IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2021 Vol.:21, Issue:2

RP-HPLC Method Development and Validation for Simultaneous Estimation of Carvedilol and Ivabradine Hydrochloride in Synthetic Mixture

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

PATEL KRUTIKA NARESHBHAI*[1], ALISHA PATEL[2]

Department of Pharmaceutical Quality Assurance at ROFEL Shri G. M. Bilakhia College of Pharmacy, Vapi, Gujarat, India-396191

Submitted: 25 April 2021
Accepted: 02 May 2021
Published: 30 May 2021

Keywords: Carvedilol, Ivabradine Hydrochloride, RP-HPLC, Simultaneous estimation, Synthetic mixture

ABSTRACT

A simple, rapid, precise and accurate high-performance liquid chromatography (HPLC) method was developed for simultaneous estimation of Carvedilol and Ivabradine Hydrochloride in a Synthetic mixture. The separation was obtained using a mobile phase consisting of Acetonitrile: Methanol: Water-0.1%TEA (75:23:2% v/v/v) adjusting the entire pH-3 with 1% OPA and using Shim-pack solar C18 (250 × 4.6 mm, 5 µm) column. A flow rate of 1.0 ml/min was used, with UV detection at 225 nm. The retention time for Carvedilol and Ivabradine Hydrochloride was 6.801 min and 1.787 min respectively. Linearity for Carvedilol and Ivabradine Hydrochloride was found to be in the range of 5-25 µg/ml and 2-10 µg/ml respectively. The research method was validated as per the ICH guidelines and the results were within the acceptance criteria for precision, linearity, specificity, and robustness.

INTRODUCTION:

Carvedilol (CAR) is a drug that treats high blood pressure and heart failure. Its chemical name is 1-(9H-carbozol-4-yloxy)-3-{[2-(2-methoxy phenoxy)ethyl]amino}propan-2-ol. Carvedilol beta-adrenergic receptor blocking (β1, β2) ability decreases the heart rate, myocardial contractility, and myocardial oxygen demand. Carvedilol also decreases systemic vascular resistance via its alpha-adrenergic receptor blocking (α1) properties. [3-4] CAR official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP) etc. and IVA not official in any Pharmacopoeia. [7-8] The High-Performance Liquid Chromatography (HPLC) method is described by the books IP and BP. Spectrophotometric, HPLC, stability-indicating RP-HPLC, and HPTLC methods for determining CAR with single and other drugs are also found in the literature. [7-18]

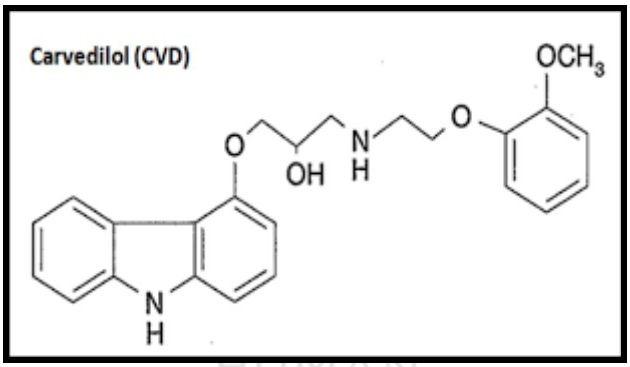

Figure No. 1: structural formula of Carvedilol

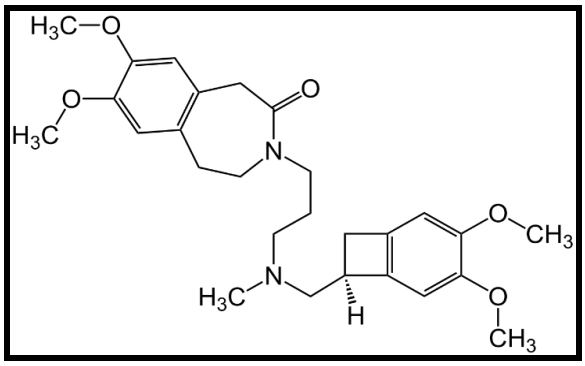

Figure No. 2: structural formula of Ivabradine Hydrochloride

Ivabradine Hydrochloride (IVA) is a 3-[3-[[(7S)-3,4-dimethoxy-7-bicyclo [4.2.0] octa-1.3.5-trienyl] methyl-methylamino]-7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-one. It's a drug and is used to treat angina pectoris (chest pain). Ivabradine reduces heart rate by blocking if channels ("funny channels") in the heart in a concentration-dependent manner while maintaining other cardiac ionic channels unaffected (including calcium and potassium).[5-6]

There is no official pharmacopeia for Ivabradine. A review of the literature reveals HPTLC and Spectro-photometric methods for determining IVA. Literature survey also reveals RP-HPLC, Spectrophotometric methods for determination of IVA with single and other drugs.[7-18]

On November 9, 2016, the EUROPEAN MEDICINES AGENCY approved a medication combination of Carvedilol and Ivabradine Hydrochloride (CARIVALAN formulation) for the treatment of heart failure and ischemic coronary artery diseases. Any pharmacopoeia does not official the combination of two drugs, CAR and IVA.

In patients treated with CAR, the most frequently used β-blocker in the trial population, CAR in combination with IVA improved cardiovascular outcomes and was safe in patients with systolic heart failure in cardiac rhythm with a resting heart rate ≥of 70 bpm.

There are few reported HPLC and other analytical methods for the determination of Carvedilol and Ivabradine Hydrochloride in single and combination with and with other formulations. The published method is having a retention time of CAR: 12.14 min. and IVA: 8.40 min. [12] But the present article uses a different mobile phase and the retention time obtained for both the drugs was less than the reported method. The drugs were thoroughly validated as per the specifications recommended in ICH Guidelines.

MATERIALS AND METHODS:

Chemicals, reagents, and samples:

Carvedilol and Ivabradine Hydrochloride drug substance were procured from Gift sample, Sun Pharmaceutical Industries Limited, Piparia, Silvassa and Gift sample, Torrent Pharma, Dahej, Bharuch respectively. Acetonitrile was procured from (HPLC grade, Rankem, Maharashtra), Methanol (HPLC grade, Rankem, Maharashtra), Water (HPLC grade, Rankem, Maharashtra), Orthophosphoric acid (HPLC grade Fisher Scientific), Potassium dihydrogen

orthophosphate (Thomas Baker), Dipotassium hydrogen orthophosphate (Thomas Baker), Triethylamine (HPLC grade) - Fisher Scientific.

Instrumentation:

The HPLC system used was gradient HPLC Shimadzu LC-2010 CHT, series equipped with a 10 µL sample loop, and UV detector. The output signal was monitored and integrated using software LC solution version 1.25. Shim-pack solar C18 (250 × 4.6 mm, 5µm) column was used for the separation.

Chromatographic Conditions:

LC solution software was used to produce chromatographic separations using an HPLC (High-Performance Liquid Chromatography) system with a SHIMADZU LC-2010 CHT with UV Detector. Acetonitrile: Methanol: Water-0.1%TEA (75:23:2% v/v/v) whole pH-3 with 1% OPA was used as a mobile phase. The study was performed on a stainless steel Shim-pack solar column 250 mm long, 4.6 mm internal diameter packed with porous silica particles of 5 mm diameter (Shim-pack solar C18, 250 mm,4.6mm, 5mm particle diameter column), which was maintained at 40°C. The mobile phase was running through the column at a flow rate of 1 ml/min and a pump was in gradient mode. The runtime was 20 min. The injection volume was 10 µl and the analyte was monitored at 225 nm. The retention time of Ivabradine Hydrochloride peak-1 is about 1.787 min and that of Carvedilol peak-2 is about 6.801 min.

Selection of Wavelength:

Aliquots of 2.5 ml from working solution of CAR (100 µg/ml) and 0.5 ml from working solution of IVA (100 µg/ml) were pipette out into two separate 10 ml of volumetric flask and volume was make up with methanol to get 25 µg/ml of CAR and 5 µg/ml of IVA. PDA Detector was used to scan each CAR and IVA solution between 200 and 400 nm. From the overlay spectra of the above solutions, a wavelength of 225 nm was selected.

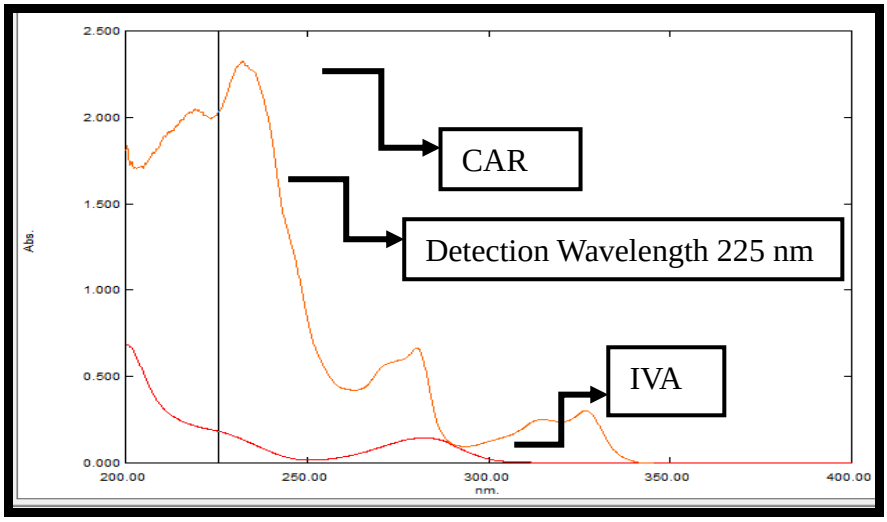

Figure no.3 UV spectrum overlay of CAR (25 µg/ml) and IVA (5 µg/ml) showing selection of wavelength detection.

Solution preparation:

1. Preparation of standard stock solutions:

A separate 10 ml volumetric flask was filled with an appropriately weighed quantity of CAR (10 mg) and IVA (10 mg), and a mobile phase (Acetonitrile: Methanol: Water) was added to both volumetric flasks. To generate a standard solution with a concentration of CAR (1000 g/ml) and IVA (1000 g/ml), the volume was adjusted up to the mark with mobile phase.

2. Preparation of Working stock solutions:

1 ml CAR (1000 g/ml) and IVA (1000 g/ml) solutions were transferred to a separate 10 ml volumetric flask and diluted with the mobile phase to a concentration of CAR (100 g/ml) and IVA (100 g/ml).

3. Preparation of TEA(Triethylamine) (0.1%):

It was made by diluting 0.1 mL concentrated TEA with 10 mL HPLC grade water.

4. Preparation of Binary mixture of CAR and IVA:

Aliquots of 2.5 ml from the CAR (100 g/ml) working solution and 0.5 ml from the IVA (100 g/ml) working solution were combined in a single volumetric flask and diluted up to 10 ml with mobile phase to make the final concentrations of CAR (25 g/ml) and IVA (5 g/ml).

5. Preparation of synthetic mixture solution:

Synthetic mixture [19] of 25 mg equivalent of CAR was taken into 10 ml of volumetric flask. It was dissolved in Methanol and sonicated for 2-3 mins and volume was made up to mark with the mobile phase. Whatman filter paper no. 42 was used to filter the water. As a result, the final solution contained 1000 g/ml CAR and 1000 g/ml IVA.From the above solution, 1.0 ml was pipette out and transferred to 10 ml volumetric flask and volume was make up with mobile phase to give a solution containing CAR (100 µg/ml) + IVA (100 µg/ml). 1.0 ml of the resultant solution was pipette out and transferred to a volumetric flask holding 10 ml of mobile phase, resulting in a solution containing CAR (25 g/ml) + IVA (5 g/ml). This solution was used for assay i.e. estimation of CAR and IVA in synthetic mixture.

METHOD VALIDATION:

1. System Suitability studies:

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution as well as asymmetric factor were evaluated.

2. Specificity:

There is no interference of mobile phase, solvents as well as placebo with the analyte peak and also the peak purity of analyte peak which specify that the method is specific for the analysis of analytes in their dosage form.

3.Accuracy study:

This experiment can be performed by the recovery test. The method's recovery was assessed using known amounts of standard placebo preparation at three different concentration levels. Three sets of duplicates were made for each concentration level and injected. The accuracy of the method was determined by recovery studies. The drug reference standards were added to the formulation (pre-analyzed sample) at levels of 0%, 80%, 100%, and 120%. The % recovery and % mean recovery for the drug were calculated three times, as shown in table 3.

4.Precision study:

The technique precision and system precision studies were combined to create a precision study. Method precision of the analytical method was determined by analyzing six sets of the

sample preparation. The mean % assay value, standard deviation, and % RSD for each of the six replicate sample preparations were determined. System precision of the analytical method was carried out to ensure that the analytical system was working properly. The standard solution was injected six times into the system and chromatograms were recorded. (table no. 5)

5. Limit of detection and Limit of quantitation study:

LOD is the smallest amount of drug content that the proposed method can detect, while LOQ is the smallest quantity that the method can quantify. For LOD, a minimum signal-to-noise ratio of (S/N) of more than 3.3 is recommended, and for LOQ, a minimum signal-to-noise ratio of (S/N) of more than 10. It can also be estimated using the supplied formula based on linearity data.

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

Where S is the slope of the regression line and σ is the residual standard deviation of the regression line.

6. Linearity:

Mixed standard test solution of Carvedilol and Ivabradine Hydrochloride were prepared with mobile phase in such a way that the final concentration of Carvedilol and Ivabradine Hydrochloride is in the range of 5-25 µg/ml and 2-10 µg/ml for CAR and IVA respectively. The peak area was recorded for all the peaks as shown in table-4 and figure no.6,7 for linearity of Carvedilol and Ivabradine Hydrochloride. The plots of peak area versus the respective concentration were found to be linear with regression coefficient (R^2 =0.9996) for Carvedilol and (R^2=0.999) for Ivabradine Hydrochloride as shown in figure 6,7, which proves the method is highly linear over the working range.

7.Robustness study:

The method's robustness was tested to ensure that the analysis was reliable when the method parameters were deliberately changed. Below are some examples of typical variations: Table 6 indicate the robustness parameters for the method when the mobile phase composition was varied by ± 2 nm volume of solvent and the flow rate was varied by ± 0.2 units.

8.Assay:

In a 10 ml volumetric flask, a synthetic mixture[19] of 150 mg equivalent of CAR was added. Methanol was added and sonicated for 2-3 minutes, after which the volume was made up to the mark. Whatman filter paper no. 42 was used to filter the water. As a result, the final solution contained 1000 g/ml CAR and 1000 g/ml IVA. 1.0 ml of the aforesaid solution was pipette out and transferred to a 10 ml volumetric flask, where the volume was made up to the mark with methanol, yielding a solution containing CAR (100 g/ml) + IVA (100 g/ml). 2.5,0.12.0.12.0.62,0.62,2.5 ml for CAR and 0.5,0.75,0.5,0.75,0.5,0.75 ml for IVA were pipette out and transferred to a 10 ml volumetric flask, where the volume was filled up to mark with methanol, yielding a solution containing CAR (25,12.5,12.5,6.25,6.25,25 g/ml) and IVA (5,7.5,5,7.5,5,7.5 µg/ml) respectively. The concentrations of CAR and IVA were determined by solving the regression equation using a chromatogram. (table no.8)

RESULTS AND DISCUSSION:

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:

Chromatographic analysis was performed on a SHIMADZU LC-2010 CHT with UV Detector using LC solution software (C18, 250 x 4.6 mm, 5µm column). Acetonitrile: Methanol: Water-0.1%TEA (75:23:2% v/v/v) whole pH-3 with 1% OPA was used as a mobile phase. The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 10 µl. Detection was performed at 225 nm.

Table no. 1: Optimized Chromatographic Conditions

Sr. No.	Parameters	Condition
1.	**Mobile Phase**	Acetonitrile: Methanol: Water-0.1%TEA (75:23:2% v/v/v) whole pH-3 with 1% OPA
2.	**Flow rate**	1.0 mL/min
3.	**Run time**	20 min
4.	**Volume of injection**	10 µl
5.	**Detection Wavelength**	225 nm
6.	**Diluent**	Mobile Phase

VALIDATION:

1. Specificity:

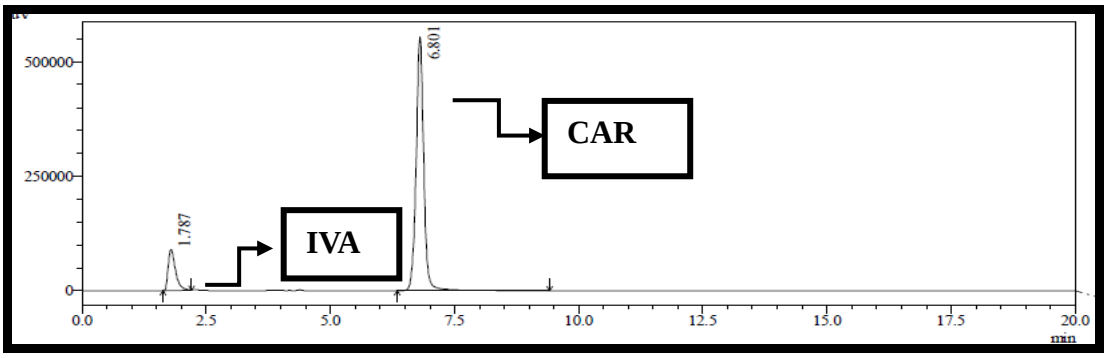

Figure no. 4: Sample chromatogram (CAR 25 + IVA 5)

2. System suitability:

The % RSD for the retention times and peak area of CAR and IVA were found to be less than 2 %. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

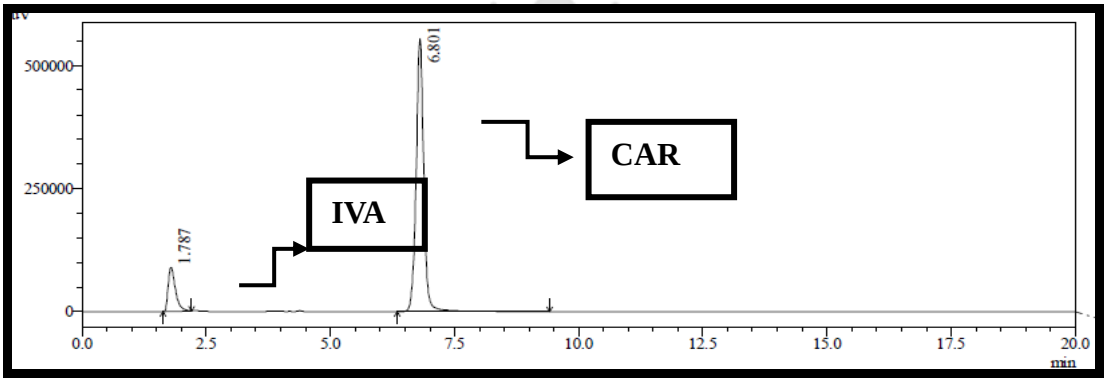

Figure no. 5: Chromatogram of sample preparation of CAR and IVA

Table no.2: Results for system suitability of CAR and IVA

Drugs	Parameters	Mean ± S.D. (n=6)	%R.S.D.
CAR	Retention Time	6.8108 ± 0.0077	0.1131
	Theoretical Plate	65382.45 ± 73.1744	0.2319
	Tailing Factor	0.9971 ± 0.0102	0.8235
IVA	Retention Time	1.7905 ± 0.0048	0.2707
	Theoretical Plate	4688.81 ± 14.5281	0.3098
	Tailing Factor	1.4351 ± 0.0152	0.7626
Resolution		18.4571 ± 0.0807	0.4377

3. Accuracy:

For accuracy, results from nine measurements were calculated and reported as recovery values for three concentrations: 0%, 80%, 100%, and 120% of expected sample concentration covering the given range. The results were shown in table 3.

Table no. 3: Accuracy data for CAR and IVA

Drugs	Level	Amount of Sample (µg/mL)	Amount of Std. Spiked (µg/mL)	Total amount (µg/mL)	Amount of Sample found (µg/mL)	%Recovery
CAR	0%	10	0	10	9.912	99.12
	80%	10	8	18	17.902	99.45
	100%	10	10	20	20.001	100.00
	120%	10	12	22	21.845	99.29
IVA	0%	4	0	4	3.962	99.05
	80%	4	3.2	7.2	7.179	99.17
	100%	4	4	8	8.001	100.02
	120%	4	4.8	8.8	8.738	99.30

4. Linearity:

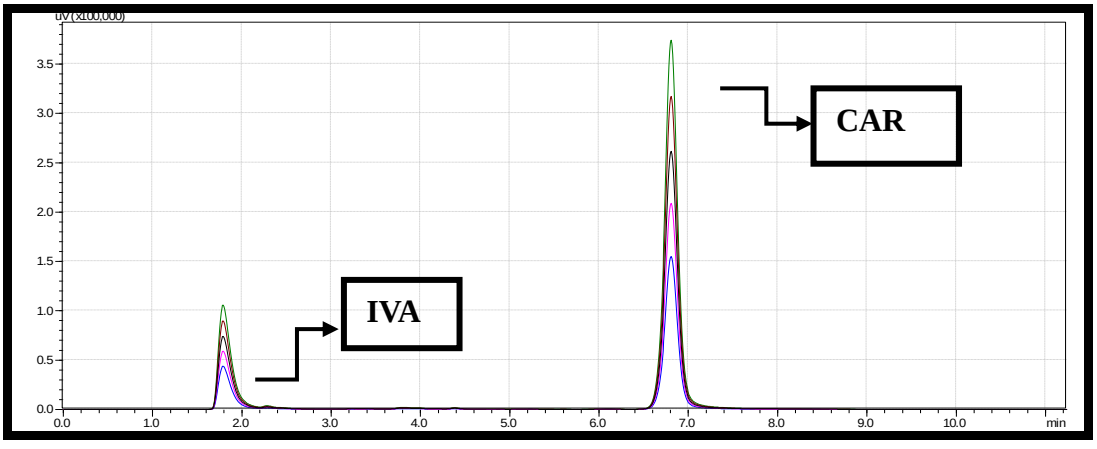

Figure no. 6: Overlays Chromatogram of CAR (5-25 µg/ml) and IVA (2-10 µg/ml)

Table no. 4: Linearity preparations of CAR and IVA:

Sr. No.	CAR Concentration (µg/ml)	IVA Concentration (µg/ml)	CAR Mean Peak Area ± S.D. (n=5)	IVA Mean Peak Area ± S.D. (n=5)	CAR % R.S.D.	IVA % R.S.D.
1.	5	2	12513 ± 6107.65	67854 ± 26095.48	0.4880	0.3845
2.	10	4	23617 ± 11307.46	78472 ± 41804.60	0.4787	0.4327
3.	15	6	34916 ± 12412.39	90270 ± 18350.35	0.3558	0.2032
4.	20	8	46399 ± 20453.20	99794 ± 34086.93	0.4408	0.3415
5.	25	10	58691 ± 24251.26	11046 ± 86617.62	0.4131	0.4840

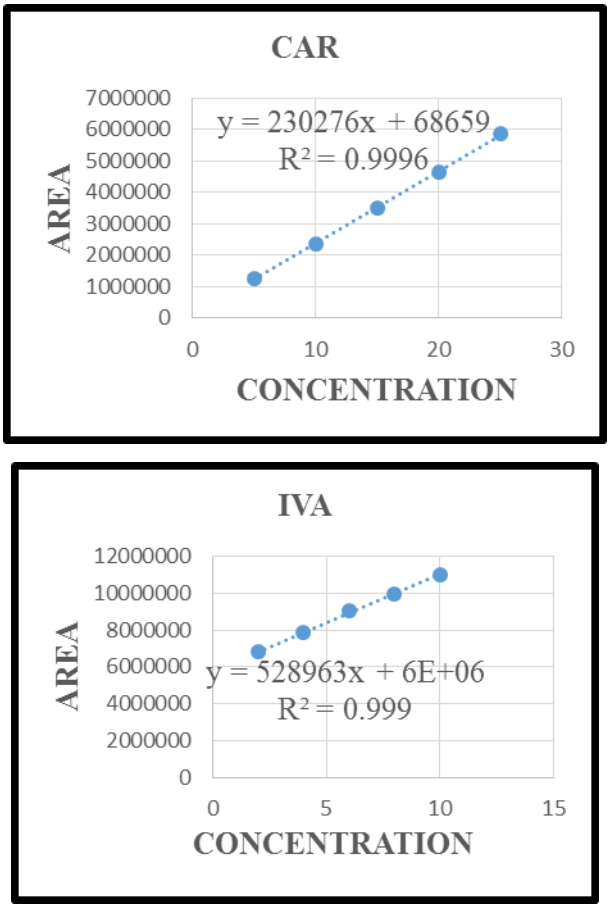

Figure no.7: Calibration curve of CAR and IVA

5. Precision:

Table no. 5: Results for method precision of CAR and IVA:

Parameter	Concentration (µg/ml)		Mean Peak Area ± S.D. (n = 3)		% R.S.D.	
	CAR	IVA	CAR	IVA	CAR	IVA
Repeatability (n=6) (% RSD)	15	6	34800 ± 13664.0	90413 ± 25625.0	0.3926	0.2834
Intraday precision (n=3) (% RSD)	10	4	23468 ± 11686.46	78498 ± 39867.04	0.4979	0.5078
	15	6	34740 ± 17471.90	90519 ± 35039.20	0.5029	0.3870
	20	8	46669 ± 28888.01	99671 ± 41866.71	0.6186	0.4200
Interday precision (n=3) (% RSD)	10	4	23432 ± 13384.63	79039 ± 60078.60	0.5931	0.7601
	15	6	34807 ± 27402.93	90685 ± 44192.55	0.7872	0.5973
	20	8	46613 ± 38799.2	99597 ± 61633.98	0.8323	0.6188

6. LOD & LOQ:

The **LOD** for CAR and IVA was found to be 0.10356 µg/mL and 0.24125 µg/mL respectively. The **LOQ** for CAR and IVA was found to be 0.31384 µg/mL and 0.73108 µg/Ml respectively.

7. Robustness:

Table.no.6 shows the outcome of the robustness of the devised test method. The results demonstrated that the assay value of the test preparation solution was unaffected by any of the variance circumstances, and that it was in good agreement with the real system suitability parameters. As a result, we can conclude that the analytical procedure is reliable.

Table no. 6: Robustness data for CAR and IVA

DRUG	Parameters	Level	Mean Peak Area ± S.D. (n=5)	%R.S.D.	Rt ± S.D. (n=5)	%R.S.D.
CAR	Mobile Phase (75:23:2 v/v)	73:25:2 v/v/v	59607 ± 24990.5	0.4192	6.809 ± 0.0076	0.2124
		77:21:2 v/v/v	59651 ± 26076.8	0.4371	6.816 ± 0.0226	0.3326
	Flow rate (1.0 mL/min)	0.8 mL/min	59809 ± 11539.8	0.4929	6.935 ± 0.0396	0.5711
		1.2 mL/min	58471 ± 28809.7	0.4927	6.695 ± 0.0436	0.6525
IVA	Mobile Phase (75:23:2 v/v)	73:25:2 v/v/v	91823 ± 1031.952	0.3112	1.789 ± 0.0042	0.2357
		77:21:2 v/v/v	91857 ± 5455.972	0.5939	1.788 ± 0.0096	0.5398
	Flow rate (1.0 mL/min)	0.8 mL/min	91808 ± 3734.97	0.4068	1.890 ± 0.0078	0.4172
		1.2 mL/min	90495 ± 4817.70	0.5323	1.783 ± 0.0096	0.5437

8. ANALYSIS OF SYNTHETIC MIXTURE:

Table no. 7: Analysis of Synthetic mixture

	Concentration (µg/ml)		Amount Obtain (µg/ml)		%Assay of CAR ± S.D (n=3)	%Assay of IVA ± S.D (n=3)
	CAR	IVA	CAR	IVA		
Synthetic mixture	25	5	24.95	4.980	99.81 ± 0.0440	99.60 ± 0.0120
	12.5	7.5	12.48	7.381	99.90 ± 0.0395	98.42 ± 0.0447
	12.5	5	12.47	4.984	99.77 ± 0.0505	99.69 ± 0.0055
	6.25	7.5	6.237	7.478	99.79 ± 0.0265	99.77 ± 0.011
	6.25	5	6.219	4.952	99.50 ± 0.0086	99.04 ± 0.0284
	25	7.5	24.93	7.423	99.72 ± 0.0635	98.97 ± 0.0105

SUMMARY OF VALIDATION PARAMETER FOR PROPOSED METHOD:

Table no. 8: Summary of RP-HPLC method

PARAMETERS	CARVEDILOL	IVABRADINE HYDROCHLORIDE
Linearity (n=5)	5-25 µg/ml	2-10 µg/ml
Regression equation	230276x + 68659	528963x + 6E+06
Regression Co-efficient (R^2)	0.9996	0.999
Correlation Coefficient (r)	0.9998	0.9995
Repeatability (n=6) (% RSD)	0.3926	0.2834
Intraday precision (n=3) (% RSD)	0.4979-0.6186	0.3870-0.5078
Interday precision (n=3) (% RSD)	0.5931-0.8323	0.5973-0.7601
LOD(µg/ml) (n=5)	0.1035	0.2412
LOQ(µg/ml) (n=5)	0.3138	0.7310
% Accuracy (n=3)	99.12-100.0%	99.05-100.02%

STATISTICAL COMPARISON FOR DEVELOPED AND REPORTED METHODS:

Table no. 9: COMPARISON FOR DEVELOPED AND REPORTED METHODS:

Methods		**% Carvedilol**	**% Ivabradine Hydrochloride**
RP – HPLC (Developed Method)		99.81	99.60
		99.90	98.42
		99.77	99.69
		99.79	99.77
		99.5	99.40
		99.72	98.97
RP – HPLC (Reported Method)		99.92	100.09
		99.79	99.04
		98.88	99.86
		99.83	100.78
		99.89	99.98
		100.5	99.94
SS	Between Group	0.0085	0.6721
	Within Group	1.4513	5.5515
DF	Between Group	1	1
	Within Group	10	10
MS	Between Group	0.0085	0.6721
	Within Group	0.1451	0.5551
$F_{Calculated}$		0.0587	1.2107
$F_{Critical}$		4.9646	4.9646

Since, $F_{calculated}$ was found to be less than $F_{critical}$ for both CAR and IVA, thus indicating that there is no significant difference observed in assay result among the two methods.

Hence it was concluded that all two methods do not differ significantly.

Rational behind selection of mobile phase:

Table no. 10: HPLC Trials for selection of mobile phase

No of Trials	Mobile Phase	Observation
Trial 1	**Acetonitrile :Phosphate Buffer(75:25% v/v)pH-3 with OPA**	Merge peak were observed
Trial 2	**Acetonitrile :0.01%TEA (50:50% v/v)whole pH-3 with 0.1% OPA**	peak were observed but not satisfactory separated
Trial 3	**Acetonitrile :0.01%TEA (75:25% v/v)whole pH-3 with 1% OPA**	peak were observed but broadening of peak and improper baseline.
Trial 4	**Acetonitrile :0.01%TEA (25:75% v/v)whole pH-3 with 1% OPA**	Peak Were Observed But Not Satisfactory Separated
Trial 5	**Acetonitrile :0.1%TEA (75:25% v/v) whole pH-3 with 1% OPA**	Single peak were observed
Trial 6	**Acetonitrile :0.1%TEA (75:25% v/v) whole pH-6.2 with 1% OPA**	peak were observed but not satisfactory separated
Trial 7	**Acetonitrile: Methanol: Water-0.1%TEA (50:25:25% v/v/v) whole pH-3 with 1% OPA**	peak were observed but broadening of peak
Trial 8	**Acetonitrile: Methanol: Water-0.1%TEA (80:10:10% v/v/v) whole pH-3 with 1% OPA**	Multiple Peak Were Observed But Broadening of Peak and improper baseline.
Trial 9	**Acetonitrile: Methanol: Water-0.1%TEA (80:10:10% v/v/v) whole pH-6.2 with 1% OPA**	Merge peak were observed
Trial 10	**Acetonitrile: Methanol: Water-0.1%TEA (80:18:2 % v/v/v) whole pH-3 with 1% OPA**	two peaks were separated, but not satisfactory
Trial 11	**Acetonitrile: Methanol: Water-0.1%TEA (75:23:2% v/v/v) whole pH-3 with 1% OPA**	Two peaks were separated satisfactory

CONCLUSION:

Development and validation of RP-HPLC method for the estimation of Carvedilol and Ivabradine Hydrochloride in synthetic mixture with the facilities and the results are incorporated in this thesis.

In conclusion, a validated RP-HPLC method has been developed for the determination of Carvedilol and Ivabradine Hydrochloride in a synthetic mixture. The results show that the method was found to be specific, simple, accurate, precise, and sensitive. The method was successfully applied for the determination of both drugs in a synthetic mixture. In the future, this method may be applied for routine analysis of both the drugs in API and synthetic mixture.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing % RSD less than 2. All statistical data supports the methods' validity and can be used to analyse pharmaceutical dose forms on a regular basis.

ACKNOWLEDGEMENTS:

The authors are thankful to Sun Pharmaceutical Industries Limited, Piparia, Silvasa, as well as Torrent Pharma, Dahej, Bharuch for distributing free samples of CAR and IVA, respectively for research. The authors are highly thankful to ROFEL Shri G. M. Bilkhia College of Pharmacy, Vapi, Gujarat, India for providing all the facilities to carry out the work.

REFERENCES:

1. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry, CBS publishers and distributors. 4th ed.; 2002: 279-300.
2. The guideline, ICH Harmonized Tripartite, Validation of analytical procedures. text and methodology Q2 (R1). International Conference on Hoarmonization (ICH), Geneva,Switzerland. 2005: 4-13.
3. Pubchem, “Carvedilol”, December 2020**,** https://pubchem.ncbi.nlm.nih.gov/compound/carvedilol.
4. Drugbank, “Carvedilol”, December 2020,https://www.drugbank.ca/drugs/DB01136.
5. Pubchem, “Ivabradine”, December 2020, https://pubchem.ncbi.nlm.nih.gov/compound/Ivabradine.
6. Drugbank, “Ivabradine”, December 2020, https://www.drugbank.ca/drugs/DB09083.
7. Indian Pharmacopoeia: The Indian Pharmacopoeia Commission Ghaziabad. Govt. of India Ministry of Health and Family Welfare**.** 8th ed.;Vol. I ; 2018: 1498.

8. British Pharmacopoeia: The department of Health, Social Service and Public Safety: The British Pharmacopoeia Commission. 8th ed.; 2016,.Vol. I: 428.
9. Sethi PD. HPLC-Quantitative analysis of pharmaceutical formulations. 3rd Ed.; CBS publishers & distributors. 1997:59-63.
10. Phani RS, Prasad KRS and Reddy U. Scientific approach for RP-HPLC method development: complete review. Int. J. Sci. Innovations Discoveries.2016;2:896-903.
11. Basaveswara Rao MV, Nagendrakumar AV, Yedukondalu M, Raman BV. New validated RP-HPLC Method for the estimation of carvedilol in pharmaceutical formulation. Int.J.Pharm.Pharma.Sci. 2012; 4(2): 353-358.
12. Patel H, Jivani N. Development of Validated RP-HPLC method for simultaneous estimation of carvedilol and ivabradine. WJPPS. 2015; 4(5): 630-639.
13. Jamil W, Shariq H, Shariq JA. Method Development and Analytical Method Validation of Carvedilol by High-Performance Liquid Chromatography. J.Pharm. Bio. Sci. 2016; 11(6): 93-97.
14. Naidu NV, Reddy MR, Naidu KB. Development and Validation of RP-HPLC Method for Determination of Carvedilol in Bulk and Pharmaceutical Dosage Forms. Der. Pharmacia Lettre.2014; 6(6): 198-206.
15. El-Adl1 SM, Abdel-Aziz1 LM, Mohamed MA. HPLC determination of Carvedilol and Atorvastatin Calcium in their bulk and dosage form. Asian J. Pharm. Medicinal Che. 2016; 4(2): 112 – 123.
16. Muzaffar-ur-Rehman M, Nagamallika G. Validated RP-HPLC method for the determination of ivabradine hydrochloride in pharmaceutical formulation. Int. J. Pharm. Sci. Drug Res. 2017; 9(5): 228-233.
17. Pundkar AS, Vyas PV. Analytical method development and validation for estimation of Ivabradine Hydrochloride by using RP-HPLC. AJRBPS. 2019; 7(2): 29-43.
18. Thete PG, Saudagar RB. Analytical Method Development and Validation for the Determination of Ivabradine HCl by RP-HPLC in Bulk and Pharmaceutical Dosage Form. Asian J. Pharm. Tech. 2019; 9(2): 89-92.
19. MIMS,Drug info. “CARIVALAN”,https://www.mims.com/myanmar/drug/info/carivalan?type=full.