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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CILNIDIPINE, METOPROLOL SUCCINATE AND TELMISARTAN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
Simultaneous Equation Method for CIL, MET and TEL was studied by UV-3000+, LABINDIA. Methanol was used as a solvent. The wavelength selected was 241 nm, 224 nm and 296 nm for CIL, MET and TEL respectively. RP-HPLC Method was studied by using Shimadzu LC-2010 AHT, Chromatographic separation was achieved using ODS C_{18} RP column (250 mm × 4.6 mm i.d., 5µm) kept at ambient temperature, using a mobile phase consisting a mixture of Phosphate buffer (pH 3.0): Acetonitrile and pH adjusted with 0.5% orthophosphoric acid at a flow rate of 1.0 ml/min. The detection was made at 220 nm.

KEYWORDS: Cilnidipine, Metoprolol succinate, Telmisartan, UV, RP-HPLC Method development and Validation.

MATERIAL AND METHOD

Cilnidipine is a Calcium channel blocker. It chemical name is 1,4-dihydro-2,6-dimethyl-4-(3nitrophenyl) -3,5-pyridinedicarboxylicacid 2-methoxy- ethyl (2E)-3-phenyl-2-propenyl ester. Metoprolol succinate is a β-adrenergic antagonist. It chemical name is 2-propanol,1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl)amino]-,(±)-, butanedioate (2:1)(salt). Telmisartan is a Angiotensin II receptor antagonist. It chemical name is 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazole]-1'yl)methyl][1,1'-biphenyl]-2-carboxylic acid.

DEVELOPMENT AND VALIDATION OF SIMULTANEOUS METHOD

Chemical and Materials

Table No. 1: Chemical and Materials.

Sr No.	Material and reagent	Supplier by
1.	Cilnidipine	Prayosha healthcare pvt.ltd.
2.	Metoprolol succinate	Ctx life sciences pvt.ltd.
3.	Telmisartan	Vapi care pharma pvt ltd
4.	Marketed dosage form	Met xl trio 25 (Ajanta pharma pvt ltd) local medical store

Literature survey revealed that no Simultaneous equation method was reported for the simultaneous estimation of cilnidipine, metoprolol succinate and telmisartan in solid dosage form. In the present investigation an attempt has been made to develop a simple, accurate and reproducible simultaneous equation method for the simultaneous estimation of cilnidipine, metoprolol succinate and telmisartan in solid dosage form. The proposed method was successfully applied for determination of cilnidipine, metoprolol succinate and telmisartan in solid dosage form.

Instrument and Apparatus

- A double beam UV-Visible spectrophotometer (LABINDIA, UV-3000+), attached to a computer software UV win 5.2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells.

Spectrophotometric Conditions

- Mode: Scan
- Scan speed: Medium
- Wavelength range: 200-400 nm
- Absorbance scale: 0.00-1.00 A
- Initial base line correction: Methanol

Preparation of stock solutions

Preparation of Cilnidipine stock solutions: A 100 mg of cilnidipine was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000µg/ml.

Working standard solution of Cilnidipine: 1 ml of stock solution was diluted to 10 ml with mobile phase to prepare 100µg/ml.

Preparation of Metoprolol succinate: A 100 mg of Metoprolol succinate was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000µg/ml.

Working standard solution of Metoprolol succinate 2.4 ml of stock solution was diluted to 10 ml with mobile phase to prepare 240µg/ml.

Preparation of Telmisartan: A 100 mg of Telmisartan was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000µg/ml.

Working standard solution of telmisartan: 4 ml of stock solution was diluted to 10 ml with mobile phase to prepare 400µg/ml.

Procedure for Determination of Wavelength for Measurement

Simultaneous Equation Method

Aliquot of 1 ml of working standard solution of cilnidipine (100µg/ml) and aliquot of 2.4 ml of working standard solution of metoprolol succinate (240µg/ml) and aliquot of 4 ml of working standard solution of telmisartan(400µg/ml) were pipetted out into three separated 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get 10µg/ml of Cilnidipine, 24µg/ml of metoprolol succinate and 40µg/ml of telmisartan. Each solution was scanned between 200-400 nm against Methanol as a blank reagent. Wavelength were selected from the overlay spectra of cilnidipine, metoprolol succinate and telmisartan.

Preparation of calibration Curve

Calibration curve for the Cilnidipine(2-10µg/ml)

Appropriate volume (0.2, 0.4, 0.6, 0.8 and 1 ml) of aliquot from working standard cilnidipine stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 2, 4, 6, 8 and10 µg/ml. the curve of each solution against the methanol was recorded. Absorbance at 240nm, 224 nm and 296 nm Was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Calibration curve for the Metoprolol succinate(5.0-24µg/ml)

Appropriate volume (0.5, 0.9, 1.4, 1.9 and 2.4ml) of aliquot from working standard metoprolol succinate stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 5, 9, 14, 19 and 24µg/ml. the curve of each solution against the methanol was recorded. Absorbance at 240nm, 224 nm and 296 nm was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Calibration curve for the Telmisartan(8-40µg/ml)

Appropriate volume (0.8, 1.6, 2.4, 3.2 and 4 ml) of aliquot from working standard Telmisartan stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 8, 16, 24, 32 and 40µg/ml. the curve of each solution against the methanol was recorded. Absorbance at 240nm, 224 nm and 296 nm Was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Validation of Proposed Method

Parameters to be considered for the validation of method are,

Linearity and Range

Procedure: The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-10µg/ml for cilnidipine, 5-24µg/ml for metoprlol succinate and 8-40 µg/ml for telmisartan respectively(n=5). The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equation for cilnidipine, metoprolol succinate and telmisartan were calculated.

PRECISION

Repeatability

Procedure: Aliquots of 1.0 ml of working standard solution of cilnidipine(100 µg/ml), metoprolol succinate(240 µg/ml) and telmisartan(400 µg/ml) were transferred to a series (n=6) of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 10 µg/ml solution of cilnidipine, 24 µg/ml solution of metoprolol succinate and 40 µg/ml solution of telmisartan. The absorbance of solution was measured spectroscopically six times and %RSD was calculated.

Intraday precision

Procedure: Aliquots of 0.5, 1 and 1.5 ml of working standard stock solution of cilnidipine(100µg/ml), metoprolol succinate(240µg/ml) and telmisartan (400µg/ml) were transferred to a series (n=3) of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 5, 10 and 15µg/ml solution of cilnidipine, 12, 24 and 36 µg/ml solution of metoprolol succinate and 20, 40 and 60 µg/ml solution of

telmisartan. The absorbance of solution was measured spectroscopically three times and %RSD was calculated.

Interday precision

Procedure: Aliquots of 0.5, 1 and 1.5 ml of working standard stock solution of cilnidipine(100µg/ml), metoprolol succinate(240µg/ml) and telmisartan (400µg/ml) were transferred to a series (n=3) of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 5, 10 and 15µg/ml solution of cilnidipine, 12, 24 and 36 µg/ml solution of metoprolol succinate and 20, 40 and 60 µg/ml solution of telmisartan. Solution was analyzed 3 times on the 3 different day using spectrophotometry and % RSD was calculated.

Accuracy

Procedure: The % recovery experiment was performed by the standard addition method. Fixed amounts of sample mixtures of cilnidipine(10µg/ml), metoprolol succinate(24µg/ml) and telmisartan(40µg/ml) and increasing amount of its working standard solution were added at 80,100 and 120% level of cilnidipine(8, 10 and 12µg/ml), metoprolol succinate(19, 24 and 28µg/ml) and telmisartan(32, 40 and 48µg/ml). Area of peak obtained with each solutions was measured for cilnidipine, metoprolol succinate and telmisartan. The mean %recovery from the peak areas was calculated.

LOD and LOQ

The LOD was estimated from the 5 calibration curves. The LOD may be calculated as

$$\mathbf{LOD = 3.3 \times (SD / Slope)}$$

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope= Mean slope of the 5 calibration curves.

The LOQ was estimated from the calibration curves used to determine method linearity. The LOQ may be calculated as

$$\mathbf{LOQ = 10 \times (SD/Slope)}$$

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD

Apparatus and Instrumentation

- HPLC: LC-2010 AHT
- Detector: UV detector
- Column: C_{18} ODS (250*4.6 mm, 5µm particle size)
- Auto Injector (Capacity Loop of 10 µl)
- Software: Class VP

Preparation of standard solutions

Preparation of Cilnidipine stock solutions: A 100 mg of cilnidipine was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000µg/ml.

Working standard solution of Cilnidipine: 1 ml of stock solution was diluted to 10 ml with mobile phase to prepare 100µg/ml.

Preparation of Metoprolol succinate: A 100 mg of Metoprolol succinate was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000µg/ml.

Working standard solution of Metoprolol succinate 2.4 ml of stock solution was diluted to 10 ml with mobile phase to prepare 240µg/ml.

Preparation of Telmisartan: A 100 mg of Telmisartan was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000µg/ml.

Working standard solution of telmisartan: 4 ml of stock solution was diluted to 10 ml with mobile phase to prepare 400µg/ml.

Preparation of ternary mixture of cilnidipine, metoprolol succinate and telmisartan: Accurately weighed 100 mg cilnidipine, metoprolol succinate and telmisartan were transferred to 100 ml volumetric flask, dissolved with sufficient mobile phase and the flask was sonicated. It was then diluted up to the mark with mobile phase to give concentration of 1000µg/ml of cilnidipine, metoprolol succinate and telmisartan. 1 ml of cilnidipine, 2.4 ml of metoprolol succinate and 4 ml of telmisartan was taken from the above solution and diluted further to 10 ml to get the concentration of 100µg/ml, 240µg/ml and 400µg/ml. Above solution was diluted further to get the concentration range of 5,7.5,10,12.5 and 15µg/ml of cilnidipine, 12,18,24,30 and 36µg/ml of metoprolol succinate and 20,30, 40, 50 and 60µg/ml of telmisartan.

Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study individual drug solutions of 10µg/ml cilnidipine, metoprolol succinate and telmisartan were prepared in methanol. These drug solutions were than scanned in the UV region of 200-400 nm and the spectrum was recorded. Wavelength for detection was selected by obtaining overlay spectra of cilnidipine, metoprolol succinate and telmisartan.

Chromatographic Conditions

Proper selection of the HPLC method depends upon the nature of the sample (ionic, ionizable or neutral molecule), its molecular weight and solubility. The drugs

selected for the present study are polar in nature and hence either reversed phase or ion-pair or ion-exchange chromatography can be used. Reversed phase HPLC was selected for the initial separations because of its simplicity and suitability.

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation.

Stationary phase: C_{18} ODS (250 mm x 4.6 mm, 5 µm)
Mobile phase: Potassium phosphate Buffer pH-3: ACN

Table No.2: Potassium Phosphate Buffer Ph-3: Can.

TIME (MINUTE)	BUFFER	ACN
0.01	90	10
5	25	75
13	25	75
14	90	10
19	90	10

pH: pH of mobile phase was adjusted to 3 using phosphoric acid.
Flow rate: 1.0 ml/min
Temperature: Ambient
Wavelength: 220nm

Chromatographic separation
Standard solutions of cilnidipine, metoprolol succinate and telmisartan were injected in column with 20 µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase Potassium phosphate Buffer pH-3: ACN. The detection was carried out at wavelength 220nm. The chromatogram was stopped after separation achieved completely.

System Suitability Test
It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Validation of RP-HPLC Method
Linearity
The linearity is expressed in term of correlation co-efficient of linear regression analysis. Aliquots of standard solutions of cilnidipine 5-15µg/ml, metoprolol succinate 12-36µg/ml and telmisartan 20-60µg/ml respectively, was prepared from working standard solution and injected to system with stated chromatographic conditions and analysed. Linear correlation was obtained between peak areas versus concentration of in the range of cilnidipine 5-15µg/ml, metoprolol succinate 12-36µg/ml and telmisartan 20-60µg/ml. The calibration curves of cilnidipine, metoprolol succinate and telmisartan at 220nm, respectively are shown in FIG.ure no. 7.3.8, 7.3.9 and 7.3.10. The graph of peak area obtained versus respective concentration was plotted. The mean area and standard deviation were calculated.

Precision
Repeatability
1 ml of combined working standard solutions 100µg/ml of cilnidipine, 240µg/ml of metoprolol succinate and 400µg/ml of telmisartan were transferred into separate 10 ml volumetric flasks and diluted up to mark with mobile phase to get 10µg/ml of cilnidipine, 24µg/ml of metoprolol succinate and 40µg/ml of telmisartan. Each concentration was prepared and analyzed. The peak are obtained with each solutions was measured and %R.S.D was calculated.

Intraday precision
Standard solution of cilnidipine (5-15µg/ml), metoprolol succinate (12-36µg/ml) and telmisartan (20-60µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed on the same day and %R.S.D was calculated.

Interday precision
Standard solution of cilnidipine (5-15µg/ml), metoprolol succinate (12-36µg/ml) and telmisartan (20-60µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed on different days and %R.S.D was calculated.

Accuracy
The % recovery experiment was performed by the Standard addition method. Fixed amounts of sample mixture of cilnidipine(10µg/ml), metoprolol succinate(24µg/ml) and telmisartan(40µg/ml) and increasing amount of its working standard solutions were added at 80, 100 and 120% level of cilnidipine(8,10 and 12µg/ml), metoprolol succinate(19, 24 and 28µg/ml) and telmisartan(32, 40 and 48µg/ml). Area of peak obtained with each solution was measured for cilnidipine, metoprolol succinate and telmisartan. The mean % recovery from the peak areas was calculated.

LOD and LOQ
The LOD was estimated from the 5 calibration curves. The LOD may be calculated as

$$\mathbf{LOD = 3.3 \times (SD / Slope)}$$

Where,
SD = Standard deviation of the Y- intercepts of the 5 calibration curves.
Slope = Mean slope of the 5 calibration curves.

The LOQ was estimated from the calibration curves used to determine method linearity. The LOQ may be calculated as

LOQ = 10 × (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Robustness

The solution containing concentration of cilnidipine(10µg/ml), metoprolol succinate(24µg/ml) and telmisartan(40µg/ml) was analyzed in different flow rate, mobile phase and pH. The peak area obtained with each solution was measured and % RSD was calculated.

Acceptance criteria: % RSD should be less than 2.

Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in presence of components that may expected to be present in the sample matrix. Specificity of developed method was established by spiking of cilnidipine, metoprolol succinate and telmisartan in hypothetical placebo (i.e. might be expected to be present) and expressing that analytes peak was not interfered from excipients.

Development of the Method

Selection of common solvent

Methanol was selected as solvent for developing spectral characteristics of cilnidipine, metoprolol succinate and telisartan. The selection was made after evaluating the solubility of cilnidipine, metoprolol succinate and telisartan in different solvent.

Determination of absorption maxima and selection of wavelength

By dilution of three standard drug solution with methanol, solutions containing 10µg/ml of cilnidipine, 25 µg/ml of metoprolol succinate and 40 µg/ml of telmisartan were scanned separately in the range of 200-400nm to determine the wavelength of maximum absorption for all the drugs. Cilnidipine, metoprolol succinate and telmisartan showed absorbance maxima at 240nm, 224nm and 296nm respectively. The overlain spectra showed λmax of all drugs.

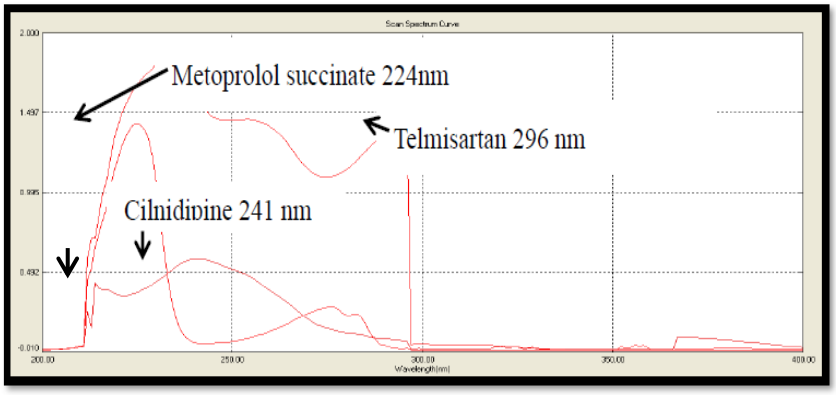

Fig. No.1: Overlain uv spectra of cilnidipine, metoprolol succinate and telmisartan showing selection of wavelength for detection.

Validation of the proposed method

Linearity and range

Linear correlation was obtained between absorbance versus concentration of cilnidipine(5-15µg/ml), metoprolol succinate(12.5-37.50µg/ml) and telmisartan20-60µg/ml). The linearity curves and calibration curves of these three drugs were shown in FIG.ure.

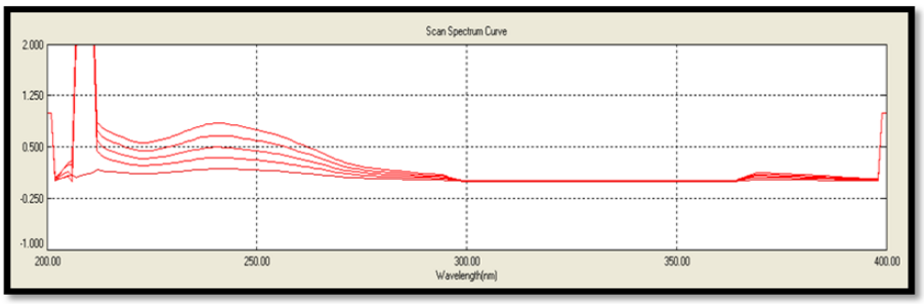

FIG. NO.2: Overlay Spectra of Cilnidipine At 241 Nm.

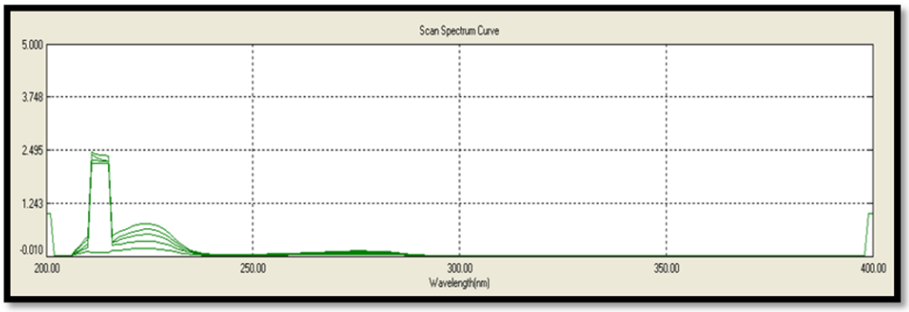

Fig. No.3: Overlay spectra of metoprolol succinate at 224 nm.

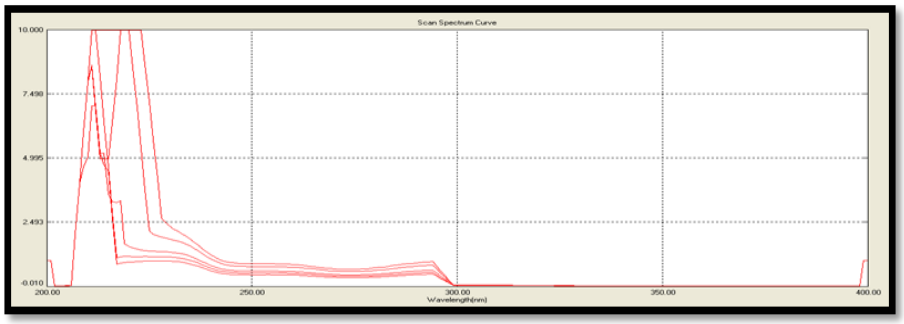

Fig. No.4: Overlay spectra of telmisartan at 296nm.

Table No.3: Calibration Curve of Cilnidipine At 224 nm.

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
2	0.121±0.00162
4	0.244±0.003033
6	0.345±0.00488
8	0.474±0.0062
10	0.603±0.00637

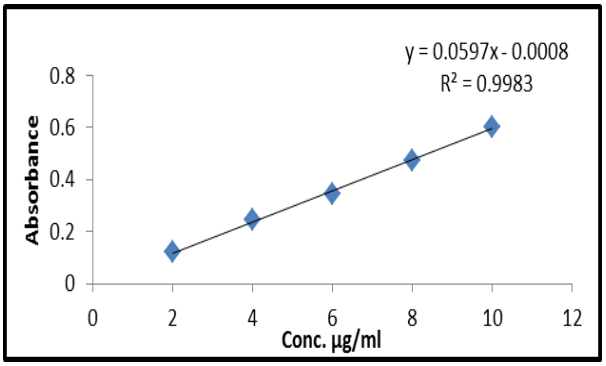

Fig. No.5: Calibration Curve of Cilnidipine at 224 nm.

Table No.4: Calibration Curve of Cilnidipine At 241 Nm.

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
2	0.183±0.0037
4	0.350±0.0064
6	0.498±0.00722
8	0.674±0.00549
10	0.853±0.00773

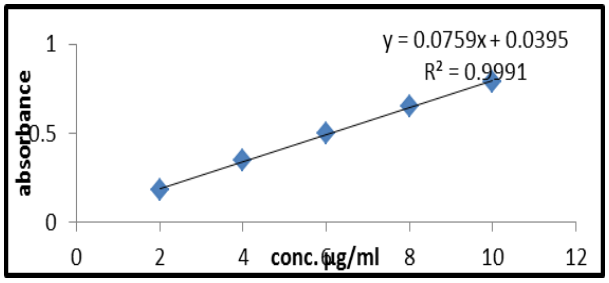

Fig. No.6: Calibration Curve of Cilnidipine at 241 nm.

Table No.5: Calibration Curve Of Cilnidipine At 296 nm.

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
2	0.047±0.000894
4	0.0682±0.000748
6	0.0854±0.00135
8	0.103±0.00135
10	0.123±0.00241

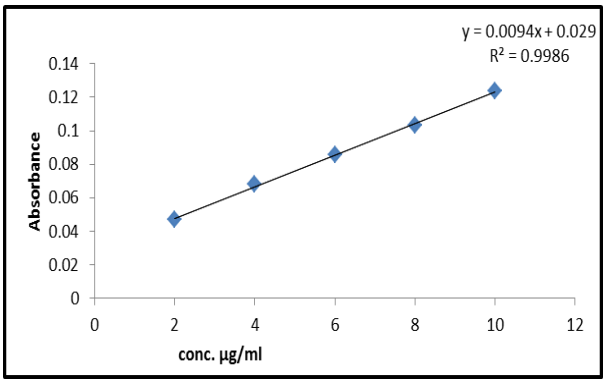

Fig. No.7: Calibration Curve of Cilnidipine 296 nm.

TABLE NO.6:Calibration of Metoprolol Succinate At 224 nm

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
5	0.172±0.002785
10	0.3454±0.003382
14	0.477±0.0053
19	0.627±0.00660
24	0.767±0.0081

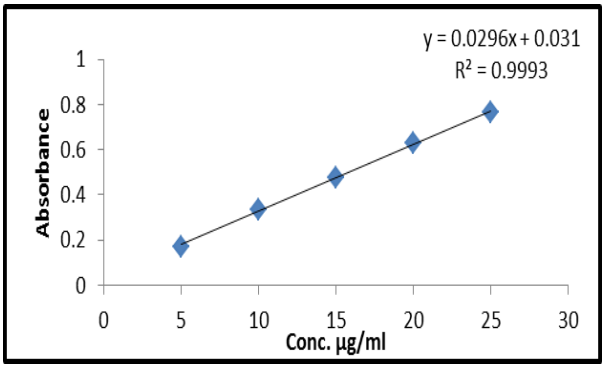

Fig. No. 8: calibration curve of metoprolol succinate at 224nm.

Table No.7: Calibration Curve of Metoprolol Succinate At 241 Nm.

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
5	0.015±0.0004
10	0.027±0.0004
14	0.041±0.0009
19	0.055±0.0013
24	0.070±0.0014

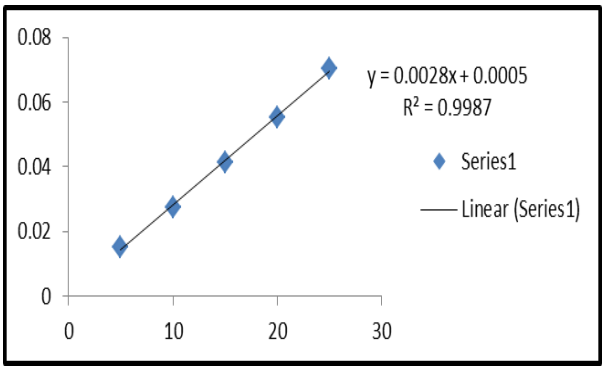

Fig. No. 9: Calibration Curve of Metoprolol Succinate At 241nm.

Table No.8: Calibration Curve of Metoprolol Succinate 296nm

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
5	0.0158±0.0004
10	0.032±0.000894
14	0.0455±0.00081
19	0.0628±0.000116
24	0.077±0.000129

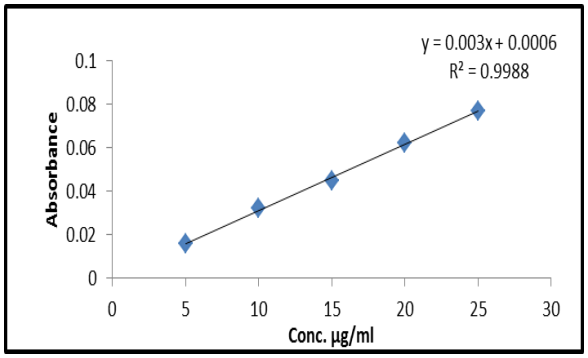

Fig. No.10: Calibration Curve of Metoprolol Succinate 296 nm.

Table No.9: Calibration Curve of Telmisartan At 224nm.

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
8	0.320±0.00581
16	0.394±0.00717
24	0.467±0.00730
32	0.532±0.00598
40	0.619±0.007088

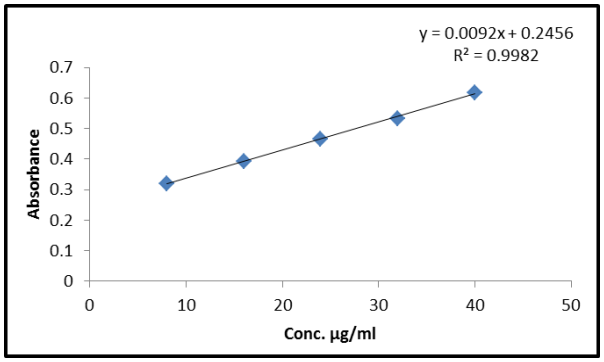

Fig.No.11: Calibration Curve of Telmisartan At 224 Nm.

Table No.10: Calibration Curve of Telmisartan AT 241 nm

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
8	0.533±0.00796
16	0.667±0.008
24	0.815±0.00828
32	0.956±0.008863
40	1.121±0.008059

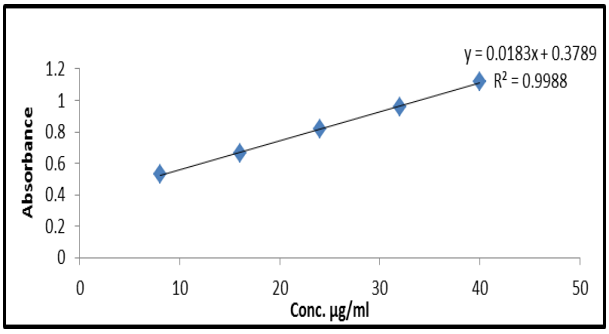

Fig. No. 12: Calibration Curve of Telmisartan at 241 nm.

Table No.11: Calibration Curve of Telmisartan AT 296 nm.

Conc. (µg/ml)	Mean abs. ± S.D. (n=5)
8	0.421±0.00454
16	0.536±0.006177
24	0.650±0.004454
32	0.744±0.00463
40	0.956±0.01003

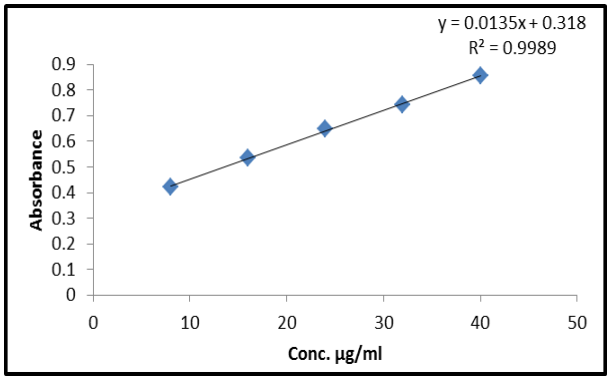

Fig. No.13: Calibration Curve of Telmisartan At 296 Nm

Accuracy (%Recovery)

The % recovery experiment was performed by the standard addition method. Known amounts of standard solutions of cilnidipine, metoprolol succinate and telmisartan were added at 80,100 and 120% level to prequantified sample solutions of cilnidipine(10µg/ml), metoprolol succinate(25µg/ml) and telmisartan (40µg/ml). Results of recovery studies are shown in table.

TABLE NO.12: Recovery Data For Cilnidipine.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	%recovery	Mean % Recovery ±S.D(n=3)
80%	10	8	18	8.130	101.63	101.36±0.2395
	10	8	18	8.139	101.74	
	10	8	18	8.175	102.18	
100%	10	10	20	10.176	101.18	101.8±0.0563
	10	10	20	10.176	101.76	
	10	10	20	10.188	101.88	
120%	10	12	22	11.884	99.03	99.18±0.2809
	10	12	22	11.872	98.93	
	10	12	22	11.949	99.57	

Table No.13: Recovery Data For Metoprolol Succinate.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % Recovery ±S.D(n=3)
80%	24	19	43	19.69	101.56	102.01±0.356
	24	19	43	19.75	102.04	
	24	19	43	19.80	102.43	
100%	24	24	48	24.41	101.65	101.10±0.874
	24	24	48	24.44	101.79	
	24	24	48	23.96	99.87	
120%	24	29	53	28.85	99.34	99.221±0.193
	24	29	53	28.10	98.94	
	24	29	53	28.76	99.37	

Table No.14: Recovery Data For Telmisartan.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	%recovery	Mean % Recovery ±S.D(n=3)
80%	40	20	60	20.499	102.49	102.09±0.254
	40	20	60	20.435	102.1775	
	40	20	60	20.560	102.800	
100%	40	40	80	40.421	101.053	101.332±0.324
	40	40	80	40.462	101.0155	
	40	40	80	40.715	101.787	
120%	40	60	100	59.036	98.393	98.74±0.250
	40	60	100	59.370	98.950	
	40	60	100	59.338	98.897	

Precision

The interday and intraday precision of the proposed method was determined by analyzing the corresponding response 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of cilnidipine(5, 10 and 15µg/ml), metoprolol succinate(12.5, 25 and 37.5 µg/ml) and telmisartan(20, 40 and 60 µg/ml) and 6 determination for the same concentration of cilnidipine(10 µg/ml), metoprolol succinate(25 µg/ml) and telmisartan(40 µg/ml) for repeatability. The results of interday and intraday precision and repeatability were shown in table.

Table No.15: Interday And Intraday Precision Data For The Estimation Of Cilnidipine, Metoprolol Succinate And Telmisartan.

Drug	Conc. µg/ml	Intraday precision		Interday precision	
		mean± S.D (n=3)	%R.S.D	mean± S.D (n=3)	%R.S.D
Cilnidipine	5	0.42±0.0097	2.31	0.427±0.008	2.02
	10	0.808±0.0110	1.36	0.805±0.010	1.25
	15	0.991±0.0151	1.51	0.986±0.012	1.13
Metoprolol succinate	12	0.365±0.0095	2.16	0.381±0.005	1.50
	24	0.764±0.0087	1.14	0.757±0.007	1.04
	36	0.984±0.0110	1.11	0.987±0.007	0.79
Telmisartan	20	0.610±0.0110	1.81	0.604±0.006	1.07
	40	0.946±0.011	1.25	0.959±0.009	1.022
	60	1.423±0.012	0.844	1.434±0.011	0.761

Table No.16: Repeatability Data For Estimation of Cilnidipine, Metoprolol Succinate And Telmisartan.

Drug	Conc. µg/ml	Abs. Mean± S.D (n=6)	%R.S.D
Cilnidipine	10	0.851±0.00943	1.107
Metoprolol succinate	24	0.758±0.00958	1.26
Telmisartan	40	0.953±0.00957	1.0046

Limit of detection(LOD) AND Limit of Quantification(LOQ)

Calibration curve was repeated for three times and the standard deviation(SD) of the intercepts was calculated. The LOD & LOQ were calculated as follows:

LOD=3.3*SD/slope of calibration curve

LOQ=10*SD/slope of calibration curve

LOD values for cilnidipine. Metoprolol succinate and telmisartan were found to be 0.5035, 0.2110 and 0.06195 µg/ml and LOQ values for cilnidipine. Metoprolol succinate and telmisartan were found to be 1.525, 0.6396 and 0.187 µg/ml at 241 nm, 224 nm and 296 nm. This data shows that this method is sensitive for the determination of cilnidipine. Metoprolol succinate and telmisartan.

Application to Pharmaceutical Dosage Form/ Applicability of the Method

Applicability of the proposed method was tested by analysing the commercially available tablet formulation met xl trio 25. The results are shown in Table.

Table No.17: Analysis of Marketed Formulation.

Formulation (tablet)	Tablet amount amount (mg)			Amount found (mg)			% Assay		
	Cil	Met	Tel	Cil	Mel	Tel	Cil± S.D (n=3)	Met± S.D (n=3)	Tel± S.D (n=3)
1	10	23.75	40	10.52	23.12	39.54	101.6±0.0282	97.866±0.225	98.866±0.032
2	10	23.75	40	10.58	23.05	39.59			
3	10	23.75	40	10.58	23.56	39.51			

The assay results were comparable to labeled value of each drug in tablet dosage form. These results indicate that the developed method is accurate, precise and simple. It can be used in the routine quality control of dosage form in industries.

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD

Selection of Wavelength

Both the components show reasonably good response at 220 nm.

Selection of Elution Mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compared to non-polar compounds. In addition to this, UV detector is used, which allows easy detection of the compounds in UV transparent organic solvents. A 250 x 4.6 mm column of 5 µm particles packing was preferred as a starting point for method development. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability. This conFIG.uration provides a large number of theoretical plates value for maximum separation. In our study various mobile phases with different ratios were used.

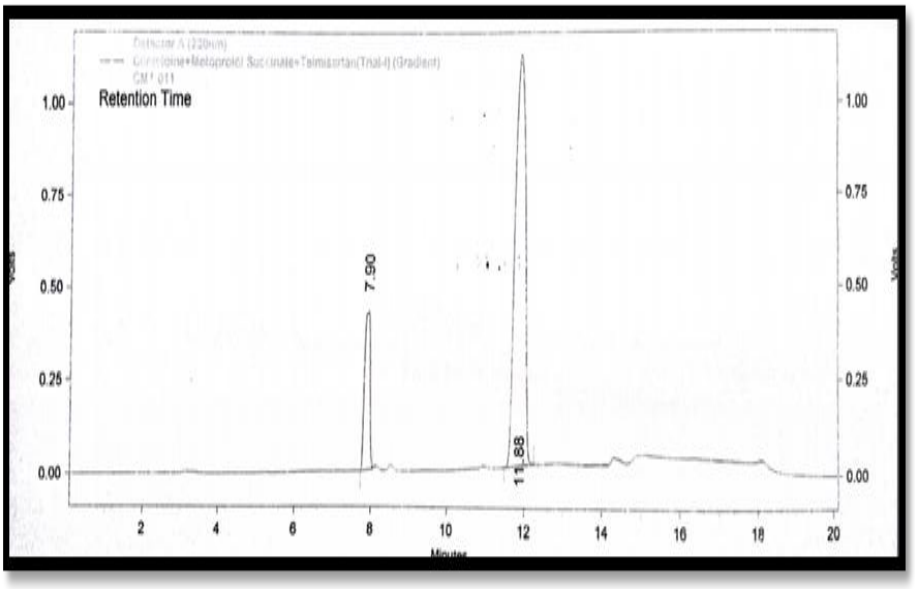

FIG. NO.14: Trial 1 Phosphate Buffer (Ph-3): Acetonitrile.

Table No.18: Trial 1 Phosphate Buffer (Ph-3): Acetonitrile.

Time (minute)	Buffer	Acn
0.01	95	5
10	30	70
15	30	70
16	95	5
20	95	5

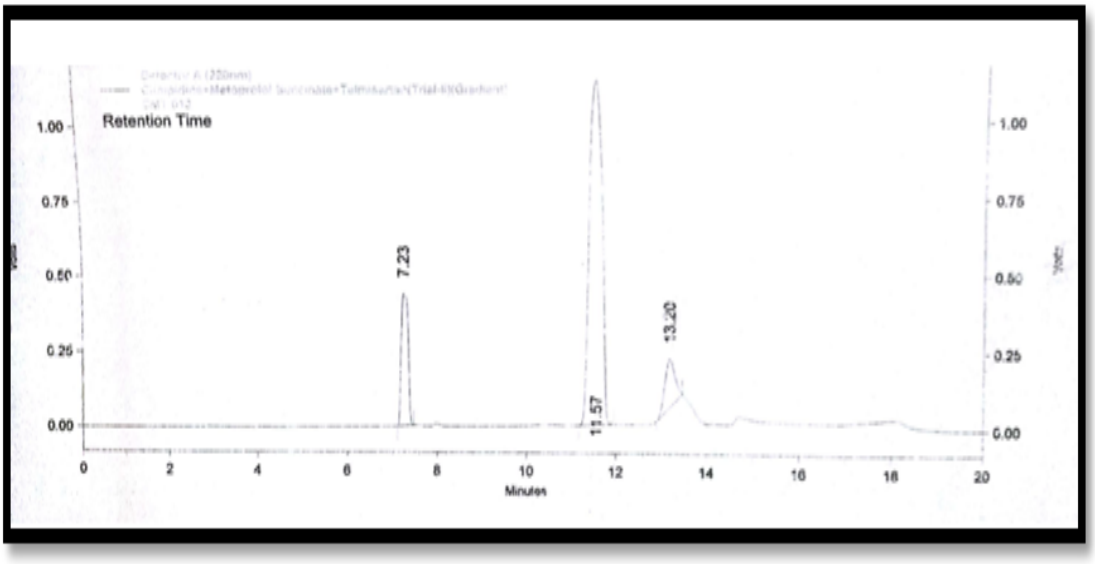

FIG NO.15: trial 2 phosphate buffer (ph-3): acetonitrile.

Table No.19: Trial 2 Phosphate Buffer (Ph-3): Acetonitrile.

Time (minute)	Buffer	Acn
0.01	90	10
10	30	70
15	30	70
16	90	10
20	90	10

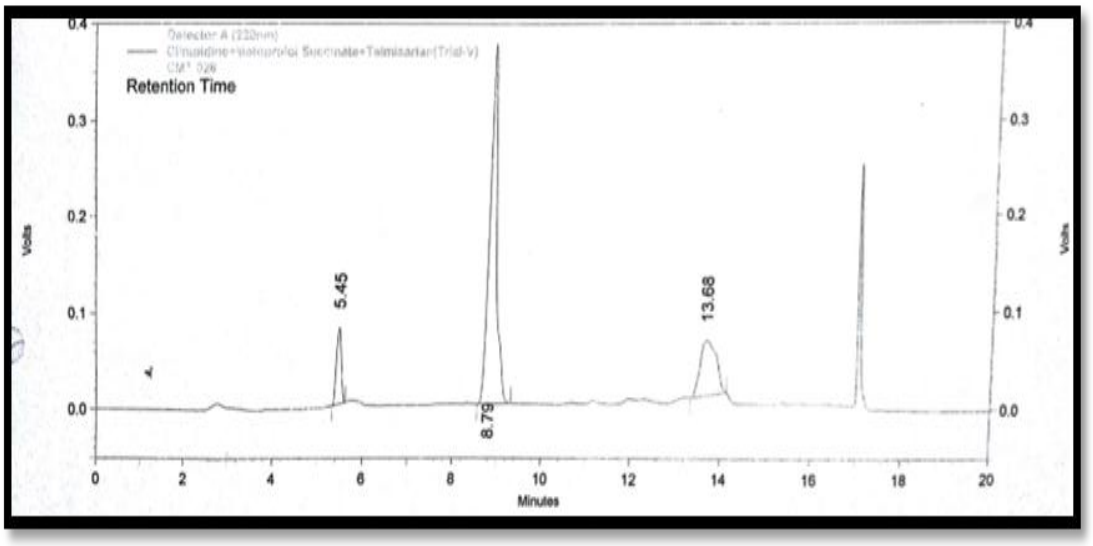

FIG. NO.16: Trial3 Phosphate Buffer (Ph-3): Acetonitrile.

TABLE NO.20: Trial 3 Phosphate Buffer (Ph-3): Acetonitrile.

Time (minute)	Buffer	Acn
0.01	80	20
8	10	90
13	10	90
14	80	20
20	80	20

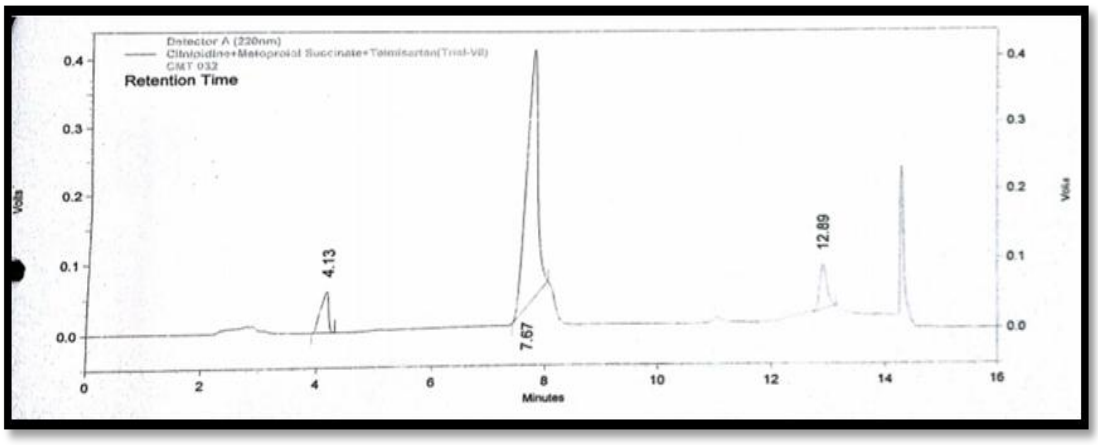

Fig. No.17: Trial 4 Phosphate Buffer (Ph-3): Acetonitrile.

Table NO.21: Trial 4 Phosphate Buffer (Ph-3): Acetonitrile.

Time (minute)	Buffer	Acn
0.01	70	30
5	10	90
10	10	90
11	70	30
16	70	30

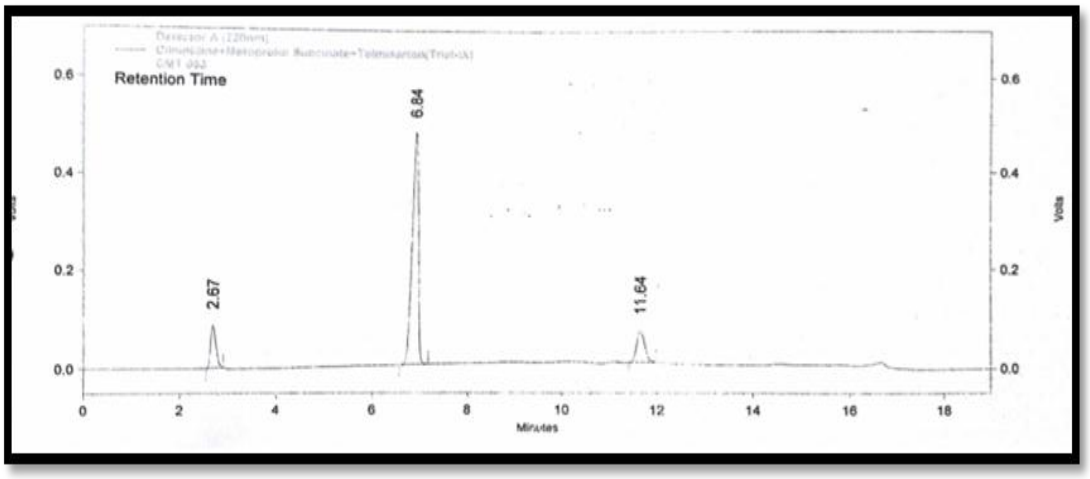

Fig. No.18: Final Mobile Phase Potassium Phosphate Buffer (Ph-3): Acetonitrile.

TABLE NO .22: Trial 5 Phosphate Buffer (Ph-3): Acetonitrile

Time (minute)	Buffer	Acn
0.01	90	10
5	25	75
13	25	75
14	90	10
19	90	10

System Suitability Parameters

TABLE NO.23: Results of System Suitability Parameters.

Parameters	Data obtained		
	Cilnidipine	Metoprolol succinate	Telmisartan
Theoretical plates	21337.8	2599.9	12440.7
Tailing factor	1.14	1.42	0.99
Retention time	11.64	2.67	6.84
Resolution	17.02	00	18.37

Method Validation

Linearity and Range

The linearity range for Cilnidipine was found to be 5-15 µg/ml, metoprolol succinate was found to be 12-36 µg/ml and Telmisartan was found to be 20-60 µg/ml respectively depicted in Table. Chromatogram of Cilnidipine, metoprolol succinate and telmisartan depicted in FIG.ure.

Correlation co-efficient for calibration curve of Cilnidipine, metoprolol succinate and telmisartan was found to be 0.999, 0.999 and 0.9993 respectively.

The regression line equation for cilnidipine is as follows:

Y= 63966x-32273

The regression line equation for metoprolol succinate is as follows:

Y=22258x+36155

The regression line equation for telmisartan is as follows:

Y=92584x+203751

Where,

Y=corresponding peak area

x=concentration of Cilnidipine, metoprolol succinate and telmisartan in µg/ml.

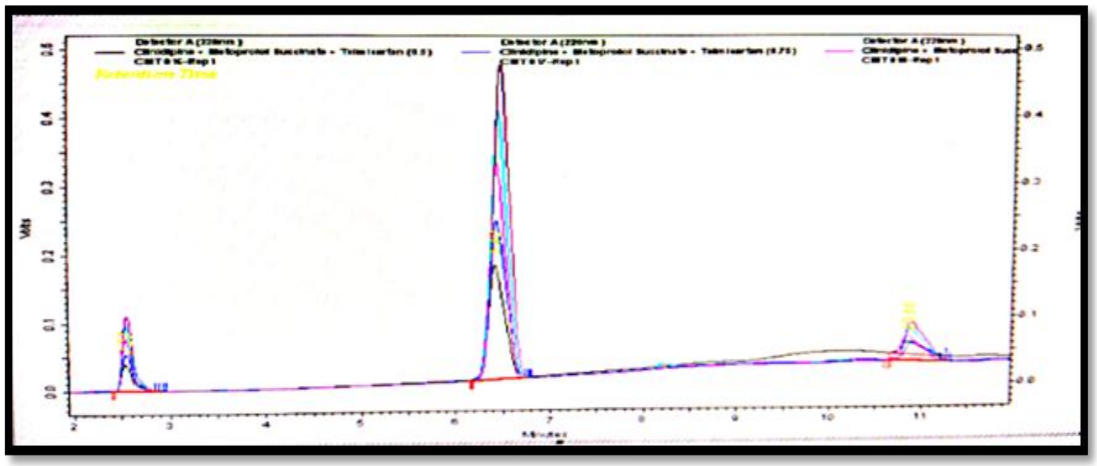

Fig. No.19: Chromatogram of Different Concentrations of Ternary Mixture of Cilnidipine, Metoprolol Succinate and Telmisartan.

Table No.24: Linearity Data For Cilnidipine.

Sr. No.	Conc. (µg/ml)	Peak area Mean±S.D (n=5)	% R.S.D
1	5	293491	1.432
2	7.5	443551	1.677
3	10	602617	1.564
4	12.5	764848	0.683
5	15	932414	0.826

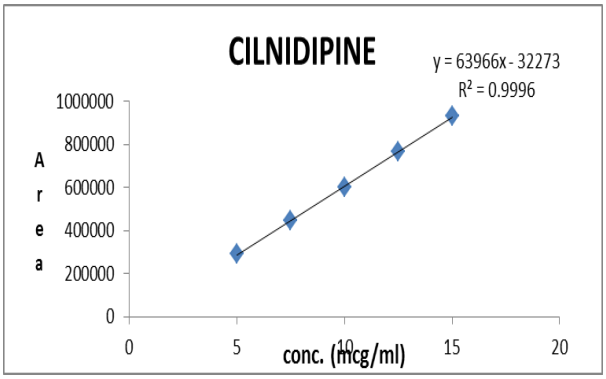

Fig. No.20: Calibration Curve of Cilnidipine.

Table NO.25: Linearity Data For Metoprolol Succinate.

Sr. No.	Conc. (µg/ml)	Peak Area Mean±S.D (n=5)	% R.S.D
1	12	309891	1.676
2	18	425935	0.983
3	24	572388	0.759
4	30	705755	0.627
5	36	837706	0.784

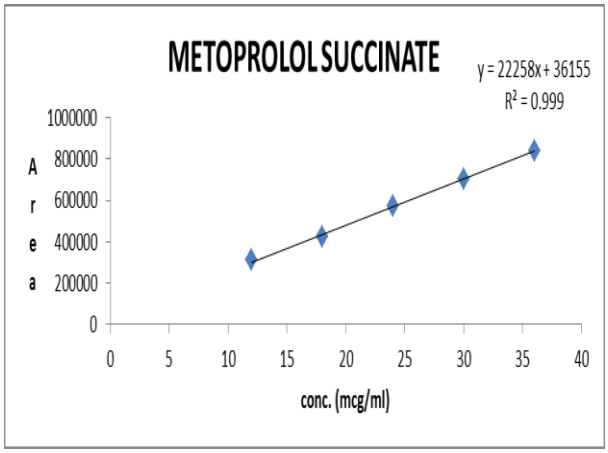

Fig. No.22: Calibration Curve of Metoprolol Succinate.

Table No.26: Linearity Data For Telmisatan.

Sr. No.	Conc. (µg/ml)	Peak Area Mean±S.D (n=5)	% R.S.D
1	20	2094018	0.954
2	30	2920055	1.482
3	40	3911868	1.303
4	50	4852844	0.847
5	60	5756842	1.057

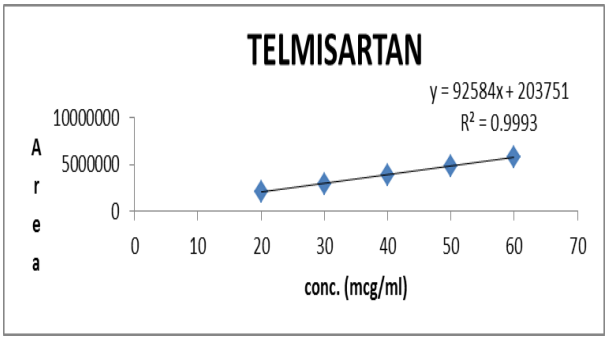

FIG. NO.23: Calibration Curve of Telmisartan.

Specificity

The specificity of the method was ascertained by analysing standard drugs and sample of Cilnidipine, metoprolol succinate and telmisartan. The results suggested that proposed method is specific, the excipient present in the formulation does not affect the result. The chromatogram taken by running only with mobile phase and after injection of the sample and standard are given in below FIG.

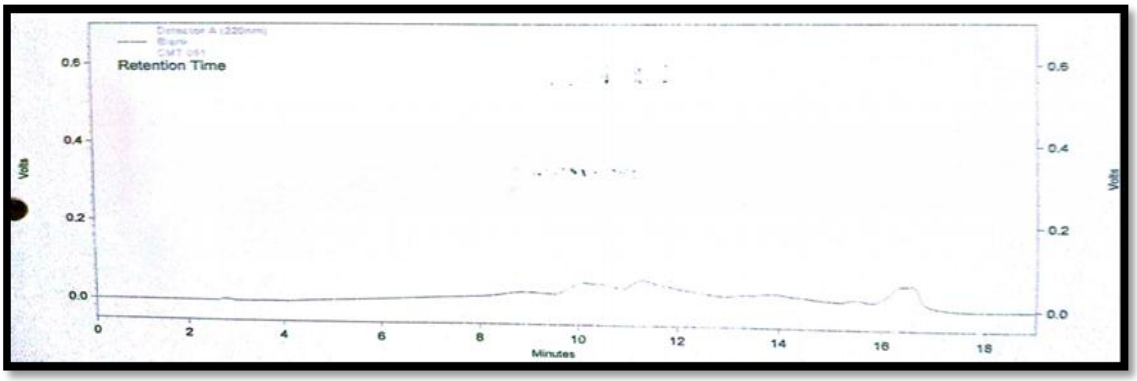

Fig. No.24: Chromatogram of Mobile Phase.

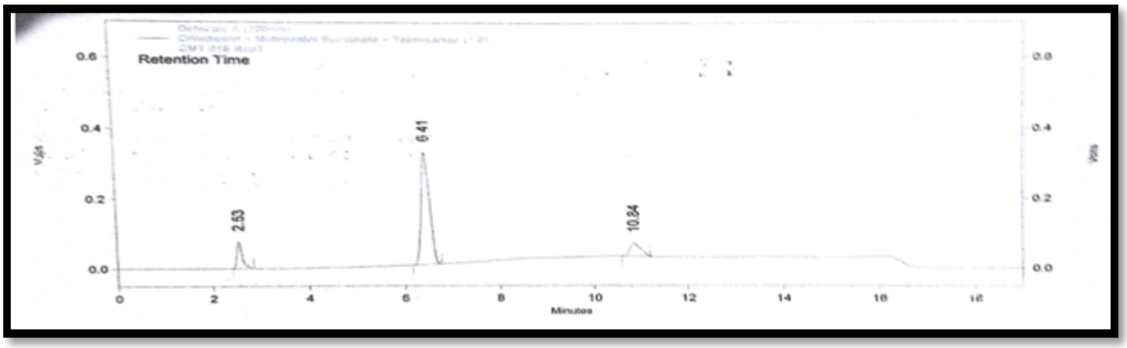

Fig. No.25: Chromatogram of Cilnidipine, Metoprolol Succinate and Telmisartan of Std. Solution.

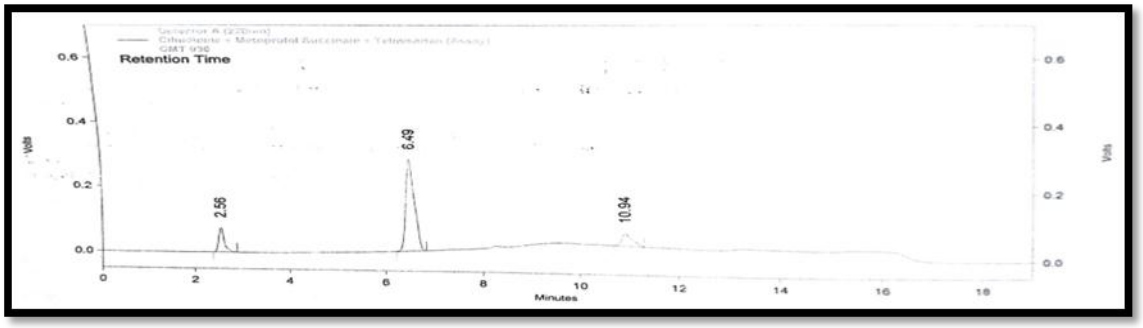

FIG. No.26: Chromatogram of Cilnidipine, Metoprolol Succinate and Telmisartan of Sample Solution.

Precision

1. Repeatability: The data for repeatability of peak area measurement for Cilnidipine, metoprolol succinate and telmisartan based on six measurements concentration of Cilnidipine, metoprolol succinate and telmisartan in linearity range are depicted in Table.

Table No.27: Repeatability Data for Cil, Met and Tel.

Drug	Conc. (µg/ml)	Peak area Mean ± S.D (n=6)	% R.S.D
Cilnidipine	10	603673	1.619
Metoprolol succinate	24	566183	1.838
Telmisartan	40	3856835	1.289

2. Intraday precision: The data for intraday precision for Cilnidipine, metoprolol succinate and telmisartan is shown in Table 6.3.5. The % R.S.D for intraday precision was found to be 0.574-0.925 for Cilnidipine, 0.484-0.869 for Metoprolol succinate & 0.564-1.118 for Telmisartan.

Table No. 28: Intraday Precision Data For Estimation Of Cilnidipine, Metoprolol Succinate And Telmisartan.

Drug	Conc. (µg/ml)	Intraday precision	
		Peak Area Mean ± S.D (n=3)	%R.S.D
Cilnidipine	5	287623	0.574
	10	596347	0.925
	15	948284	0.615
Metoprolol succinate	12	312954	0.484
	24	562420	0.869
	36	834921	0.680
Telmisartan	20	2087457	0.765
	40	3894228	0.564
	60	5717383	1.118

3. Interday precision: The data for intraday precision for Cilnidipine, metoprolol succinate and telmisartan is shown in Table. The % R.S.D for interday precision was found to be 0.742-1.550for Cilnidipine, 1.099-1.133 for metoprolol succinate & 1.384-1.573 for Telmisartan.

Table No.29: Interday Precision Data For Estimation Of Cilnidipine, Metoprolol Succinate And Telmisartan.

Drug	Conc. (µg/ml)	Interday precision	
		Peak Area Mean ± S.D (n=3)	% R.S.D
Cilnidipine	5	294458	1.550
	10	627421	1.487
	15	969085	0.742
Metoprolol succinate	12	326951	1.099
	24	588575	1.123
	36	842894	1.133
Telmisartan	20	2164368	1.384
	40	3970316	1.573
	60	5804389	1.502

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation (Met XL trio) at three level of standard addition. The results are shown in Table.

Table No. 30: Recovery Data For Cilnidipine.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Std. Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)
80%	8	10	18	8.02	100.28	101.17±0.77
	8	10	18	8.14	101.71	
	8	10	18	8.12	101.53	
100%	10	10	20	9.85	98.46	99.31±31
	10	10	20	10.01	100.14	
	10	10	20	9.93	99.33	
120%	12	10	22	11.85	98.71	99.79±1.36
	12	10	22	12.16	101.32	
	12	10	22	11.92	99.33	

Table No.31: Recovery Data For Metoprolol Succinate.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Std. Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)
80%	19	24	43	19.40	101.03	101.05±0.67
	19	24	43	19.27	100.38	
	19	24	43	19.53	101.73	
100%	24	24	48	24.22	100.92	100.81±1.00
	24	24	48	23.94	99.75	
	24	24	48	24.42	101.76	
120%	29	24	53	29.08	100.97	100.46±1.69
	29	24	53	29.33	101.85	
	29	24	53	28.39	98.56	

Tabale No.32: Recovery Data For Telisartan.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Std. Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)
80%	32	40	72	31.80	99.37	100.99±1.39
	32	40	72	32.55	101.71	
	32	40	72	32.60	101.87	
100%	40	40	80	39.44	98.59	99.84±1.55
	40	40	80	39.75	99.37	
	40	40	80	40.63	101.58	
120%	48	40	88	48.32	100.66	101.48±0.70
	48	40	88	48.87	101.81	
	48	40	88	48.95	101.97	

LOD and LOQ

Calibration curve was repeated for three times and the standard deviation (SD) of the intercepts was calculated. The LOD & LOQ were calculated as follows:

LOD=3.3*SD/slope of calibration curve

LOQ=10*SD/slope of calibration curve

TABLE NO.33: Lod And Loq Data For Cilnidipine, Metoprolol Succinate And Telmisartan.

Parameters	Cilnidipine(µg/ml)	Metoprolol succinate(µg/ml)	Telmisartan(µg/ml)
Mean slope	32273	36155	203751
Intercept	63966	22258	92584
LOD	0.608	0.686	0.703
LOQ	1.845	2.080	2.131

Robustness

All the three drugs were analyzed in different flow rate, % of Mobile phase and pH. The peak area obtained shown in Table.

Table No. 34: Robustness Data for Cilnidipine.

Sr. No	Cilnidipine (10µg/ml)					
	pH:3		Mobile Phase		Flow Rate:1 ml/min	
	pH 3.2 (+0.2 units)	pH 2.8 (-0.2 units)	Mobile phase (+2%)	Mobile phase (-2%)	Flow rate 1.2 ml/min (+0.2 units)	Flow rate 0.8 ml/min (-0.2 units)
1	538947	582368	606014	641677	493953	787567
2	540077	581742	602122	618831	493482	778524
3	549756	579756	598149	628695	496198	771861
Mean	542926	581288	602095	629734	494544	779317
S.D	5941.3	1363.7	3932.5	11458.4	1451.3	7882.9
%R.S.D	1.094	0.235	0.653	1.820	0.293	1.012

Table No.35: Robustness Data For Metoprolol Succinate

Sr. No	Metopolol succinate(24µg/ml)					
	pH:3		Mobile Phase		Flow Rate:1 ml/min	
	pH 3.2 (+0.2 units)	pH 2.8 (-0.2 units)	Mobile phase (+2%)	Mobile phase (-2%)	Flow rate 1.2 ml/min (+0.2 units)	Flow rate 0.8 ml/min (-0.2 units)
1	615714	581541	589596	581234	479544	711886
2	613419	601485	582168	559886	479965	711938
3	620158	589894	580156	569635	469562	709568
Mean	616430	590973	583973	570251	476357	711130
S.D	3426.1	10015.7	4972.2	10687	5888.4	1353.5
%R.S.D	0.556	1.695	0.851	1.874	1.236	0.190

Table No.36: Robustness Data For Telmisartan.

Sr. No	Telmisartan (40 µg/ml)					
	pH:3		Mobile Phase		Flow Rate:1 ml/min	
	pH 3.2 (+0.2 units)	pH 2.8 (-0.2 units)	Mobile phase (+2%)	Mobile phase (-2%)	Flow rate 1.2 ml/min (+0.2 units)	Flow rate 0.8 ml/min (-0.2 units)
1	970094	3834365	3979739	4148464	3235293	4950978
2	4017510	3820662	4025390	4097349	3249814	5106549
3	3954269	3901356	3987542	4185462	3301953	5092628
Mean	3980624	3852127	3997557	413758	326253	5050051
S.D	32909.2	43180.0	24417.7	44244.5	35054.4	86082.1
%R.S.D	0.826	1.120	0.610	1.067	1.074	1.704

Application to Pharmaceutical Dosage Form/ Applicability of the Method

Applicability of the proposed method was tested by analysing the commercially available tablet formulation Met XL Trio. The results are shown in Table no. 7.3.16.

Table No.37: Analysis of Marketed Formulation.

Formulation (tablet)	Tablet amount (mg)			Amount found (mg)			% assay		
	Cil	Met	Tel	Cil	Met	Tel	Cil± s.d (n=3)	Met± s.d (n=3)	Tel± s.d (n=3)
1	10	24	40	9.99	23.71	39.63	99.35±0.85	99.07±0.65	99.70±0.76
2	10	24	40	9.98	23.67	39.61			
3	10	24	40	9.84	23.95	40.21			

The assay results were comparable to labelled value of each drug in tablet dosage form. These results indicate that the developed method is accurate, precise and simple. It can be used in the routine quality control of dosage form in industries.

SUMMARY AND CONCLUSION

Spectrophotometric Method

TABLE NO.38: Summary of The Validation Parameters Of Proposed Method.

Result		Cil	Met	Tel
Wavelength for measurement		241 nm	224 nm	296 nm
Beer's law limit		2-10 µg/ml	5-24 µg/ml	8-40 µg/ml
Regression equation		y = 0.0759x + 0.039	y = 0.0296x + 0.031	y = 0.0135x + 0.318
Slope		0.0395	0.031	0.318
Intercept		0.0759	0.0296	0.0135
Correlation coefficient (r^2)		0.9991	0.9993	0.9989
% recovery	80%	101.63±0.2395	102.015±0.3568	102.49±0.2544
	100%	101.8±0.0563	101.10±0.874	101.332±0.3246
	120%	99.18±0.2809	99.221±0.193	98.74±0.25075
LOD (µg/ml)		0.5035	0.2110	0.0619
LOQ (µg/ml)		1.5258	0.6396	0.1877
Repeatability (% RSD, n=6)		1.1079	1.2639	1.0046
Interday Precision (%RSD) (n=3) at 3 diff.conc.		1.367-2.316	1.119-2.616	0.844-1.816
Intraday Precision (%RSD) (n=3) at 3 diff.conc.		1.138-2.023	0.794-1.502	0.761-1.072

- The values of percentage recovery and standard deviation show that the proposed method was reproducible, accurate and precise.
- The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug.
- No interference of the excipients with the absorbance of interest analyte appeared; hence the proposed method is applicable for the routine estimation of CIL, MET and TEL in pharmaceutical combined dosage form.

RP-HPLC Method

Table No. 39: Summary of The Validation Parameters of Proposed Method.

Result		Cil	Met	Tel
Wavelength for measurement		220 nm		
Beer's law limit		5-15 µg/ml	12-36 µg/ml	20-60 µg/ml
Regression equation (Y)		Y=63966x-32273	Y=22258x+36155	Y=92584x+203751
Correlation coefficient (r)		0.999	0.999	0.9993
% recovery	80%	101.17±0.77	101.05±0.67	100.99±1.39
	100%	99.31±31	100.81±1.00	99.84±1.55
	120%	99.79±1.36	100.46±1.69	101.48±0.70
LOD (µg/ml)		0.608	0.686	0.703
LOQ (µg/ml)		1.845	2.080	2.131
Repeatability (% RSD, n=3)		1.619	1.838	1.289
Interday Precision (%RSD) (n=3) at 3 diff.conc.		0.742-1.550	1.099-1.133	1.384-1.573
Intraday Precision (%RSD) (n=3) at 3 diff.conc.		0.574-0.925	0.484-0.869	0.564-1.118
Robustness (%RSD)	pH (+0.2 units)	1.094	0.556	0.826
	pH (-0.2 units)	0.235	1.695	1.120
	Flow rate (+2units)	0.293	1.236	1.074
	Flow rate(-2 units)	1.012	0.190	1.704
	Mobile phase (+0.2%)	0.653	0.851	0.610
	Mobile phase (-0.2%)	1.820	1.874	1.067

- RP-HPLC method has been developed and validated for the determination of CIL, MET and TEL in pharmaceutical dosage form.
- The method is found to be specific as there was no interference of excipients and impurities.
- The proposed method is found to be simple, accurate, and precise.
- The values of percentage recovery and standard deviation show that the proposed method was reproducible, accurate and precise and can be used in routine analysis.
- Both the developed method can be used for routine estimation of Cilnidipine, Metoprolol succinate and Telmisartan respectively in their combined pharmaceutical preparation.

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