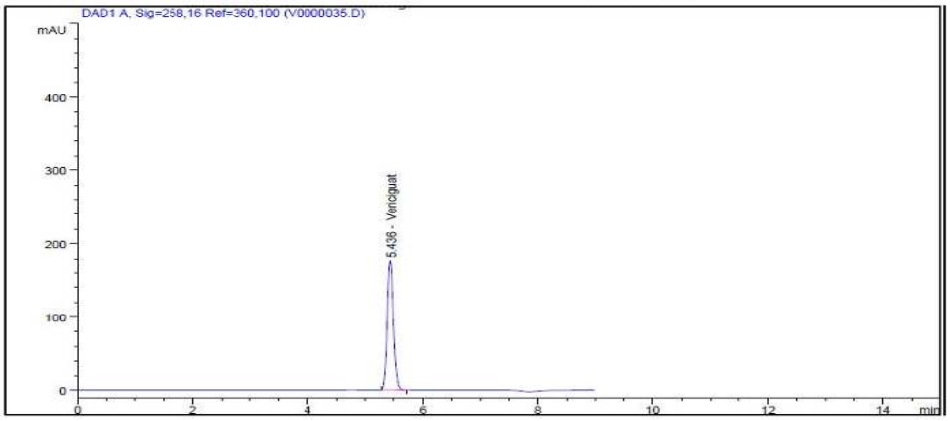


Fig. 22: Chromatogram of System suitability No. 1

Table 28: Result for Chromatogram of System suitability No. 1

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.436	1313.92358	13050	0.85	-

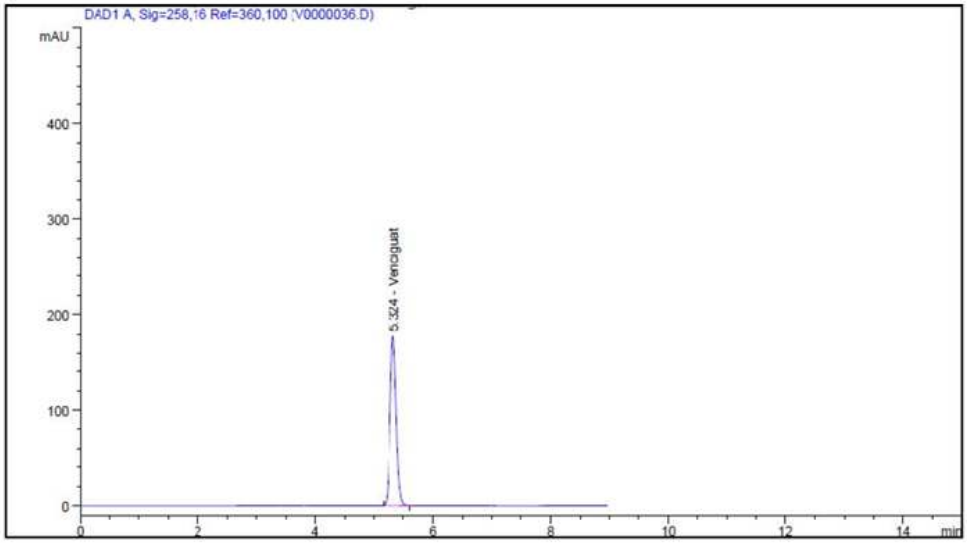


Fig. 23: Chromatogram of System suitability No. 2

Table 29: Result for Chromatogram of System suitability No. 2

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.324	1310.95288	12224	0.85	-

Table 30: Repeatability studies on Vericiguat (HPLC)

Sr.No.	Conc. (µg/mL)	Peak area	Amt found (mg)	% Amt found
1	40	1313.9235	40.41	101.02
2	40	1310.9528	40.39	101.00

Mean	—	—	40.40	101.01
SD	—	—	2.10	2.10
%RSD	—	—	0.16	0.16

5.5 Precision

The method was established by analyzing various replicates of Vericiguat. Solutions were analyzed to record intra-day and inter-day variation.

Chromatogram of Intra-day Precision:

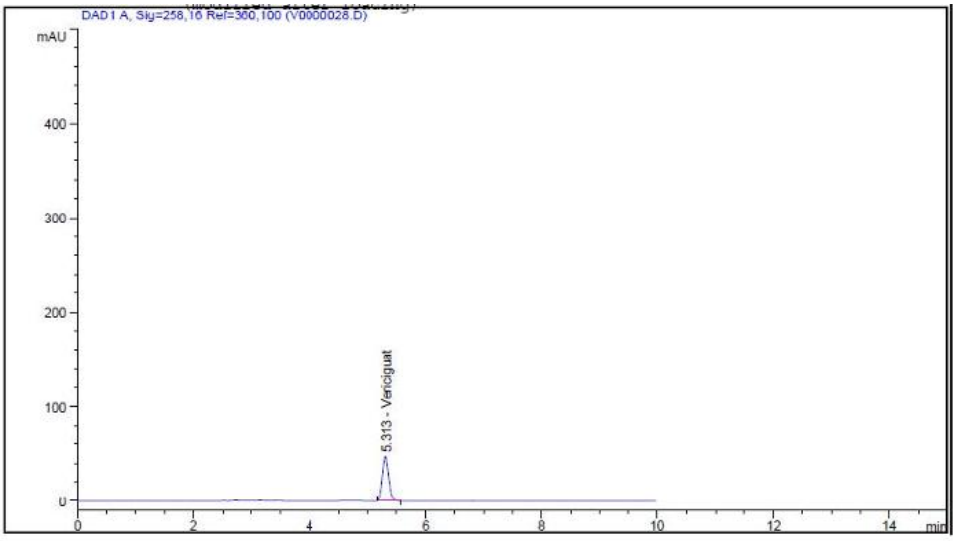


Fig. 24: Chromatogram Intra-day precision (10 mcg)-01

Table 31: Result for Chromatogram of Precision (10 mcg)

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.313	352.98010	12175	0.86	-

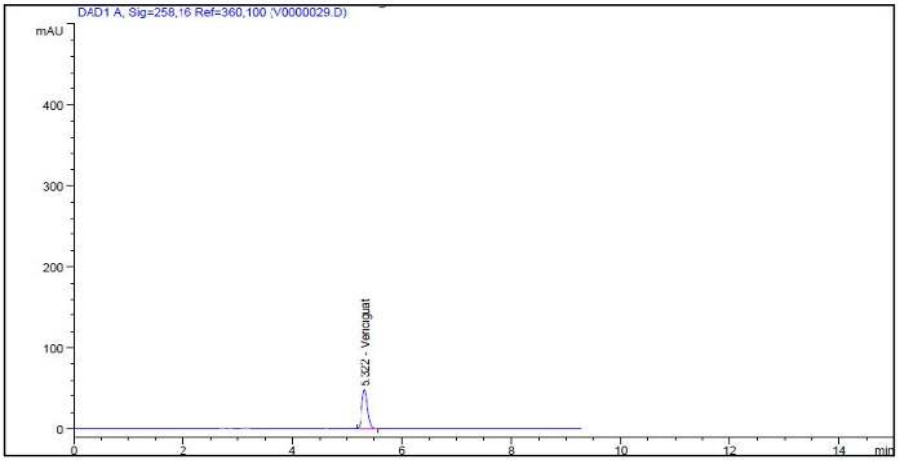


Fig. 25: Chromatogram Intra-day precision (10 mcg)-02

Table 32: Result for Chromatogram of Precision (10 mcg)-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.322	355.04132	12215	0.85	-

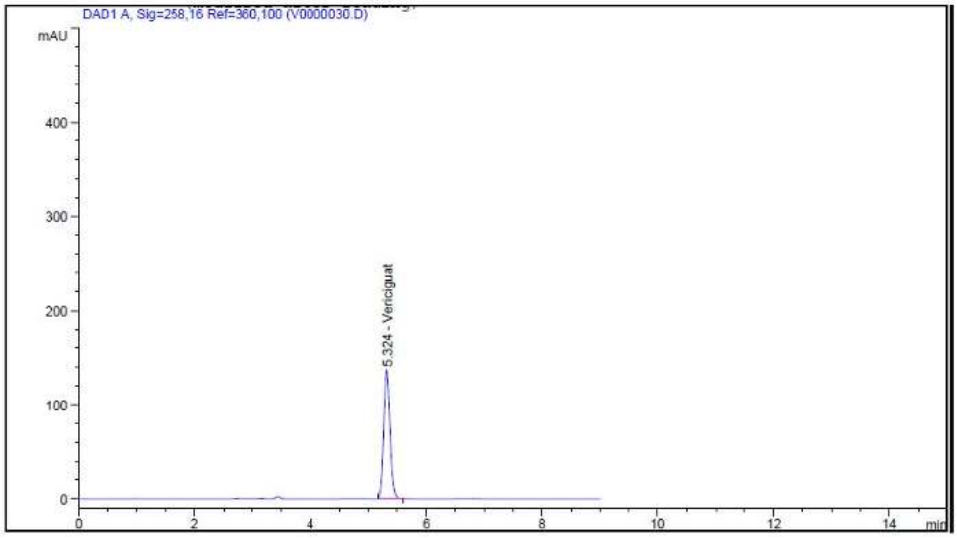


Fig. 26: Chromatogram Intra-day precision (30 mcg)

Table 33: Result for Chromatogram of Precision (30 mcg)

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.324	985.0011	12227	0.86	-

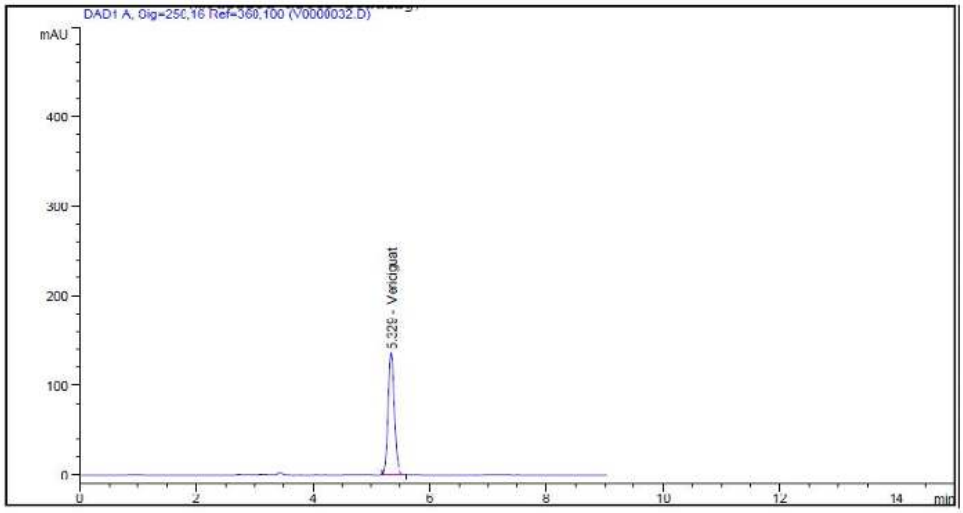


Fig. 27: Chromatogram Intra-day precision (30 mcg)-02

Table 34: Result for Chromatogram of Precision (30 mcg)-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.329	998.00055	12251	0.86	-

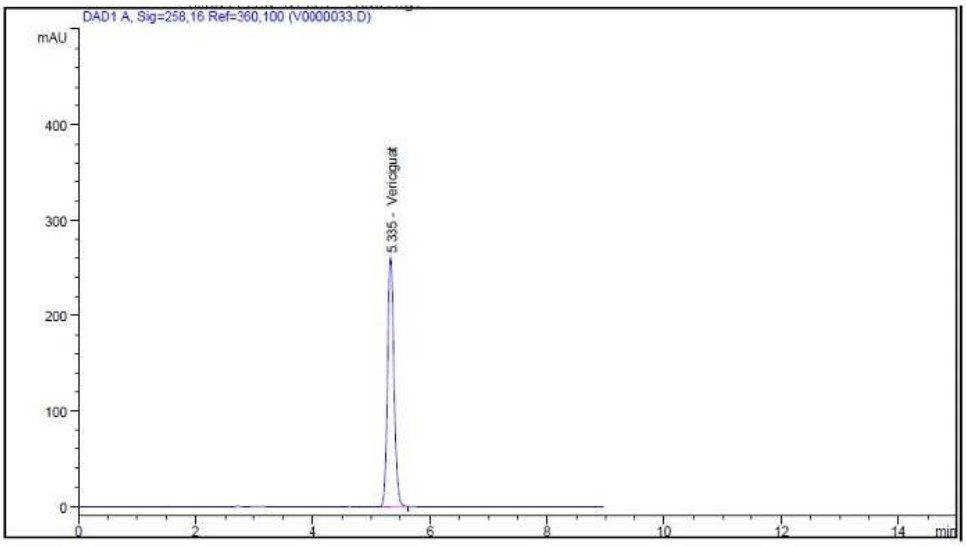


Fig. 28: Chromatogram Intra-day precision (50 mcg)

Table 35: Result for Chromatogram of Precision (50 mcg)

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.335	1606.45911	12276	0.86	-

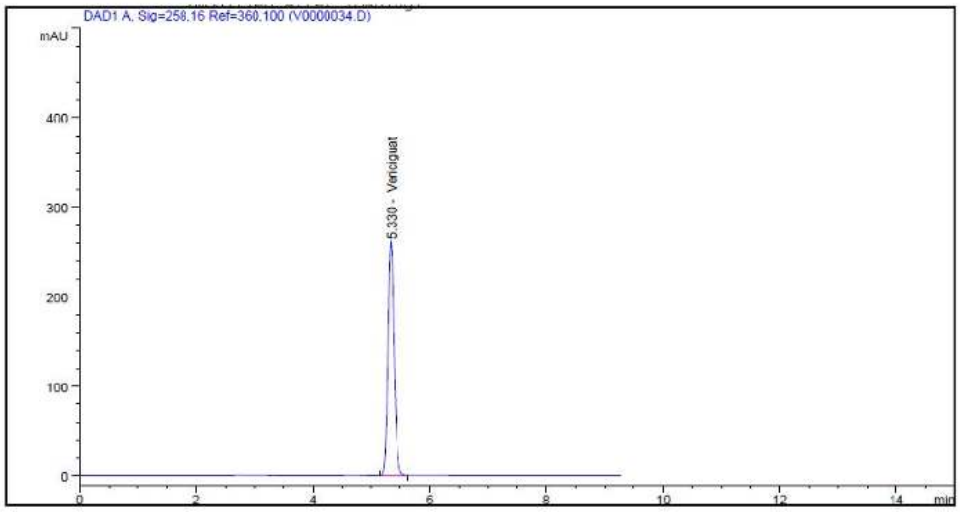


Fig. 29: Chromatogram Intra-day precision (50 mcg)-02

Table 36: Result for Chromatogram of Precision (50 mcg)-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.330	1602.14404	12253	0.86	-

Inter-day Precision:

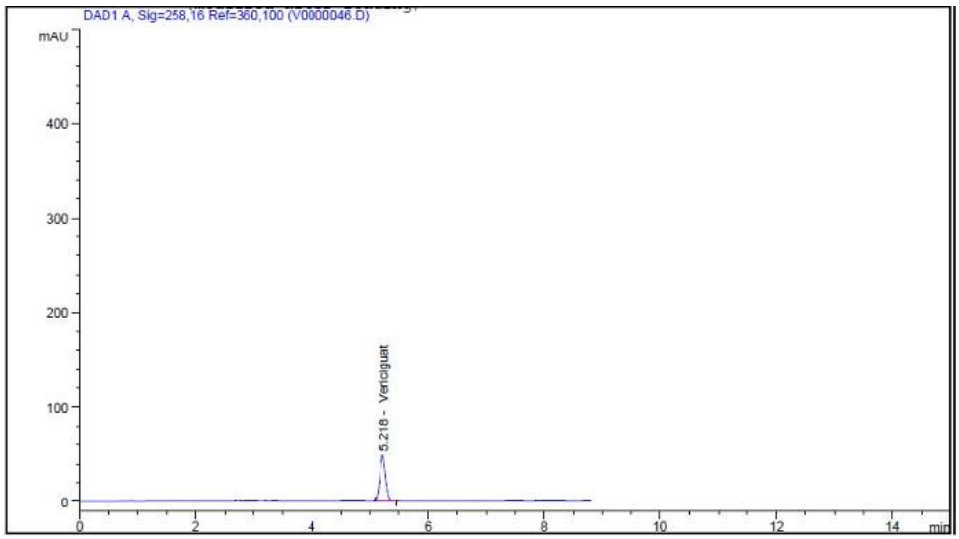


Fig. 30: Chromatogram Inter-day precision (10mcg)

Table 37: Result for Chromatogram of Precision (10mcg)

No.	RT[min]	Area[mV*s]	TP	TF	Resolution

1	5.218	354.42664	12617	0.85	-

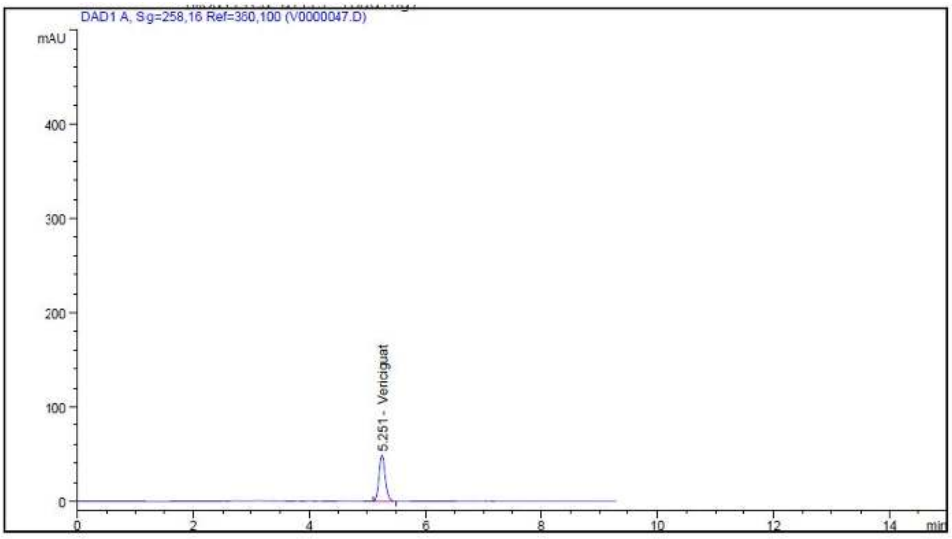


Fig. 31: Chromatogram Inter-day precision (10mcg)-02

Table 38: Result for Chromatogram of Precision (10mcg)-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.251	354.18427	12177	0.85	-

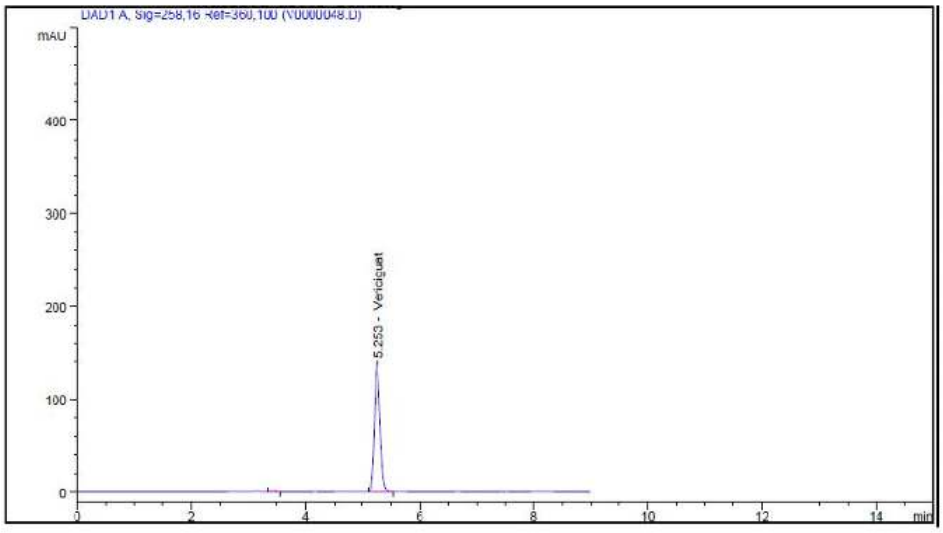


Fig. 32: Chromatogram Inter-day precision (30mcg)-01

Table 39: Result for Chromatogram of Precision (30mcg)-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.253	990.411	12787	0.86	-

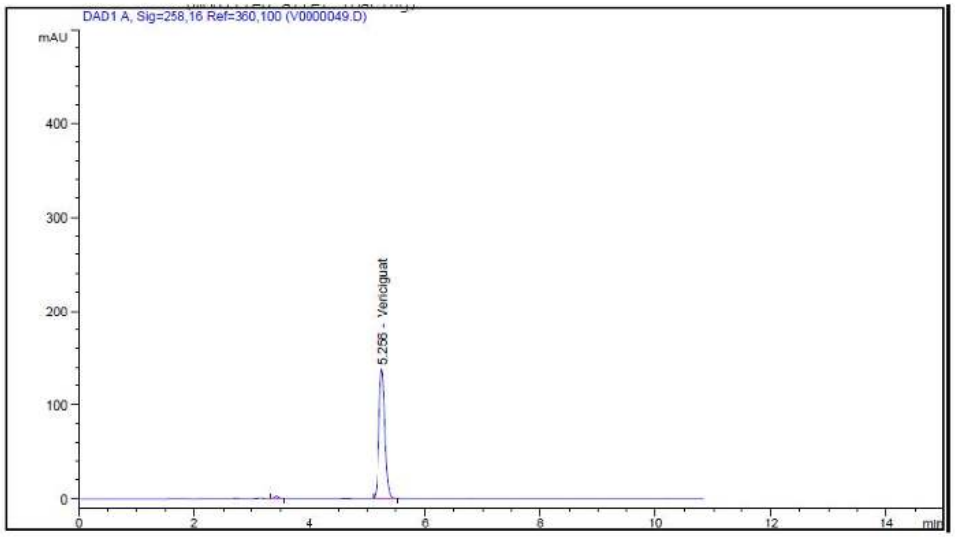


Fig. 33: Chromatogram Inter-day precision (30mcg)-02

Table 40: Result for Chromatogram of Precision (30mcg)-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.256	1001.332	12201	0.85	-

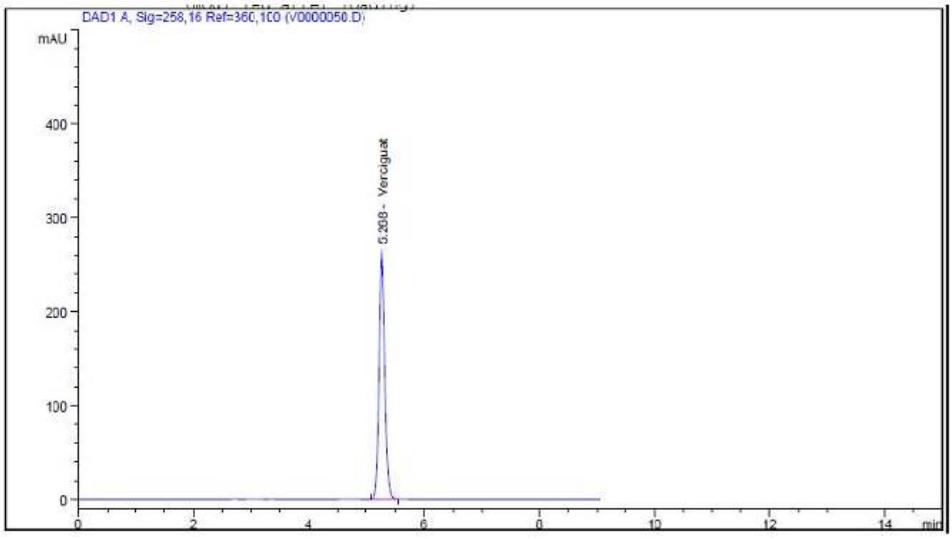


Fig. 34: Chromatogram Inter-day precision (50mcg)

Table 41: Result for Chromatogram of Precision (50mcg)

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.268	1640.70581	12256	0.86	-

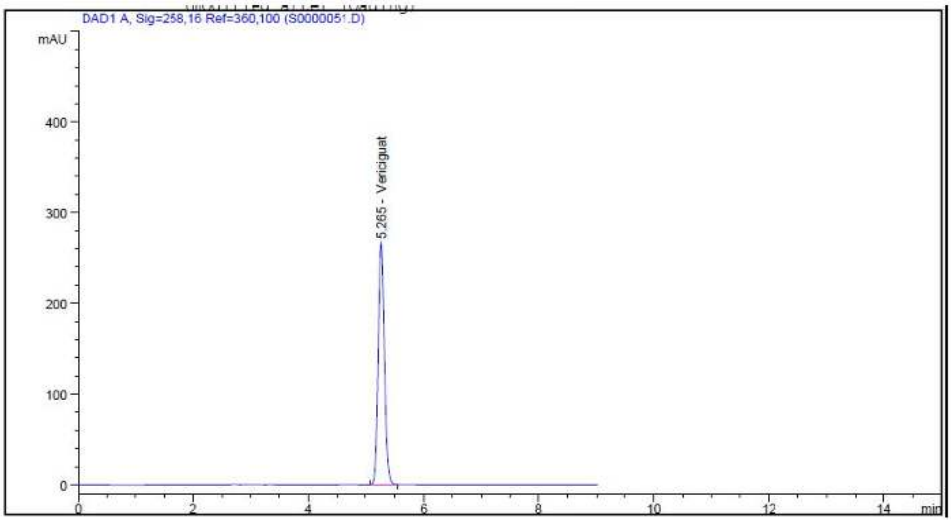


Fig. 35: Chromatogram Inter-day precision (50mcg)-02

Table 42: Result for Chromatogram of Precision (50mcg)-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.265	1620.30737	12245	0.85	-

Table 43: Result of Intraday and Inter day Precision for Vericiguat HPLC

Conc. (µg/mL)	Intraday Mean±SD	% Amt	%RSD	Interday Mean±SD	% Amt	%RSD
10	354.01±1.46	98.15	0.41	354.31±0.17	98.25	0.05
30	991.50±9.19	100.54	0.93	995.87±7.72	101.01	0.78
50	1604.30±0.19	99.44	0.19	1630.51±14.42	101.12	0.88

5.6 Robustness

Small deliberate variations in flow rate, mobile phase composition and wavelength were made to evaluate robustness.

1) Flow Rate Change 0.7 mL:

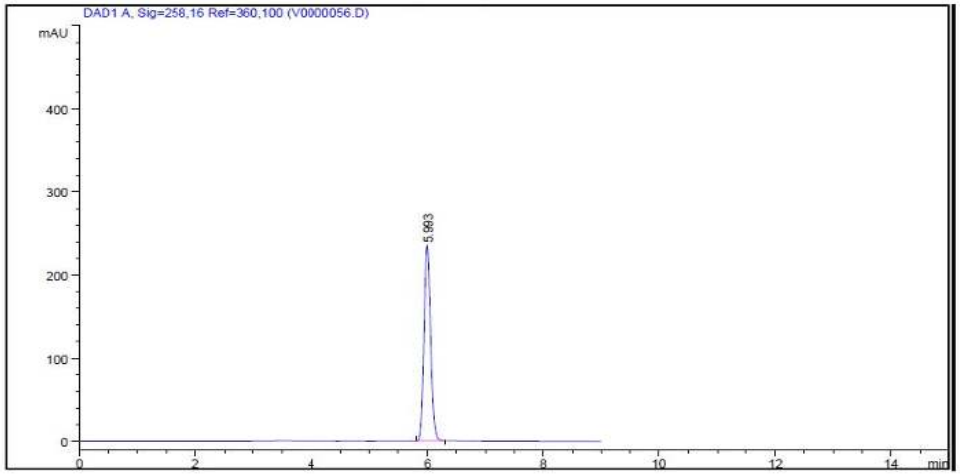


Fig. 36: Chromatogram of Flow rate change 0.7ml-01

Table 44: Result for Chromatogram of Flow rate change 0.7 ml-01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.993	1840.79297	14129	0.85	-

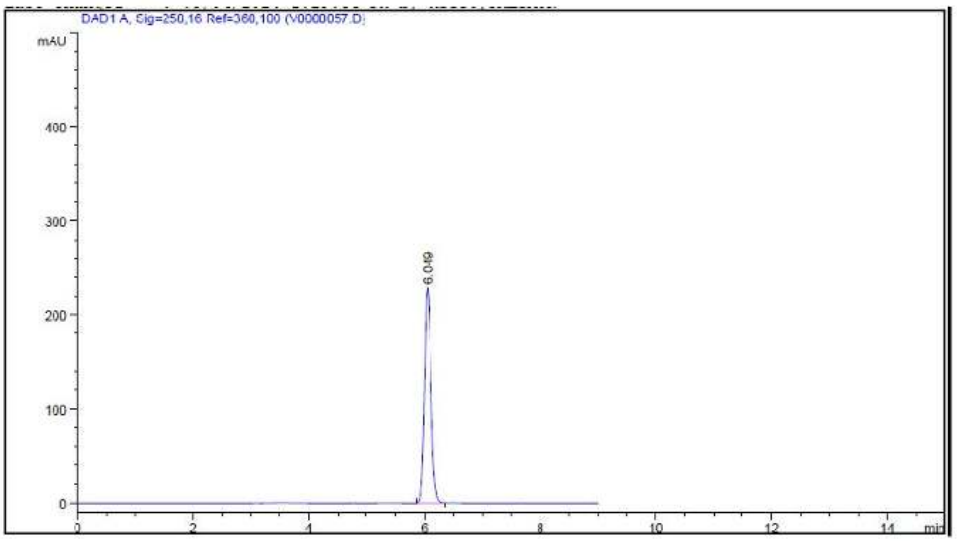


Fig. 37: Chromatogram of Flow rate change 0.7 ml-02

Table 45: Result for Chromatogram of Flow rate change 0.7 ml-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	6.049	1843.96606	13471	0.86	-

2) Flow Rate Change 0.9 mL:

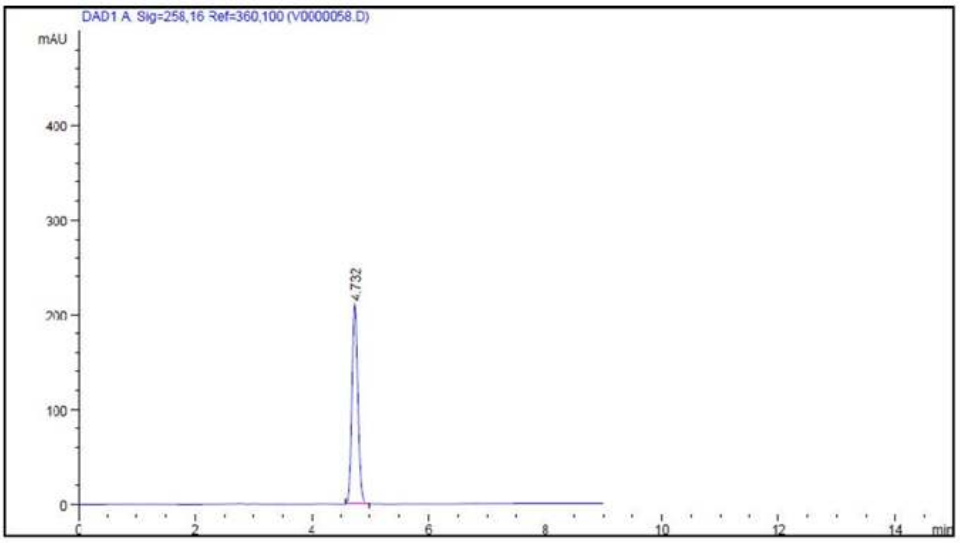


Fig. 38: Chromatogram of Flow rate change 0.9 ml

Table 46: Result for Chromatogram of Flow rate change 0.9 ml

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.732	1432.00659	11769	0.87	-

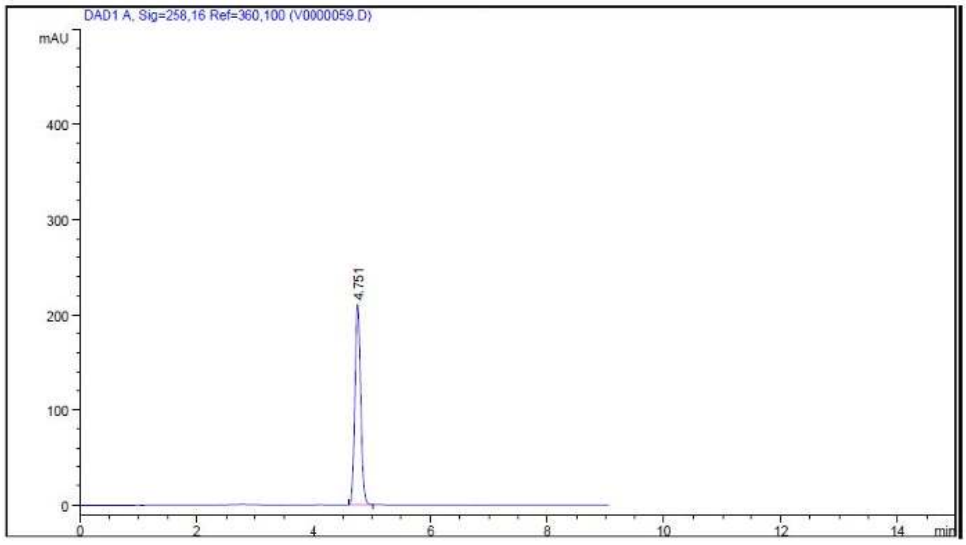


Fig. 39: Chromatogram of Flow rate change 0.9 ml-02

Table 47: Result for Chromatogram of Flow rate change 0.9 ml-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.751	1432.00659	11769	0.87	-

3) Mobile phase composition: 49 mL MeOH + 0.1% acetic acid 51 mL Water:

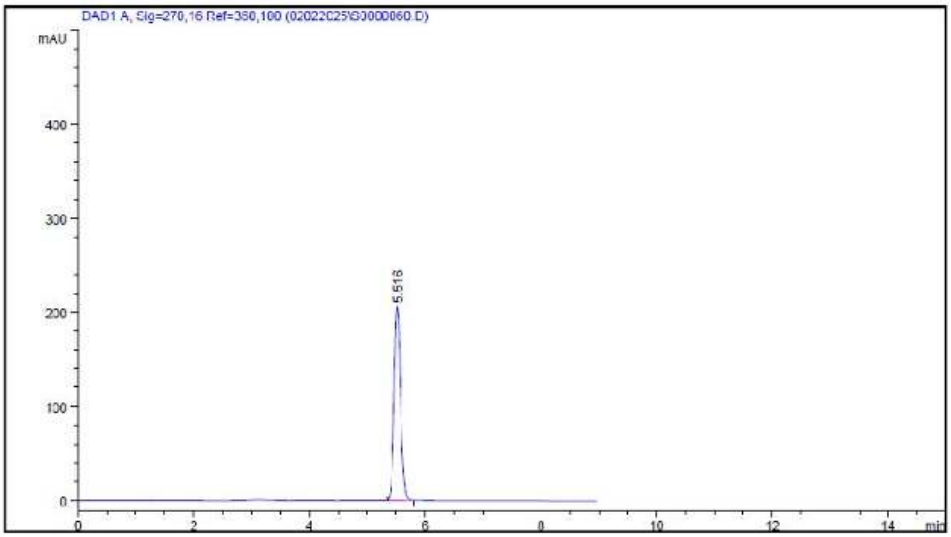


Fig. 40: Chromatogram of Mobile phase composition change (49:51)

Table 48: Result for Chromatogram of Mobile phase composition change

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.516	1609.83984	11707	0.86	-

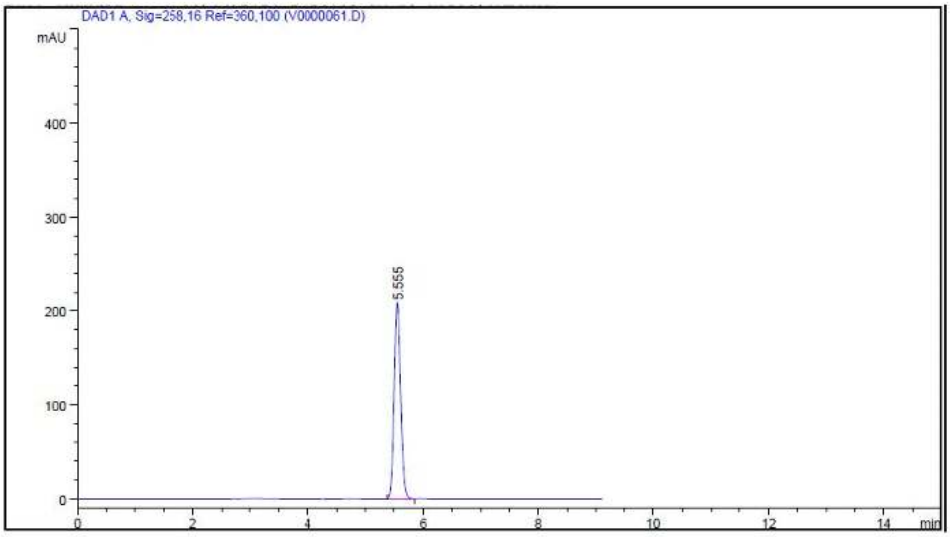


Fig. 41: Chromatogram of Mobile phase composition change (49:51)-02

Table 49: Result for Chromatogram of Mobile phase composition change-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.555	1614.18884	12139	0.87	-

4) Mobile phase composition: 51 mL MeOH + 0.1% acetic acid 49 mL Water:

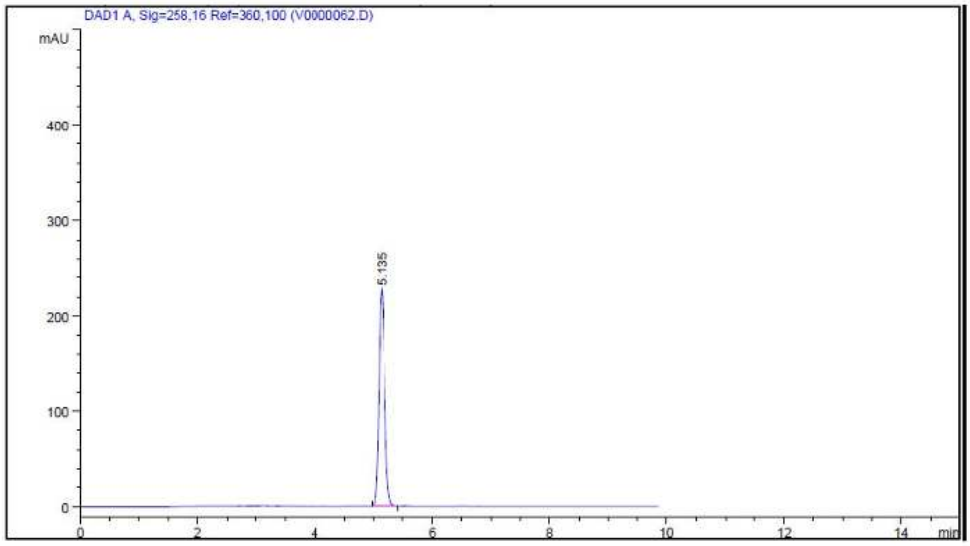


Fig. 42: Chromatogram of Mobile phase composition change (51:49)

Table 50: Result for Chromatogram of Mobile phase composition change

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.135	1613.71716	12526	0.87	-

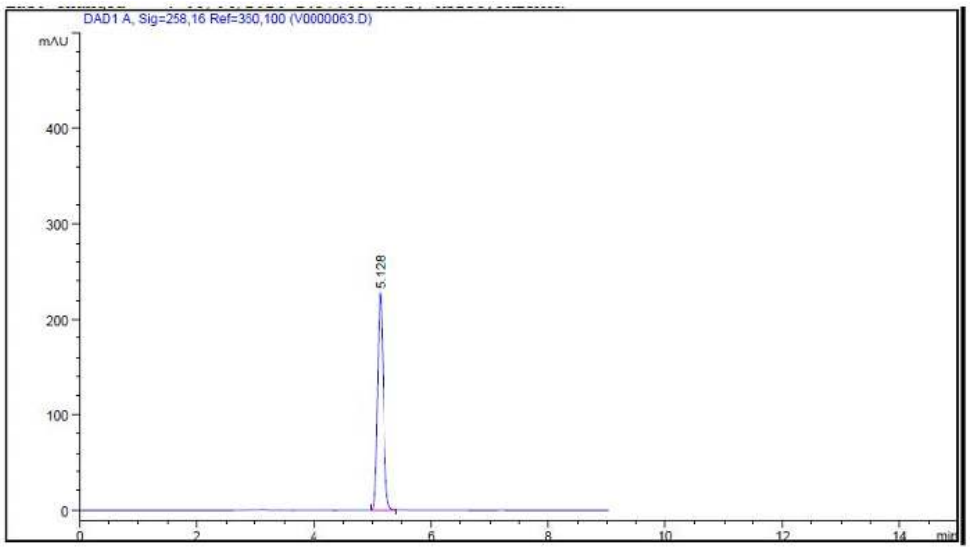


Fig. 43: Chromatogram of Mobile phase composition change (51:49)-02

Table 51: Result for Chromatogram of Mobile phase composition change-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.128	1617.48083	12448	0.86	-

5) Wavelength Change 257 nm:

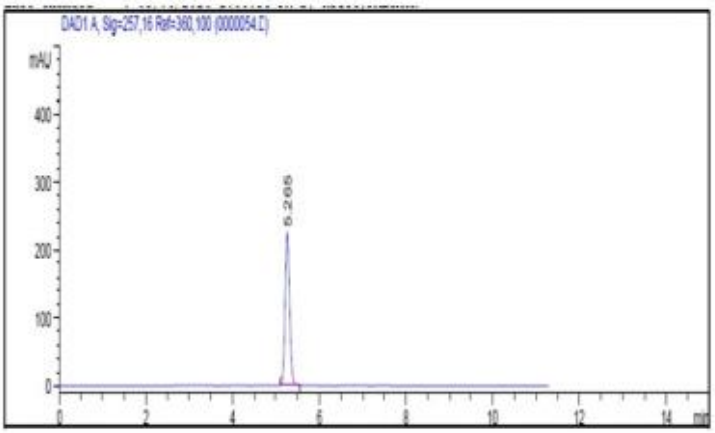


Fig. 44: Chromatogram of wavelength change 257nm

Table 52: Result for Chromatogram of wavelength change 257 nm

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.265	1632.62476	12540	0.86	-

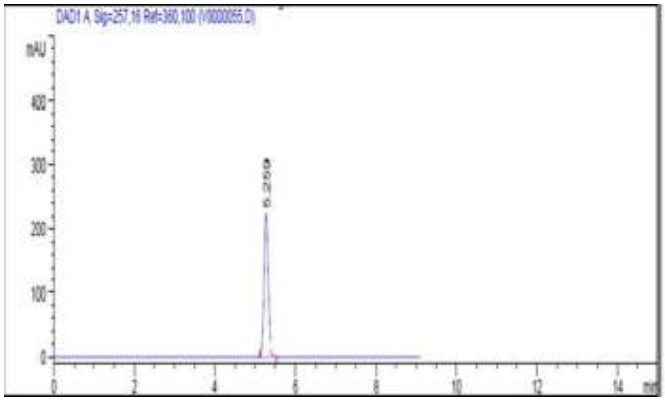


Fig. 45: Chromatogram of wavelength change 257nm-02

Table 53: Result for Chromatogram of wavelength change 257 nm-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.259	1615.85889	12512	0.86	-

6) Wavelength Change 259 nm:

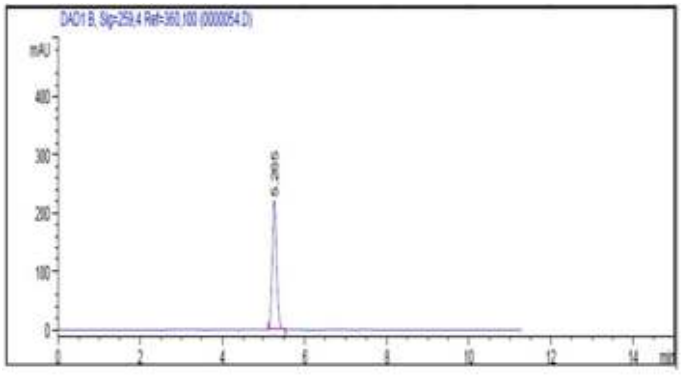


Fig. 46: Chromatogram of wavelength change 259nm

Table 54: Result for Chromatogram of wavelength change 259 nm

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.265	1596.38977	12540	0.86	-

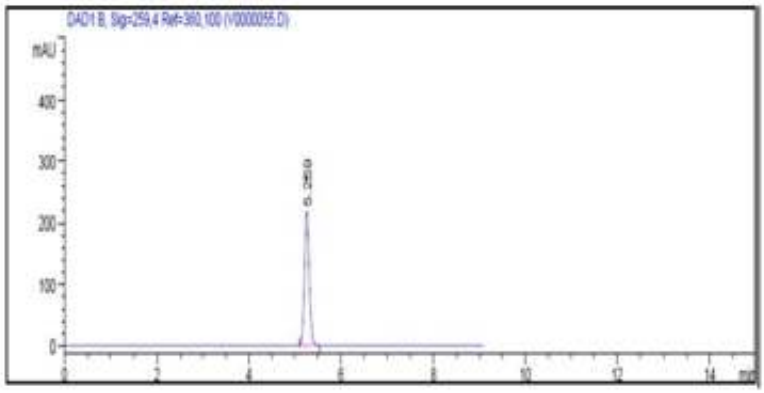


Fig. 47: Chromatogram of wavelength change 259nm-02

Table 55: Result for Chromatogram of wavelength change 259 nm-02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.259	1581.1506	12512	0.86	-

Table 56: Result of Robustness Study of Vericiguat

Parameter	Conc.	Amount detected (Mean±SD)	%RSD
Mobile phase (51:49)	50	1612.0±3.08	0.19
Mobile phase (49:51)	50	1615.6±2.66	0.16
Wavelength 257 nm	50	1623.7±11.15	0.69
Wavelength 259 nm	50	1588.77±10.78	0.68
Flow rate 0.7 mL	50	1842.38±2.24	0.12
Flow rate 0.9 mL	50	1431.78±0.32	0.02

5.7 Limit of Detection and Limit of Quantitation

LOD = 3.3 × SD / Slope = 3.3 × 1.25 / 31.33 = 0.1320 µg/mL

LOQ = 10 × SD / Slope = 10 × 1.25 / 31.33 = 0.3949 µg/mL

5.8 Specificity and Selectivity

No interfering peaks were observed at or near the retention time of Vericiguat, confirming specificity [36-41].

6. CONCLUSION

A reverse-phase HPLC method for the estimation of Vericiguat, a cardiovascular drug, was developed and validated. Separation used an Agilent C18 column with methanol and 0.1% acetic acid water (50:50 v/v, pH 3.2) at 0.8 mL/min and 258 nm, giving a retention time of 5.268 min. Validation per ICH guidelines confirmed linearity, accuracy, precision, robustness and specificity within acceptable limits.

The method offers shorter retention time, isocratic elution, an economical mobile phase, and good peak resolution compared to several previously reported methods. The proposed method is simple, accurate, precise, economical and reproducible, and can be adopted for the routine quality-control analysis of Vericiguat.

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CONFLICT OF INTEREST

The authors declare no known competing financial interests or personal relationships that could have influenced this work.

AUTHOR CONTRIBUTIONS

All authors contributed to study conception and design. Method development and data acquisition were performed by the first author. Validation and statistical analysis were carried out jointly. The corresponding author supervised and critically revised the manuscript. All authors approved the final version.

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