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Analytical method Development and validation of Ivabradine by using RP-HPLC

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ABSTRACT

The RP-HPLC method, which makes use of an XTERR C8 column (150mm × 4.6mm, 3.5µm) and a mobile phase of acetonitrile:0.01N KH2PO4(60:40) of HPLC grade, became created and accepted to be used in Ivabradine analysis. Buffer's pH became stored at 3. After being subjected to a 10-minute sonication, the use of a 0.45µm Nylon clear out, A 0.9mL/min waft charge became used to clear out the cellular section. After 260 nm of detection, it became observed that Ivabradine had a 1.846-minute retention period. Linearity became found with equation y=14200x+106895, from 80–120 µg/mL (R2 became the coefficient of determination). ICH hints had been accompanied with inside the validation of the approach.

INTRODUCTION

A new drug referred to as Ivabradine HCL is used to deal with strong angina pectoris symptomatically. Ivabradine works otherwise from betablockers in that it lowers coronary heart rate. It impacts the incredibly expressed If ion present day withinside the sinoatrial node. [1,2] It is an inward present day this is combined Na+ and K+ this is regulated through the autonomic frightened gadget and generated through hyperpolarization. Ivabradine in evaluation to beta-blockading medications, does now no longer seem to have any vasodilatory impact on inotropic characteristics, in step with initial animal research. [3-6]. Initially, techniques of liquid chromatography (LC) have been confirmed to quantify chemical substances in urine and plasma, respectively. These techniques used mass spectrometric detection and fluorometric detection, respectively. [7,8] There is presently no posted technique for figuring out and quantifying Ivabradine in pharmaceutical dosage bureaucracy and bulk drugs. [9,10] Consequently, it's miles essential to have an affordable, precise, dependable, and selective technique for figuring out the quantity of Ivabradine in pharmaceutical and bulk medicinal drug dosage bureaucracy. In this work, we document a totally straightforward, fast, accurate, and incredibly touchy HPLC and spectrophotometric method for figuring out Ivabradine that does not require pattern treatment. Additionally, we've got emphasised the HPLC assay technique 's balance indicator.

During the literature review, some analytical and bioanalytical strategies for Ivabradine dedication had been observed. Ivabradine and its low focused metabolites in urine and plasma had been usually observed using excessive - overall performance liquid chromatography (HPLC) and ultra- excessive overall performance liquid chromatography (UHPLC) methods using quite a few detection strategies in courses which includes organic samples. [11,12] Furthermore, only a few investigations targeting growing analytical strategies for Ivabradine identification, in addition to for figuring out its impurities and/or breakdown merchandise. In particular, most effective posted papers [13, 14]

deal with the optimization of the stability-indicating excessive - overall performance thin-layer chromatographic method (HPTLC) and the reversed- segment excessive - overall performance liquid chromatographic method (RP-HPLC), in addition to the spectrophotometric method, for the separation of Ivabradine and capacity degradation merchandise shaped below acidic and primary conditions. [15,16]

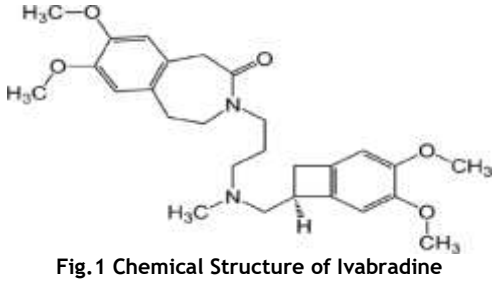


Fig.1 Chemical Structure of Ivabradine

Mechanism of Action

Ivabradine lowers coronary heart failure via way of means of appropriately and selectively inhibiting the cardiac pacemaker contemporary (If), a blended sodium-potassium inward contemporary that controls the Sinoatrial (SA) node's spontaneous diastolic depolarization, that is how the coronary heart rhythm is regulated. [17, 18] The molecular channel belongs to the HCN family. Ion waft is impeded while this channel is blocked, delaying diastolic depolarization, slowing down the SA node's firing, and in the long run reducing coronary heart fee.[19] Ivabradine handiest influences the SA node's coronary heart; it has no impact on blood pressure, intracardiac conduction, myocardial contractility, or ventricular repolarization. [20, 21] Furthermore, Ivabradine inhibits retinal contemporary (Ih), which stocks homes with cardiac If. [22, 23] It improves the temporal decision of the visible device via way of means of lessening retinal reactions to sturdy mild stimuli. [24, 25] When Ih is in part blocked, sufferers may also enjoy phosphenes, or luminous phenomena, because of surprising adjustments in brightness or different cause conditions. [26.27]

MATERIALS AND METHODS:

Materials

Ivabradine was purchased from Lairus Pvt. Ltd. in Ambad MIDC in Nashik, Maharashtra, India. The remaining chemicals were all purchased from Sidhi Laboratories and were of HPLC grade.

Chemical:

Reagents	Grade
Potassium di hydrogen Orthophosphate	AR
Orthophosphoric Acid	HPLC
Acetonitrile	HPLC
Water	HPLC
Triethlyamine	HPLC

Equipment

The digital balance (Skytech), the sonicator (Leelasonic,4 L), and the virtual pH meter (Equip-Tronics) have been the gadgets applied withinside the investigation. Empower three software programs become used to reveal and integrate the HPLC (Alliance Waters 2695 Separation Module) and detector (Water 2487 Dual Absorption Detector). UV (double beam Shimadzu 1800), Membrane filter (0.455 µm nylon).

Methods of Ivabradine:

Selection of chromatographic method: This is contingent upon the drug's molecular weight, solubility, and kind. Chromatography in opposite segment has been employed.

Selection of mobile Phase: A variety of cellular stages had been examined in various proportions to make this decision. Ivabradine became injected the use of numerous cellular stages at various ratios and glide costs till a point height with a spectrum-containing height became achieved freed from interference. The diverse cellular stages overed acetonitrile, water, methanol, KH2PO4, ammonium acetate, or any mixture of or 3 of the foregoing solvents. Despite attempting diverse ratios, no high-quality consequences had been discovered. However, the cellular segment containing 0.1% KH2PO4 and acetonitrile in a 60:40 ratio produced a suitable height with a 1.87-minute retention length.

Trial. 1: A cellular segment containing methanol and 0.1% ammonium acetate buffer (75:25) at specific ratios became attempted as a way to acquire the height. Temperature: room temperature, wave length: 281; run time: 10 minutes; injection volume: 10µL; buffer pH: 6.8; glide fee: 1.2 millilitres in step with minute. However, Ivabradine Peak became determined to show off tailing.

Trial. 2: Methanol, acetonitrile, and 0.1% ammonium acetate buffer made up the cellular segment of this experiment (75:23:2). Injection volume: 10µL, run time: 20min, temperature: ambient, wavelength: 225, glide fee: 1 ml/min. However, Ivabradine Peak became determined to show off tailing.

Trial. 3: A cellular segment containing methanol and 0.1% ammonium acetate buffer (50:50) at specific ratios became attempted as a way to reap the height. Temperature: room temperature, wave length: 281; run time: 10 minutes; injection volume: 10µL; buffer pH: 6.8; glide fee: 1.2 millilitres in step with minute. However, Ivabradine Peak became determined to show off tailing.

Trial. 4:

To attain the height, numerous ratios of acetonitrile to KH2PO4 (55:45) had been tried within side the cellular segment. Temperature outside, wavelength of 260, length of run (five minutes), injection volume (10µL), buffer pH adjusted at 3, and glide fee of one ml/min. Still, it became discovered that Ivabradine Peak confirmed tailing.

Impact of Mobile Phase ratio

The ratio of the cell segment changed into adjusted to maximise the height as soon as the cell segment changed into validated. the 50:50, 70:30 ratio. Then, 60:40v/v presentations a terrific decision and retention time.

Effect of flow Rate

The cell segment float price changed into changed as soon as the cell segment ratio changed into verified. With a top fronted at 1.2 ml/min, it has an extended retention time of 0.8 ml/min. The 0.9ml/min float price produced a pleasant outcome.

Selection of column

The literature studies validated a way to decide Ivabradine the use of the C8 column. The C8 column, XTERR C8 (150mm×4.6mm, 3.5µm) changed into selected, and its famous excellent performance.

Selection of detector wavelength

The proper preference of wavelength determines the sensitivity of the UV detector-primarily based totally HPLC technique. The wavelength that offers the fine reaction and maximum absorbance for drug detection is taken into consideration optimum.

The maximum broadly used kind of detector is the UV one considering it could pick out a huge kind of substances, has a few diplomas of selectivity for precise analytes, and is useful for several HPLC applications. The analytes chemical shape should have the perfect chromophore, which includes an fragrant ring, for detection, and the solvent used should be UV-grade or non-UV-absorbing.

Ivabradine's UV spectrum changed into captured. The cautioned examine will awareness at the λmax at 260 nm that changed into selected from this spectrum.

Method Development

Stationary phase	C8 column, XTERRA@ RP8 3.5 µm, C8 (150mm×4.6mm,3.5µm)
Mobile phase	Acetonitrile:0.01NKH2PO4 (60:40)
Ratio	60:40
Flow rate	0.9ml/min
Operating temperature	Ambient
Detection wavelength	260

Run Time	5min
Detector	UV Detector
Mode	Isocratic
Injection Volume	10µl
Diluent	Acetonitrile:0.01N%KH_2PO_4

Table. 1 Chromatographic conditions

SOLUTION PREPARATIONS:

Preparation of System suitability test (Ivabradine standard solution):

In order to dissolve the 25 mg of Ivabradine reference or running well known, exactly weigh it after which fill a 25 ml volumetric flask with 15 ml of diluent sonicate. Next, use diluent to regulate the volume. Fill a ten-millilitre volumetric flask with one millilitre of the same old inventory answer the use of a pipette. then dilute with diluent to the important volume. To filter, use a 0.45µ membrane filter. Chromatograms have been recorded.

The Pharmacopeia calls for device appropriateness, which verifies that the chromatographic device is suitable for the deliberate analysis. Data for the checks have been received the use of 5 reflect injections of a well-known pharmaceutical answer, and the effects have been recorded.

Acceptance Criteria:

The well-known 's 5 reflect injections' RSD should not be greater than 2.0%.

PREPARATION OF BUFFER SOLUTION:

Buffer: 0.01N orthophosphate of potassium dihydrogen (3.0pH)

A 1000ml volumetric flask turned into packed with exactly weighed 1.36 grams of potassium dihydrogen orthophosphate and approximately 900 ml of HPLC water. The water turned into then applied to make up the quantity through degassing the HPLC water and sonicating it. After that, one millilitre of triethylamine turned into introduced, and a diluted orthophosphoric acid answer turned into used to convey the pH right all the way down to 3.0.

PREPARATION OF MOBILE PHASE:

Create a 60:40 aggregate of acetonitrile and buffer. Remove fuel line after passing through a 0.45µ membrane clear out

DILUENT PREPARATION:

The diluent of preference is mobile phase.

STANDARD STOCK SOLUTION:

To dissolve the 25 mg of Ivabradine reference or running standard, weigh it precisely and upload 15 ml of diluent sonicate to a 25 ml volumetric flask. Next, regulate the quantity with diluent.

SAMPLE STOCK SOLUTION PREPARATION:

To dissolve approximately 25 mg of the Ivabradine reference/ running standard, weigh it exactl and switch it right into a 25 ml volumetric flask. Next, upload 15 ml of diluent sonicate. Then, use diluent to get the quantity as much as par.

STANDARD SOLUTION:

One millilitre of the usual inventory answer must be pipetted into a 10milliliter volumetric flask. Then, diluent must be introduced to acquire the specified quantity of diluting. Use a 0.45µ membrane clear out for filtering.

PREPARATION OF SAMPLE SOLUTIONS:

Pipette one millilitre of the pattern inventory answer right into a volumetric flask with ten millilitres, after which dilute to the important power the usage of diluent. To clear out out, use a 0.45µ membrane clear out.

Sample	Sample (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Standard	25	25	1	10
Sample	25	25	1	10

Table. 2 Summary of standard and sample preparation

PREPARATION OF CALIBRATION CURVE

Standard inventory answers had been aliquoted and diluted with cellular segment to the correct degree in a ten mL volumetric flask. In this manner, the drug's very last concentrations ranged from eighty to a hundred and twenty µg/mL. Singlet injections containing 10 µL had been created and subjected to chromatograph evaluation withinside the formerly referred to circumstances. The effectiveness of the drugs changed into evaluated and top areas had been measured. Plotting the height vicinity at the y-axis as opposed to the correct medicine awareness at the x-axis ended in calibration curves. The coefficient of determination (R2) changed into used to evaluate the calibration curve.

PROCEDURE:

Record the chromatograms after injecting 10µl quantities of the sample, standard, and blank solutions into the chromatograph. For each of the main peaks, note the peak responses.

Sr. No	Description	Number of Injection
1	Blank	1
2	Standard	1
3	Sample	1

Table. 3 Injection Sequence

Method Validation-

The evolved approach became confirmed through comparing the subsequent factors: detection limit, quantification limit, robustness, specificity, accuracy, linearity, precision, stability, and robustness. With the exception of the quantification limit in which those values had been set at 2% in keeping with the literature's recommendations, coefficients of version and relative mistakes of much less than 2% had been seemed as acceptable.

System Suitability

The percent RSD for the height location and tailing thing for ivabradine had been decided after 5 mirror injections of the same old answers had been made.

Specificity

A standard, clean answer is brought into the HPLC apparatus. At the Ivabradine height retention time, there has been no interference from the clean. Peak purity shows that there had been no co-eluting peaks all through the Ivabradine height 's retention period, indicating that the height became homogenous.

Accuracy

The diploma of settlement among the price found and the price diagnosed as both a traditional proper price or a suitable reference price shows whether or not the analytical technique became correct. The assessment variety for accuracy is 80% to 120% of running concentration. For each answer the share RSD for the general restoration in addition to the share RSD for

every accuracy degree had been calculated 3 times. Range parameters and linearity are the resets of accuracy.

Linearity and Range

The capability of an analytical technique to provide check findings which might be precisely proportionate to the concentration (amount) of analyte withinside the sample, inside a targeted variety is referred to as linearity. The variety of the analytical method is the variety among the sample's best and lowest analyte concentration (amount), inclusive of concentrations, in which the analytical technique has been proven to have the precise diploma of linearity, accuracy, and precision. There had been 5 wonderful linearity ranges used, starting from 80% to 120% of running concentration

Sr. No.	Level (%)	mL of stock solution	Diluted to with Mobile phase (mL)	Ivabradine Conc. (ppm)
1	80	0.8	10	80
2	90	0.9	10	90
3	100	1.0	10	100
4	110	1.1	10	110
5	120	1.2	10	120

Table. 4 Linearity

Precision

The degree of settlement among numerous measurements taken from a couple of samplings of the identical homogenous check carried out beneathneath the required situations is the definition of precision in analytical methods. Repeatability and intermediate precision are the 2 classes of precision. The technique is completed on a check specimen. To attain reproducibility, chromatographic situations had been adjusted and one injection of every of the subsequent changed into made: one of the pattern solutions (six duplicates), one in all the same old solution (repetitions), and the blank. Furthermore, for every of the six mirror samples the proportion of the relative trend deviation (RSD) of the assay that should not move above 1% changed into determined. In order to assure consistency, a couple of analysers had been required to uphold best chromatographic situations and offer one injection of the blank, one replication of the same old solution, and pattern solution (six repeats). The six mirror samples' relative trendy deviation for the assay changed into additionally computed. in which the proportion RSD should not exceed 1%.

Repeatability

Sr. No.	mL of stock solution	Diluted to with Mobile phase (mL)	Ivabradine Concentration (ppm)
1	1.0	10	100
2	1.0	10	100
3	1.0	10	100
4	1.0	10	100
5	1.0	10	100
6	1.0	10	100

Table. 5 Repeatability

Robustness

Robustness is a statistic that describes how nicely an analytical process play beneathneath standard running situations and the way resistant it's miles to tiny, intentional adjustment in technique parameters. adjustments in wavelength (± 1 nm), pH, cell segment ratio, and float rate (±10%, or 0.1 ml/min). The recuperation percent and rsd for Ivabradine had been noted.

SR.NO.	PARAMETERS	NORMAL CONDITION	HIGHER SIDE	LOWER SIDE
1	Flow rate	0.9ml/min	1ml/min	0.8ml/min
2	Wavelength	260nm	261nm	259nm

Table. 6 Robustness

The Limit of Quantitation (LOQ) and the Limit of Detection (LOD)

Detection limit: The detection restriction of the analytical procedure is the bottom awareness of analyte in a pattern that may be detected however isn't always always measurable as a precise quantity.

Quantitation Limit:

The lowest awareness of analyte in a pattern that may be quantitatively recognized with suitable precision and accuracy is referred to as the quantitation restriction of a selected analytical procedure.

The procedure changed into accomplished according with ICH Q2R1 guidelines to calculate LOD and LOQ. The following method changed into used to the calibration curve, which changed into used to calculate the residual general deviation of a regression line, that allows you to achieve the LOD and LOQ:

LOD equals 3.3 σ / S.

LOQ is identical to 10σ / S.

Were, σ = a regression line's residual general deviation S is the regression line's slope.

Solution Stability

In order to confirm the stableness of the analytical answer, in addition to the preliminary examination, the same old and filtered pattern answer became additionally evaluated at diverse durations as proven below. After that, it became saved withinside the HPLC system' pattern field at room temperature. calculated the Ivabradine top areas' cumulative % RSD for each the pattern and the reference answer.

Sr. No	Time	Chromatogram ID	No. of Injections
01	0 hour	Standard Solution Test Solution	01 01

02	1st hour	Standard Solution Test Solution	01 01
03	2nd hour	Standard Solution Test Solution	01 01
04	3rd hour	Standard Solution Test Solution	01 01
05	6th hour	Standard Solution Test Solution	01 01
06	12th hour	Standard Solution Test Solution	01 01
07	24th hour	Standard Solution Test Solution	01 01

Table. 7 Solution Stability

RESULT:

Method development

Chromatographic separation

A variety of HPLC chromatographic systems were examined in order to maximize Ivabradine separation. Table No. 1 displays the retention duration for the Ivabradine function of the mobile phase, stationary phase (XTERRA C8 reversed-phase column), and other optimized chromatographic settings.

Calibration Curve

The coefficient of determination (R2), slope, and intercept values for Ivabradine were 0.999, 14200, and 106895, in that order. Ivabradine had a retention period of 1.846 minutes. Fig. 2 displays the Ivabradine HPLC overlay chromatogram at 260 nm together with the calibration curve.

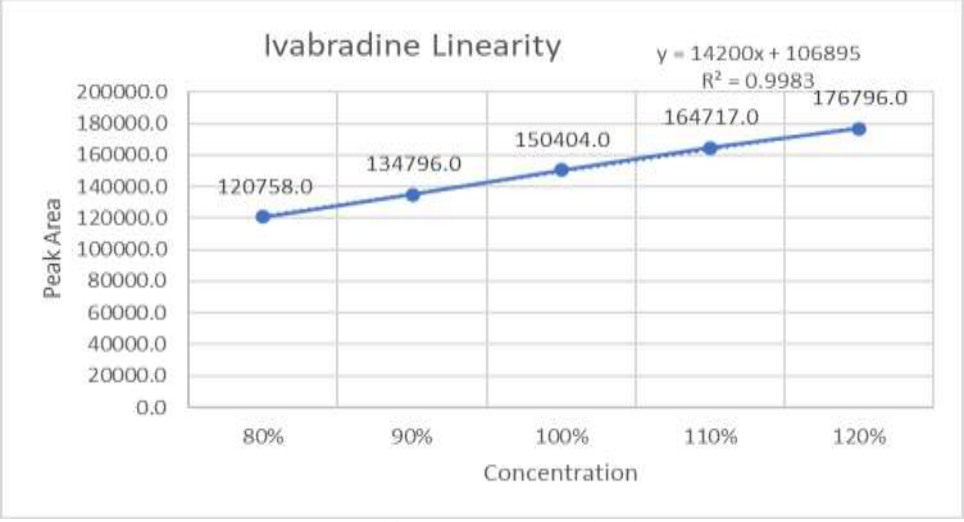


Fig. 2 calibration Curve of Ivabradine

Method Validation

System Suitability

The analyte height area's relative widespread deviation in widespread chromatograms must now no longer exceed 2.0%. The final results are displayed in Table No.8.

Specificity

The potential to actually discover the analyte within side the presence of probably predicted additives is referred to as specificity. A blank, widespread answer is made and injected to verify height purity. The consequences are displayed in Table No. 9.

Accuracy

Within the variety of 80% to 120% of operating concentration, accuracy might be measured. The percent RSD for every accuracy degree and the share RSD for the whole restoration have been computed in triplicate for every answer. The restoration installed and tested the method's accuracy. Accuracy is referred from Linearity and variety parameters. The consequences are displayed in Table No. 10.

Linearity

The coefficient of determination (R2) for Ivabradine turned into 0.99 as proven in Fig. 2.

Precision

The precision records concerning the repeatability and reproducibility of the RP-HPLC method for Ivabradine are supplied in Tables 12 and 13.

Robustness

Intentional manipulation of the analytical parameters, which includes the wavelength and glide rate (0. five ±0.1 mL/min), turned into used to check the robustness of the approach. The effects may be proven in Tables 14 and 17

LOD and LOQ

The following formulation is used to compute the LOD and LOQ. three. three σ / S is the LOD, even as 10 σ / S is the LOQ. It turned into installed that Ivabradine's LOD and LOQ have been three .083 µg/mL and 9.343 µg/mL, respectively.

Stability

After comparing the usual and filtered pattern answer, the analytical answer 's balance turned into showed at diverse intervals, as indicated below, even as the pattern turned into stored at room temperature within side the HPLC equipment's pattern compartment. computed the pattern and widespread cumulative % RSD for the Ivabradine height locations. The effects are proven in Table No. 18.

System Suitability

Sample	Area	% Assay	Mean Assay
Sample 1	153997	99.73	99.63
Sample 2	154393	99.54	

Table. 8 System Suitability

Specificity

Sr. No	Component	Retention time	Area	%Area	Height
1	Standard	1.846	154048	100.00	29169
2	Sample	1.839	154506	100.00	29274

Table. 9 Results of Specificity

Accuracy

Sr. No.	Concentration (%)	Peak Area	Amt Found	% Recovery	Mean recovery	%RSD
1	80	120620	79.909	99.89	100	0.1151
2	80	120756	79.999	100.00		
3	80	120898	80.093	100.12		
4	100	150402	99.999	100.00	100	0.0559
5	100	150489	100.057	100.06		
6	100	150321	99.945	99.94		
7	120	176792	119.997	100.00	100	0.0026
8	120	176795	119.999	100.00		
9	120	176801	120.003	100.00		

Table. 10 Accuracy

Sr. No.	Concentration (%)	Peak Area
1	80	120758.0
2	90	134796.0
3	100	150404.0
4	110	164717.0
5	120	176796.0
STD Deviation		**62.3703**
Correlation Coefficient		**0.999**
Slope (m)		**14200**
R^2		**0.998**
Intercept		**106895**
% RSD		**0.04670**

linearity of Ivabradine

Table. 11 Linearity

Repeatability

Sr. No.	NAME	AREA	%ASSAY
1	Test sample1	153997	**99.73**
2	Test sample2	154393	**99.54**
3	Test sample3	154092	**99.99**
4	Test sample4	154418	**99.59**
5	Test sample5	156678	**98.36**
6	Test sample6	155466	**100.58**
Average			**99.63257671**
Std. Relative deviation			**0.630442854**
%RSD			**0.63276779**

Table. 12 Repeatability

Reproducibility

Sample No.	Test	Area	% ASSAY
1	Precision (Analyst1)	153997	99.8713
2		154393	100.0566
3		154092	99.6054
4		154418	100.0111
5		156678	101.2606
6		155466	99.0280
7	Precision (Analyst2)	154822	99.3866

8		154012	99.2779
9		155380	100.6865
10		155254	99.7191
11		157211	101.0580
12		154678	98.1920
Average			**99.8461**
Std. Relative deviation			**0.8666**
% RSD			**0.868**

Table. 13 Reproducibility

Result of Robustness study of Ivabradine

Flow Rate 0.8ml

SR. NO.	NAME	RT	AREA
1	STD	2.091	176572
2	TEST 1	2.091	176091
3	TEST 2	2.09	176300
Average			**176321**
Std. Relative Deviation			**241.19**
% RSD			**0.14**

Table. 14 Flow Rate 0.8ml

Flow Rate 1ml

SR. NO.	NAME	RT	AREA
1	STD	1.694	143337
2	TEST 1	1.688	144397
3	TEST 2	1.69	142338
Average			**143357**
Std. Relative Deviation			**1029.65**
% RSD			**0.72**

Table. 15 Flow Rate 1ml

Wavelength 259nm

SR. NO.	NAME	RT	AREA
1	STD	1.852	141080
2	TEST 1	1.847	139563
3	TEST 2	1.849	142527
Average			**141057**
Std. Relative Deviation			**1482.138**
% RSD			**0.011**

Table. 16 Wavelength 259nm

Wavelength 261nm

SR. NO.	NAME	RT	AREA
1	STD	1.847	171732
2	TEST 1	1.847	172106
3	TEST 2	1.846	172565
Average			**172134**
Std. Relative Deviation			**417.222**
% RSD			**0.002**

Table. 17 Wavelength 261nm

The limits of detection (LOD) and quantitation (LOQ):

The slope is equal to 14200, and the residual standard deviation of a regression line is

σ = 13267.75.

Limit of detection (LOD): LOD = 3.3 σ / S

3.3 x 13267.75 / 14200 is the LOD.

3.083 µg/mL is the LOD.

Limit of quantification (LOQ):

LOQ is equal to 10σ / S.

10 x 13267.75 / 14200 is the LOQ.

9.343 µg/mL is the LOQ.

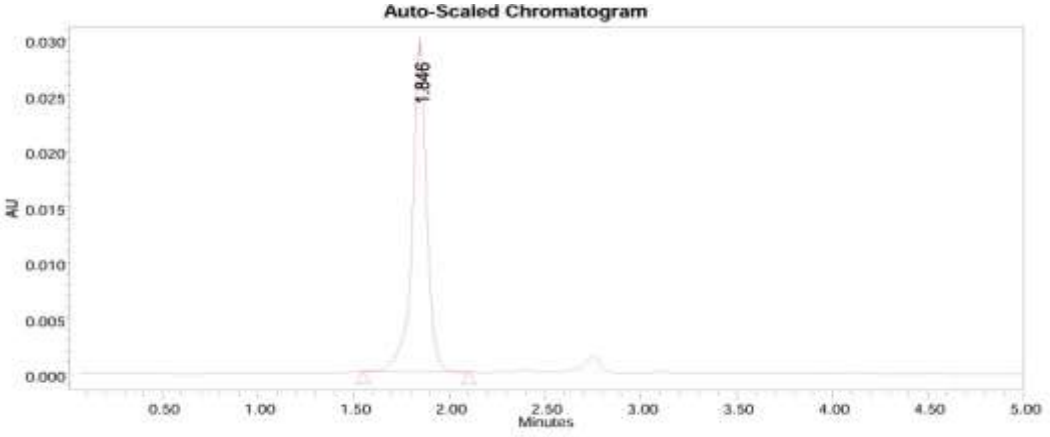
Solution Stability

Sr. No.	Time	Sample	RT	Area	Cumulative Std Relative Deviation	Cumulative % RSD
1	0 hr	STD	1.841	154929		
		SPL	1.844	155593		

2	1 hr	STD	1.841	153205	1219.05	0.80
		SPL	1.844	152654	2078.19	1.36
3	2 hr	STD	1.841	154221	718.42	0.47
		SPL	1.841	153751	775.70	0.50
4	3 hr	STD	1.846	153954	188.80	0.12
		SPL	1.846	153883	93.34	0.06
5	6hr	STD	1.841	153376	408.71	0.27
		SPL	1.841	153640	171.83	0.11
6	12 hr	STD	1.839	154309	659.73	0.43
		SPL	1.839	153113	372.65	0.24
7	24 hr	STD	1.84	153773	379.01	0.25
		SPL	1.84	154837	1219.05	0.79

Table. 18 Solution Stability

Specificity

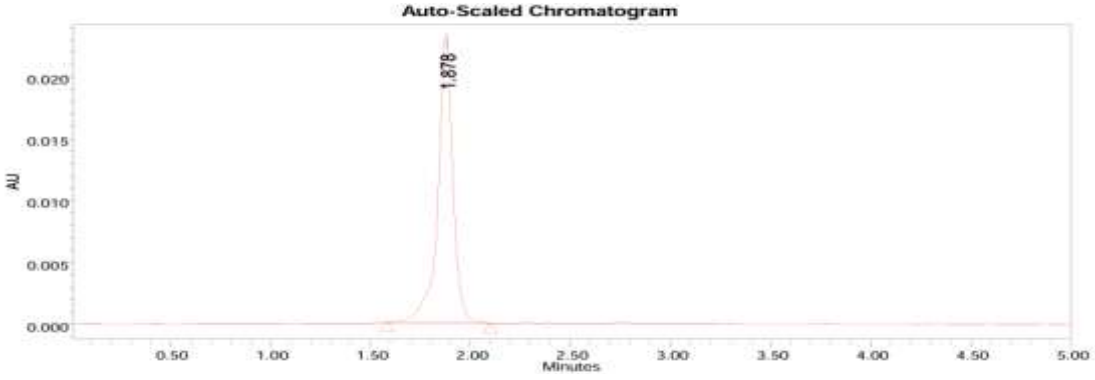


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.846	154048	100.00	29169	0.92	3430

Fig. 3 Typical Chromatogram of Specificity

Accuracy 80%

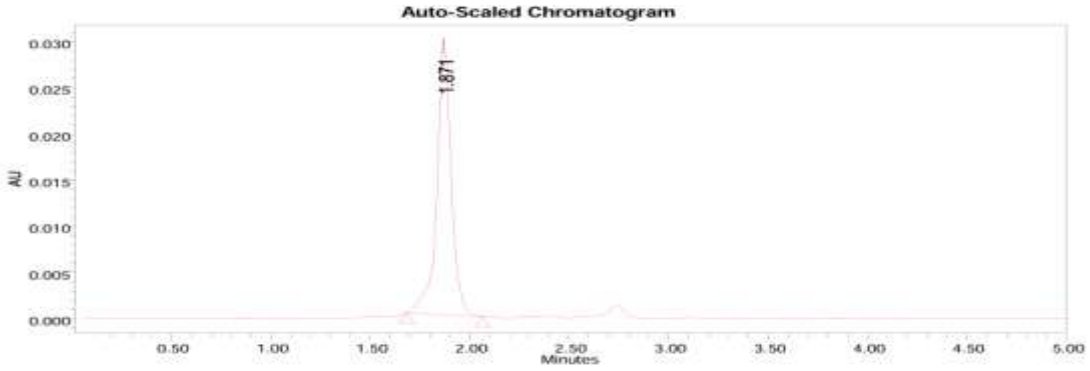


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.878	120620	100.00	22746	0.91	3627

Fig. 4 Typical Chromatogram of Accuracy 80%

Accuracy 100%

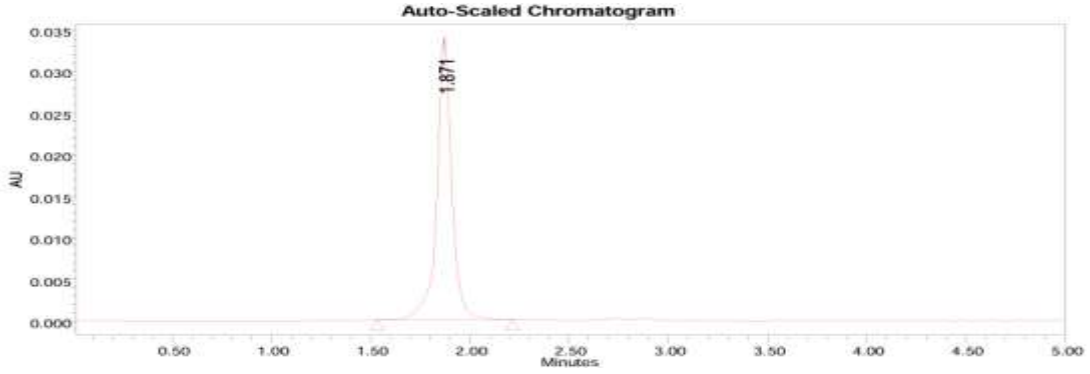


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.871	150404	100.00	29226	0.93	3610

Fig. 5 Typical Chromatogram of Accuracy 100%

Accuracy 120%

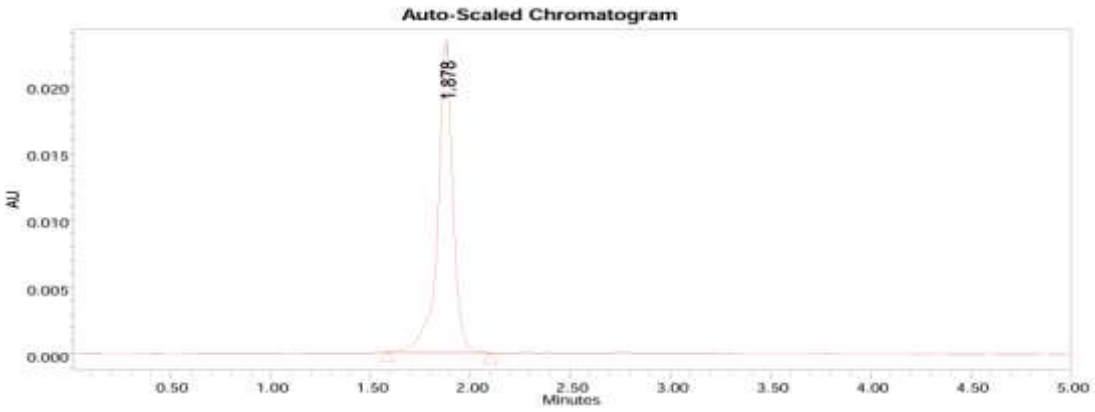


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.871	176796	100.00	33117	0.93	3533

Fig. 6 Typical Chromatogram of Accuracy 120%

Linearity 80%

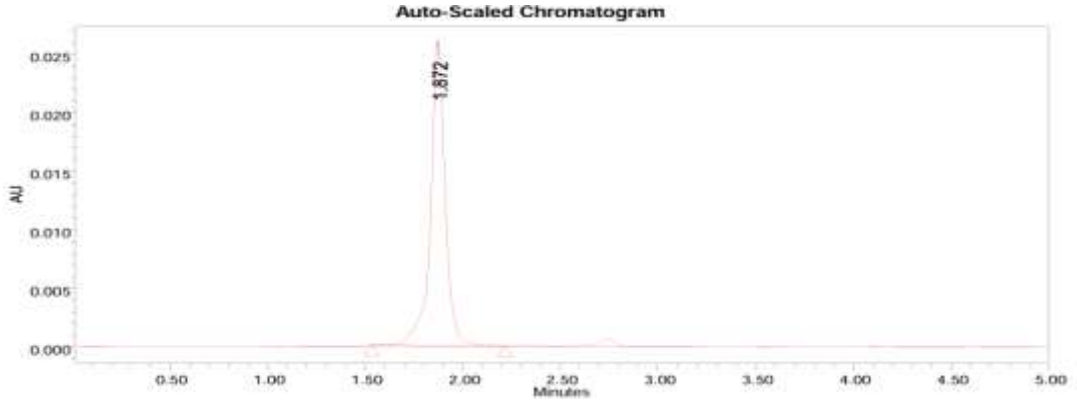


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.878	120620	100.00	22746	0.91	3627

Fig. 7 Typical Chromatogram of Linearity 80%

Linearity 90%

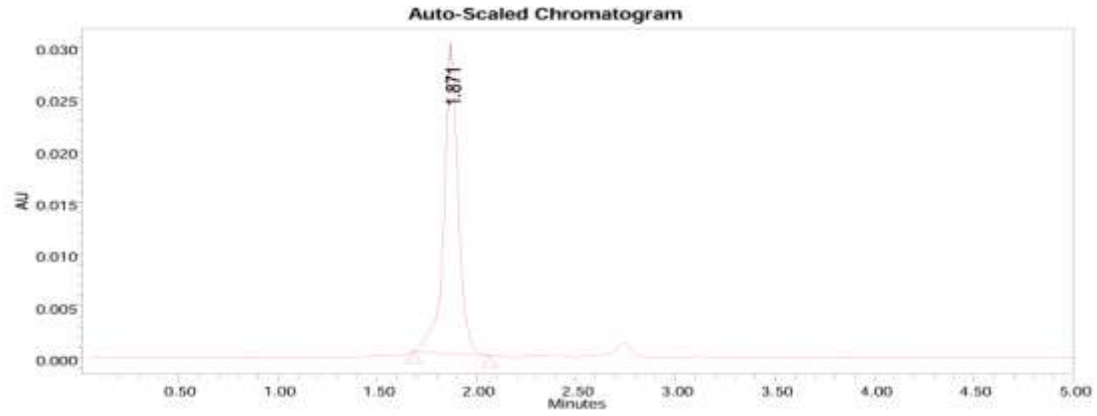


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.872	134796	100.00	25493	0.91	3602

Fig. 8 Typical Chromatogram of Linearity 90%

Linearity 100%

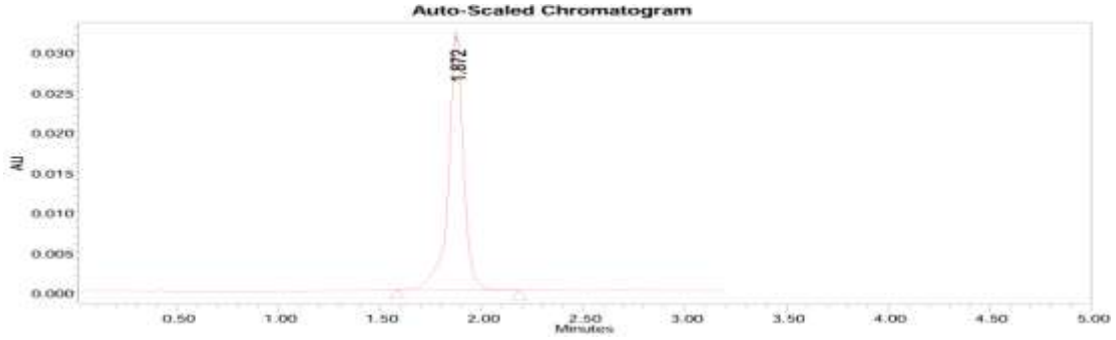


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.871	150404	100.00	29226	0.93	3610

Fig. 9 Typical Chromatogram of Linearity 100%

Linearity 110%

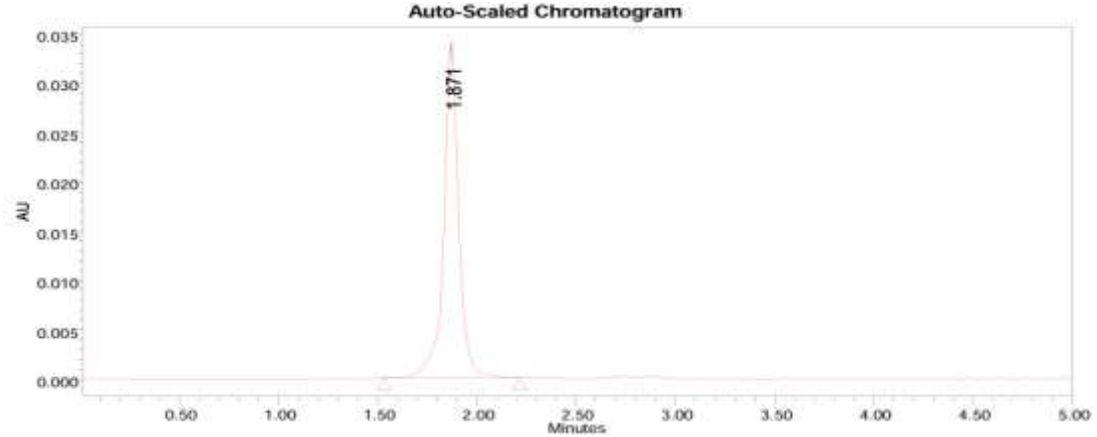


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.872	164696	100.00	31494	0.91	3692

Fig. 10 Typical Chromatogram of Linearity 110%

Linearity 120%

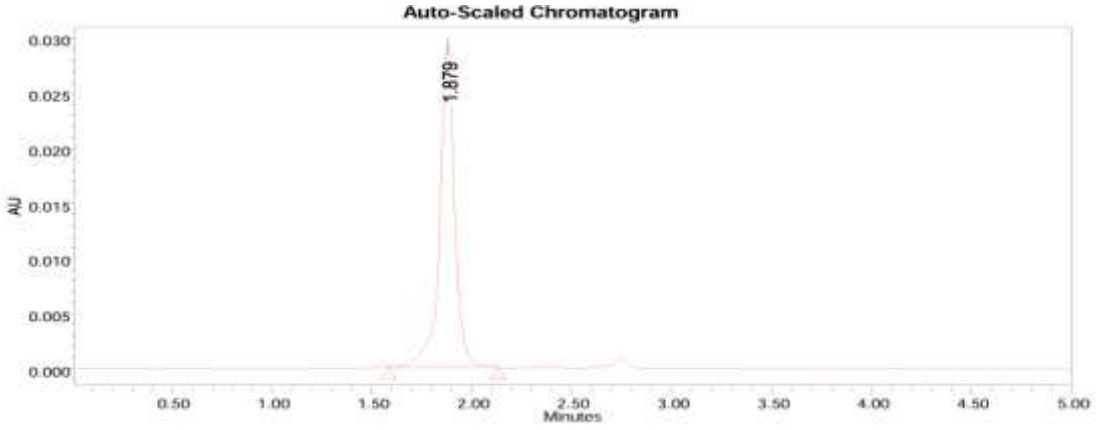


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.871	176796	100.00	33117	0.93	3533

Fig. 11 Typical Chromatogram of Linearity 120%

Precision
Repeatability

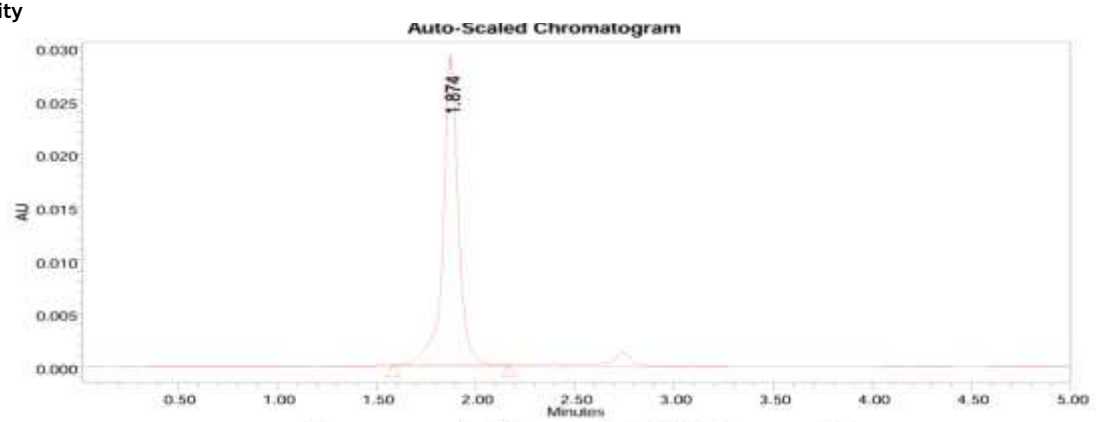


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.879	153997	100.00	29050	0.94	3671

Fig. 12 Typical Chromatogram of Repeatability

Reproducibility

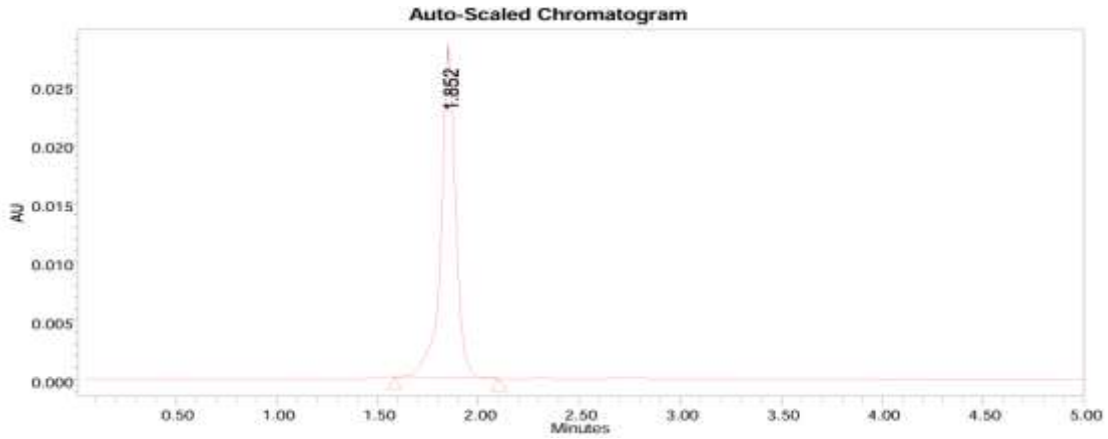


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.874	154822	100.00	28662	0.92	3559

Fig. 13 Typical Chromatogram of Reproducibility

Robustness
259nm Wavelength

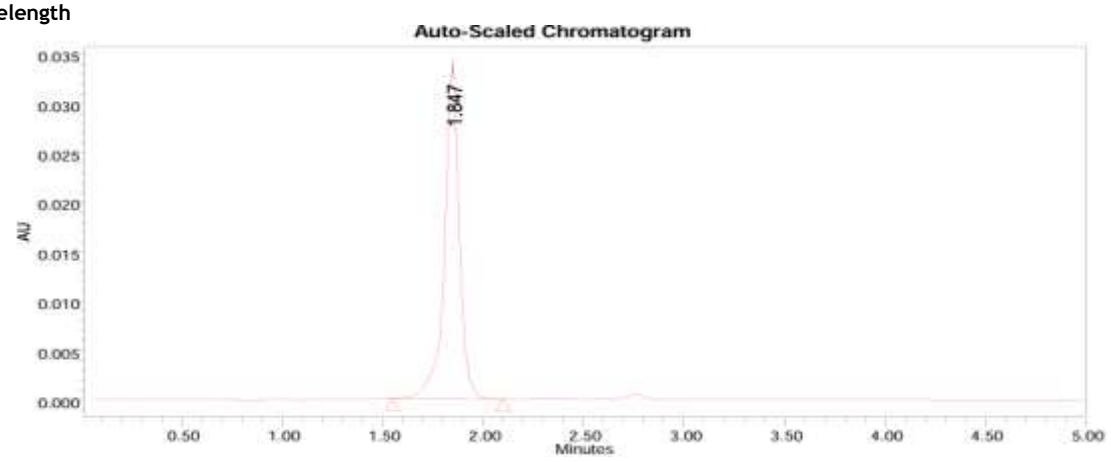


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.852	141080	100.00	27479	0.92	3834

Fig. 14 Typical Chromatogram of 259nm Wavelength

261nm Wavelength

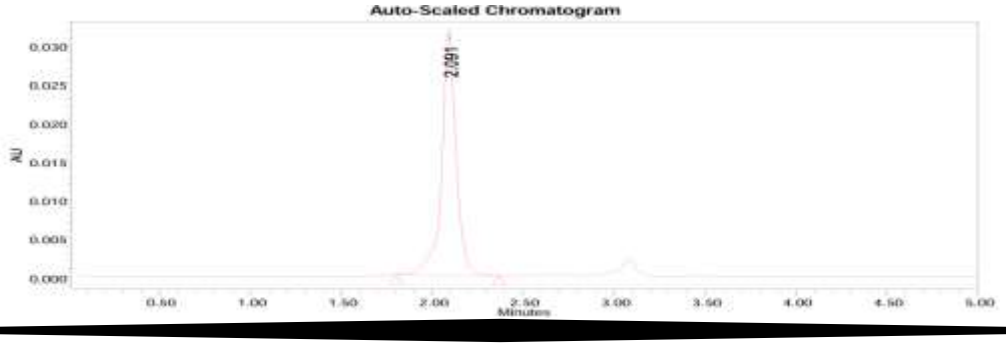


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.847	171732	100.00	33168	0.92	3646

Fig. 15 Typical Chromatogram of 261nm Wavelength

Flow Rate 0.8ml

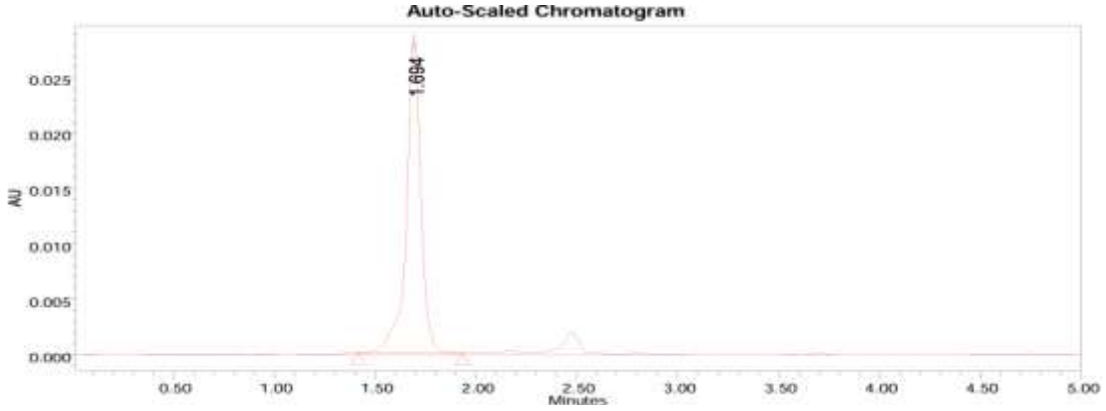


Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	2.091	176572	100.00	31291	0.92	4119

Fig. 16 Typical Chromatogram of Flow Rate 0.8ml

Flow Rate 1ml



Processed Channel: 2487Channel 1

	Processed Channel	Retention Time (min)	Area	% Area	Height	USP Tailing	USP Plate Count
1	2487Channel 1	1.694	143337	100.00	27976	0.92	3165

Fig. 17 Typical Chromatogram of Flow Rate 1ml

DISCUSSION

The linearity of this RP-HPLC approach became discovered to be among 80-120µg/mL, the approach became efficaciously proven below most efficient circumstances, and it became determined that the validation parameters fell in the restrictions. By using this chromatographic approach, it became determined that immiserate had a LOQ of 9.343 µg/mL and a LOD of 3.083 µg/ml. According to ICH criteria, Ivabradine underwent balance investigations below diverse settings within side the contemporary inquiry.

CONCLUSION

It became observed that the mounted RP-HPLC easy to use, dependable, sensitive, affordable, and particular all features that made it suitable for the observe of Ivabradine. As a result, the RP-HPLC approach may be utilized in number of laboratories to quantitatively decide the quantity of Ivabradine in pharmaceutical dosage forms.

CONFLICT OF INTEREST:

With relation to this investigation, the writers have no conflicts of interest.

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