

DEVELOPMENT AND VALIDATION OF HPLC METHOD OF DISSOLUTION TEST FOR METOPROLOL SUCCINATE AND CILNIDIPINE

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ABSTRACT

A reverse phase high performance liquid chromatographic (RP-HPLC) method suitable for simultaneous determination of Metoprolol Succinate and Cilnidipine dissolution of solid dosage forms in pharmaceuticals has been developed. Chromatographic separation was performed on a Empower 2 software. ELIPSE Phenomenax C18, 150X4.5mm, 5 μ , (Make: Waters) column using a mobile phase of buffer utilizing OPA buffer (pH 3.0): Methanol (70:30%v/v). The effluent flow rate monitored at 1.0mL/minute, injection volume was 10 μ L and detected by ultraviolet at 285nm. The retention times of metoprolol succinate and cilnidipine 2.590 and 4.047minutes, respectively. The total run time was 6minutes within which the drug product. The developed method has been validated for specificity, precision, linearity, accuracy, ruggedness and robustness. Additionally, the conditions of the dissolution test for metoprolol succinate and cilnidipine tablets were presented by using: paddle at 50rpm stirring speed; medium volume of 900mL; temperature at 37 \pm 0.5 $^{\circ}$ C; and pH 6.8 phosphate buffer used as dissolution medium. The average percentage drug release was found to be in between 95% to 105% within 30minutes for both drugs. The proposed analytical and dissolution method can be applied successfully for the quality control of commercial metoprolol succinate and cilnidipine tablets and the comparison of in vitro dissolution of combination drug products.

Keywords: metoprolol succinate, cilnidipine, Dissolution test; Method development, validation.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and Food Drug Administration (FDA) Guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation. It is rare today that an HPLC-

based method is developed that does not in some way relate (or) compare to existing, literature based approaches. Today HPLC (High performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV

spectroscopy has been chosen for these proposed methods.

Newer analytical methods are developed for these drugs or drug combinations of the below reasons

- There may not be suitable method for a particular analyte in the specific matrix.
- Existing method may be too error prone or unreliable (have poor accuracy and precision).
- Existing method may be expensive, time consuming, energy intensive and may not be provide sensitive or analyte selectivity, and not easy for automation.
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods.
- There may be need for an alternate method to confirm, for legal and scientific reasons.

The newly developed analytical methods having their importance in different fields that include, research and development centre (R&D), Quality control department (QC). Approved testing laboratories, chemical Analysis laboratories etc. For analysis of these drugs different analytical methods are routinely being used.

CHROMATOGRAPHY IN THE PHARMACEUTICAL WORLD

In the modern pharmaceutical industry, chromatography is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The development of new chemical entities (NCEs) is comprised of two major activities. Drug discovery and development. The goal of the drug discovered is to investigate a plethora of compounds employing fast screening approaches, leading to generation of lead compounds and then narrowing the selection through targeted synthesis and selective screening (lead optimization). The main functions of drug development are to completely characterize candidate compounds by performing drug metabolism, preclinical and clinical screening, and clinical trials. Throughout this drug discovery and development paradigm, rugged analytical HPLC separation methods are developed, at each phase of development to analyses of a myriad of samples are performed to adequately control and monitor the quality of the prospective drug candidates, excipients, and final products. Effective and fast method development is of paramount importance

throughout. This drug development life cycle. This requires a thorough understanding of HPLC principles and theory which have solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization.

MOST COMMONLY USED METHODS IN HPLC

Normal phase chromatography

For a polar stationary bed like silica we need to choose a relatively non-polar Mobile phase. This mode of operation is termed as Normal phase chromatography. Here the least polar component elutes first, and increasing the mobile phase polarity leads to decrease in elution time. Non-polar solvents like pentane, Hexane, isooctane, cyclohexane, etc. are more popular. It is mainly used for separation of nonionic, non-polar to medium polar substances.

Reverse phase chromatography

In 1960s, chromatographers started modifying the polar nature of the silanol group by chemically reacting silicon with organic silanes. The object was to make silica less polar or non-polar so that polar solvents can be used to separate water-soluble polar compounds. Since the ionic nature of the reverted, the chromatographic separation carried out with such silica is referred to as Reverse- phase chromatography. Here the most post components elutes first. Increasing mobile phase polarity leads to decrease In elution time. Common solvents used in this mode include Methanol /Acetonitrile /Isopropanol etc. Mostly used for separation of ionic and polar substances. The parameters that govern the retention in reversed phase system are the following:

- a. The chemical nature of the stationary phase surface.
- b. The type of solvents that compose the mobile phase.
- c. pH and ionic strength of the mobile phase.

Isocratic elution: A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning constant composition).

Gradient elution: The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution.

MATERIALS AND METHODS

Metoprolol is obtained as gift sample from Dr Reddys Labs Pvt Ltd, Hyderabad. Cilnidipine was procured from Dr Reddys Labs Pvt Ltd, Hyderabad. Potassium dihydrogen phosphate was obtained from Dr Reddys Labs Pvt Ltd, Hyderabad. Methanol and OPA were obtained from Finar chemicals Pvt Ltd.

OPTIMIZED METHOD FOR HPLC

Chromatographic parameters (fig. 1)

Mobile phase : OPA: Methanol (70:30).
Column : Phenomenax C18, 150X4.5mm, 5 μ
Auto sample temperature : 25°C
Column temperature : 30°C
Injection volume : 10 μ L
Detector wavelength : 285nm
Flow rate : 1 mL/min

Procedure for UV and HPLC

Inject 10 μ L of standard, sample into chromatographic system and measure the areas for the metoprolol succinate and cilnidipine peaks and calculate the % assay by using the formula.

BUFFER PREPARATION

OPA: Methanol (70:30) Used as diluent.

Preparation of Metoprolol succinate Standard Solution

Accurately weigh and transfer 100mg of Metoprolol succinate into 100 ml of volumetric flask and add 60ml of mobile phase and sonicate to dissolve. Dilute to volume with mobile phase and mix.

Transfer each 5.0 ml of Metoprolol succinate standard solution into a 25ml volumetric flask, dilute to volume with mobile phase and mix.

Preparation of Clinidipine Standard Solution

Accurately weigh and transfer 10mg of Clinidipine into 100 ml of volumetric flask and add 60ml of mobile phase and sonicate to dissolve. Dilute to volume with mobile phase and mix.

Transfer each 5.0 ml of Clinidipine standard solution into a 25ml volumetric flask, dilute to volume with mobile phase and mix.

PREPARATION OF SAMPLE SOLUTION

Commercially available 20 tablets are weighed and powdered equivalent to the 100mg of Metoprolol succinate and 10mg of Clinidipine into a 100ml volumetric flask, add about 60ml of mobile phase and sonicate for 30min with intermediate shaking (maintain the sonicator bath

temperature between 20-25°C). Make up to the volume with mobile phase and mix. Filter a portion of the solution through 0.45 μ m membrane filter and discard first few ml of the filtrate.

Transfer each 5.0 ml of above sample solution into a 25ml volumetric flask, dilute to volume with mobile phase and mix.

METHOD VALIDATION

SYSTEM SUITABILITY

Solution of standard sample and placebo were prepared as per test procedure. Equilibrate the column with mobile phase for not less than 30minutes at a flow rate of 1ml/min.

Separately inject 10 μ L of blank, standard solution (5 times) and sample solution into the chromatographic system.

Tailing factor for the peaks due to metoprolol succinate and cilnidipine standard solution should not be more than 2.0. Theoretical plates for the metoprolol succinate and cilnidipine peaks in standard solution should not be less than 3000.

SPECIFICITY

Solution of standard sample and placebo were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria

Chromatogram of standard and sample should be identical with near Retention time.

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Acceptance criteria

Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

LINEARITY

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in μ g/ml and determine the coefficient of correlation and basis for 100% response.

Acceptance criteria

Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained

from the linearity data (without extrapolation through origin 0, 0) should be within ± 2.0 .

Statistical Evaluation

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

PRECISION

Preparation of sample

- Commercially available 20 tablets are weighed and powdered equivalent to the 100mg of Metoprolol and 10mg cilnidipine into a 100ml volumetric flask, add about 60ml of mobile phase and sonicate for 30min with intermediate shaking (maintain the sonicator bath temperature between 20-25°C). Make up to the volume with mobile phase and mix. Filter a portion of the solution through 0.45µm membrane filter and discard first few ml of the filtrate.
- Transfer 5 ml of the filtered solution into a 25ml volumetric flask, dilute to volume with mobile phase and mix.
- The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peak areas from 6 replicate injections.

Acceptance criteria: The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention times is not more than 2.0.

RECOVERY/ACCURACY

Recovery study can be performed in the concentration range of 50% to 150% of the target concentration of the test. Minimum 3 concentrations are recommended.

Acceptance criteria

The average percentage recovery was between 97-103% and Relative standard deviation of these recovery concentrations was less than 2%

ROBUSTNESS

Effect of variation in flow rate

Prepare the system suitability solution as per the test method and inject into the HPLC system with ± 0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1 ml/min and it was changed to 0.8 ml/min and 1.2 ml/min

and inject into HPLC and system suitability was checked.

Effect of variation in Temperature

Prepare the system suitability solution as per the test method and injected into the HPLC with $\pm 5^\circ\text{C}$ of the method temperature. Evaluate the system suitability values as required by the test method for both temperatures.

LIMIT OF DETECTION

The sensitivity of measurement of metoprolol succinate and cilnidipine method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level.

$$\text{LOD} = 3.3 \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

LIMIT OF QUANTIFICATION

The sensitivity of measurement of metoprolol succinate and cilnidipine by the use of proposed method was estimated in terms of limit of quantification (LOQ). The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

$$\text{LOQ} = 10 \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

DISSOLUTION OF METOPROLOL AND CILNIDIPINE BY RP-HPLC

Preparation of dissolution medium

Dissolved 6.8g of mono basic potassium phosphate in 200mL of water and added 173.5mL of 0.2M sodium hydroxide and diluted with water to 1000mL.

Bath preparation

150rpm speed of the motor, the constant temperature bath at 37°C. Placed 900mL volume of dissolution me-dium in each of six vessels of dissolution apparatus, which previously have been immersed in the constant temperature bath, and allowed the medium to come to a temperature of $37.0 \pm 0.5^\circ\text{C}$.

DISSOLUTION TEST OF TABLETS

In vitro dissolution studies of CTL tablets was carried out in 900mL of 0.1N HCl using USP type-II (paddle method) Dissolution Rate test apparatus (DISSO 8000, Lab India). The tablet was directly placed in the dissolution medium. A speed of 100

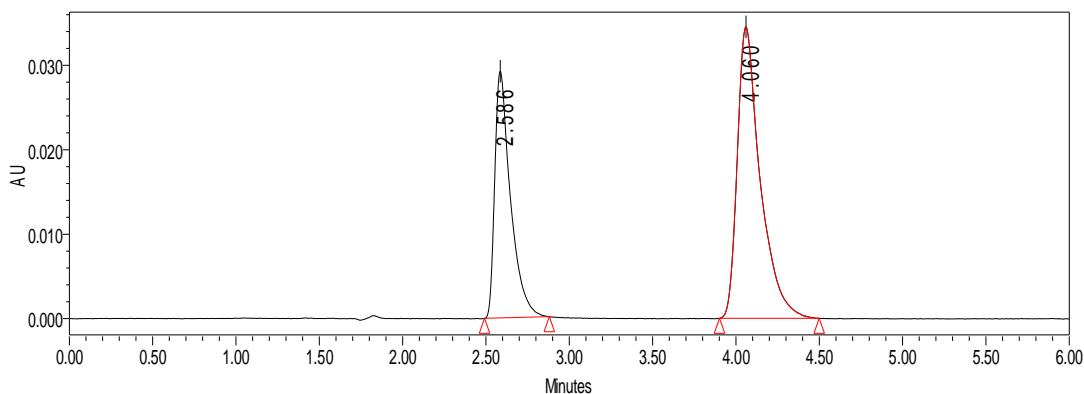
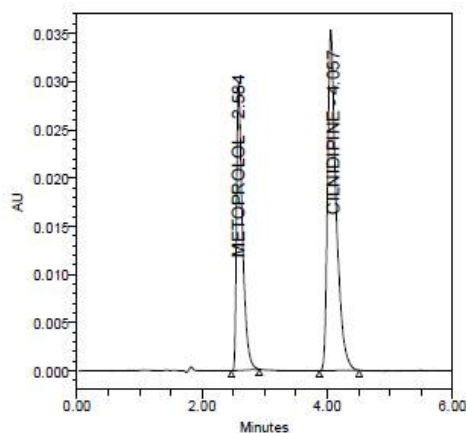
rpm and a temperature of $37 \pm 0.5^\circ\text{C}$ were used in the test. A 5mL aliquot was withdrawn at different time intervals and replaced with 5mL of fresh dissolution medium; the samples were filtered using a $0.45\mu\text{m}$ nylon disc filter. The filtered samples were suitably diluted if necessary and analysed by HPLC method as described above.

RESULTS AND DISCUSSION**SYSTEM SUITABILITY****RESULT**

Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Table 1: System suitability data of Metoprolol and Cilnidipine

Parameter	Metoprolol	Cilnidipine	Acceptance criteria
Retention time	2.584	4.057	± 10
Theoretical plates	3666	4514	>2500
Tailing factor	1.52	1.44	<2.00
% RSD	0.11	0.07	<2.00

**Fig. 1: Chromatogram for optimized method****Fig. 2: System suitability chromatogram of Metoprolol and Cilnidipine**

**SPECIFICITY
RESULT**

Chromatograms explain that retention time for standard, sample and commercial product of Metoprolol and Cilnidipine are same. This proves that, excipients have no effect on the

analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective (Table 2).

3. ACCURACY (Table 3-6)**Table 2: Specificity data for Metoprolol and Cilnidipine**

S no	Sample name	Metoprolol area	Rt	Cilnidipine Area	Rt
1	Standard	197383	2.597	338937	4.056
2	Sample	193681	2.587	332082	4.134
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Table 3: Accuracy data for Metoprolol

S.NO	Accuracy level	injection	Sample area	RT
1	50%	1	97327	2.592
		2	97402	2.589
		3	97269	2.587
		4	97327	2.592
		5	97402	2.589
		6	97269	2.587
2	100%	1	192797	2.586
		2	193681	2.587
		3	192574	2.587
3	150%	1	291144	2.588
		2	291981	2.588
		3	291438	2.586
		4	291144	2.588
		5	291981	2.588
		6	291438	2.586

Table 4: Accuracy (%recovery) results of Metoprolol

Spiked Level	Sample Weight	µg/ml added	µg/ml found	% Recovery	% Mean
50%	59.05	49.500	49.68	100	100
50%	59.05	49.500	49.72	100	
50%	59.05	49.500	49.65	100	
100%	118.10	99.000	98.41	99	100
100%	118.10	99.000	98.86	100	
100%	118.10	99.000	98.29	99	
150%	897.99	297.000	177.15	100	100
150%	897.99	297.000	177.15	100	
150%	897.99	297.000	177.15	100	

Table 5: Accuracy data for Cilnidipine

S.NO	Accuracy level	Sample name	Sampl area	RT
1	50%	1	166619	4.080
		2	166961	4.081
		3	166454	4.090
		4	166619	4.080
		5	166961	4.081
		6	166454	4.090
2	100%	1	332502	4.111
		2	332082	4.134
		3	332630	4.161
3	150%	1	499435	4.185
		2	499199	4.210
		3	499115	4.234
		4	499435	4.185
		5	499199	4.210
		6	499115	4.234

Table 6: Accuracy (%recovery) results of Cilnidipine

Spiked Level	Sample Weight	$\mu\text{g/ml}$ added	$\mu\text{g/ml}$ found	% Recovery	% Mean
50%	59.05	10.000	9.98	100	100
50%	59.05	10.000	10.00	100	
50%	59.05	10.000	9.97	100	
100%	118.10	20.000	19.92	100	100
100%	118.10	20.000	19.89	99	
100%	118.10	20.000	19.93	100	
150%	177.15	30.000	29.92	100	100
150%	177.15	30.000	29.91	100	
150%	177.15	30.000	29.90	100	

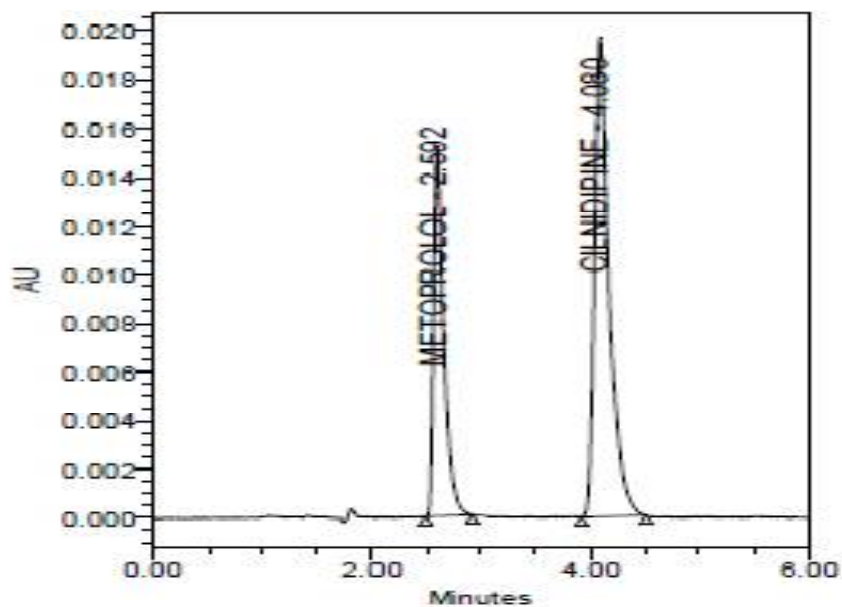


Fig. 3: Typical chromatogram for Accuracy 50 %

RESULT

Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount.

% Recovery was 100.00% for Metoprolol and 100.00% for Cilnidipine. All the results indicate that the method is highly accurate.

4. PRECISION:**RESULT**

Results of variability were summarized in the above table. % RSD of peak areas was calculated

for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise.

LINEARITY**RESULT**

A linear relationship between peak areas versus concentrations was observed for Metoprolol and Cilnidipine in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both Metoprolol and Cilnidipine which prove that the method is linear in the range of 50% to 150%.

Table 7: Precision data for Metoprolol

S.no	RT	Area	%Assay
Injection1	2.587	194398	99
Injection2	2.584	194595	99
Injection3	2.587	194688	99
Injection4	2.584	194200	99
Injection5	2.587	194434	99
Injection6	2.587	194797	99
Mean			99
Std. Dev.			0.11
% RSD			0.11

Table8: Precision data for Cilnidipine

S.no	RT	Area	%Assay
Injection1	4.056	332660	100
Injection 2	4.057	332399	100
Injection 3	4.060	332242	100
Injection 4	4.058	332471	100
Injection 5	4.066	332882	100
Injection 6	4.056	332281	100
Mean			100
Std. Dev.			0.07
%RSD			0.07

Table 9: Linearity data for Metoprolol

S.no	Conc($\mu\text{g/ml}$)	RT	Area
1.	50	2.587	97675
2.	75	2.584	145044
3.	100	2.587	194470
4.	125	2.584	242957
5.	150	2.587	291390
Correlation coefficient (r^2)			0.999

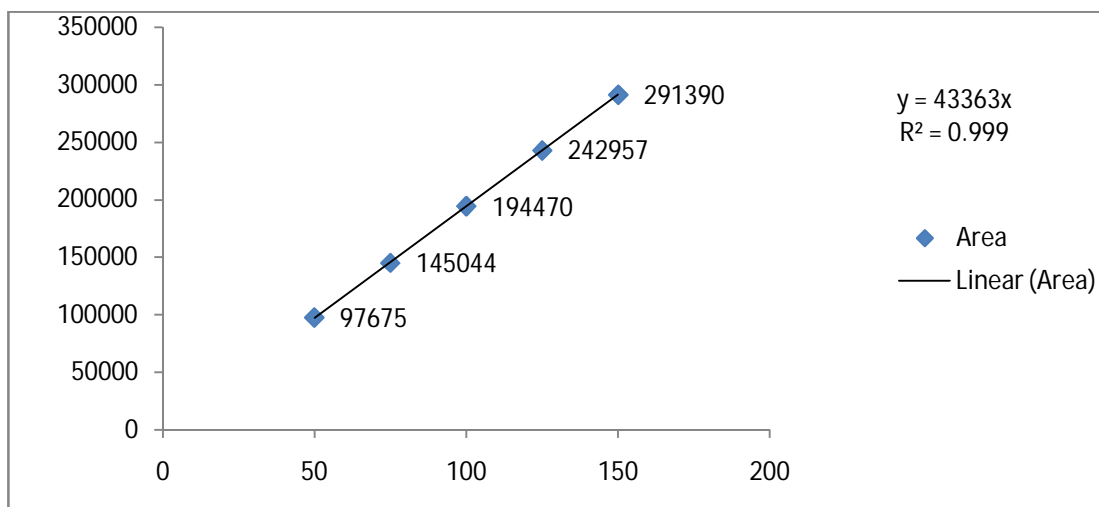


Fig. 4: Linearity plot of Metoprolol

Table 10: Linearity data for Cilnidipine

s.no	Conc(µg/ml)	RT	Area
1.	50	4.056	164470
2.	75	4.057	249362
3.	100	4.060	332541
4.	125	4.058	416018
5.	150	4.066	499411
Correlation coefficient (r ²)			0.999

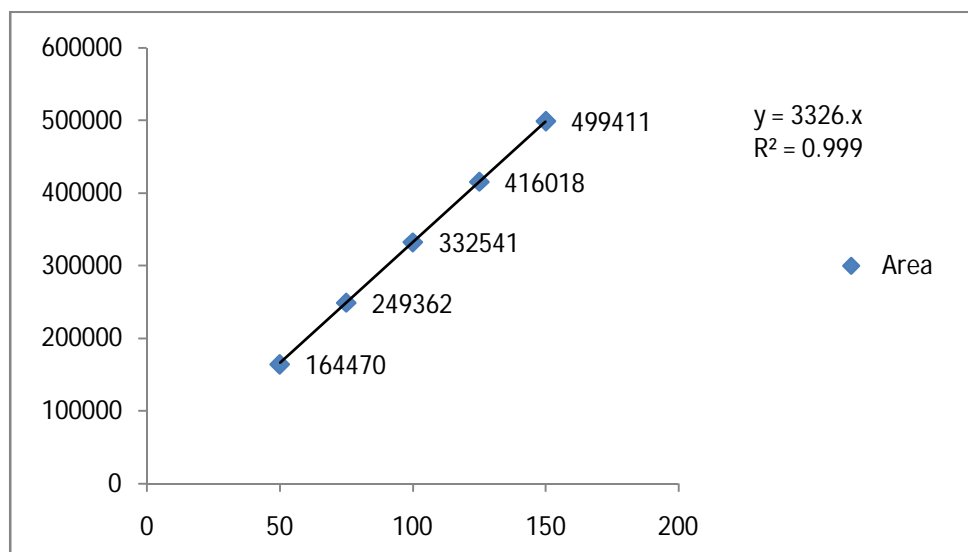


Fig. 5: Linearity plot of Cilnidipine

Dissolution studies

In vitro dissolution studies of combination of Metoprolol succinate and cilnidipine tablets was carried out in 900mL of PH 6.8 buffer using USP

type-II (paddle method) Dissolution Rate test apparatus (DISSO 8000, Lab India). The tablet was directly placed in the dissolution medium. A speed of 100 rpm and a temperature of $37 \pm 0.5^\circ\text{C}$ were

used in the test. A 5mL aliquot was withdrawn at different time intervals and replaced with 5mL of fresh dissolution medium; the samples were filtered using a 0.45 μ m nylon disc filter. The filtered samples were suitably diluted if necessary and analysed by HPLC method as described above. The percent release of drug was found to be within the limits, indicating that the present LC conditions can be used for the dissolution analysis of Metoprolol succinate and cilnidipine in different commercially available formulations.

Acceptance Criteria

The percentage release of drug from the formulation should be more than 85% within 30 minutes as per the ICH Guidelines.

Observation

The percentage of drug release for Metoprolol succinate and cilnidipine was found to be 95.72% within 30 minutes (Table 11 & Fig. 6).

SUMMARY AND CONCLUSION

The study is focused to develop and validate HPLC methods for simultaneous determination Metoprolol Succinate and Cilnidipine in dissolution test of the formulation.

The dissolution test developed and validated for Metoprolol Succinate and Cilnidipine tablets was considered satisfactory. The conditions that allowed the dissolution determination were 900ml of pH 6.8 at 37°C \pm 0.5°C, paddle apparatus, 100 rpm stirring speed and filtration with quantitative filter. In these conditions, the Metoprolol Succinate and Cilnidipine was more stable. It can be concluded that the proposed method was fully validated and it was found to be simple, sensitive, accurate, precise, reproducible and relatively inexpensive and they gave an acceptable recovery of the analyte. Hence, the developed method can be recommended for routine quality control analysis of Metoprolol Succinate and Cilnidipine in tablet formulation.

Table 11: Dissolution parameters

S.NO	Time (min)	Peak area	Concentration (μ g/mL)	Cum drug release	% of drug release	% of drug unreleased
1.	0	-	-	-	-	-
2.	5	20753	13.41	13.41	36.12	63.88
3.	10	32979	16.88	16.94	57.56	42.44
4.	15	41509	17.86	18.01	72.15	27.85
5.	30	46556	17.93	18.17	81.06	18.94
6.	45	50663	18.72	19.05	88.55	11.45
7.	60	54753	13.12	19.51	95.72	4.28

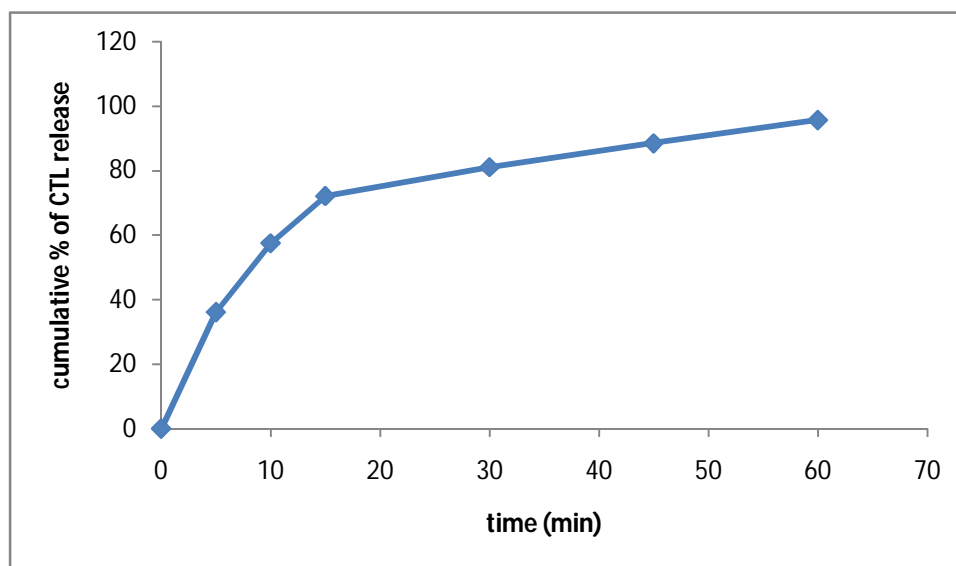


Fig. 6: Dissolution profile of Metoprolol succinate and cilnidipine

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