MANTECH Publications

Journal of Pharmaceutical Analysis and Drug Research
Volume 2, Issue 1, January-April, 2020

Stability Study of Cilnidipine by UV-Visible Spectrophotometric Method using Quality by Design Approach

Dr. Pintu B Prajapati[1*], Hitika B. Patel[1], Dr. Kunjan Bodiwala[1], Dr. Shailesh Shah[2]

Assistant Professor[1], Professor[2]

Department of Quality Assurance

Maliba Pharmacy College, Maliba Campus, Uka Tarsadia University, Bardoli, Gujarat, India

Corresponding Author's email id: *pintu.prajapati@utu.ac.in[1*]*

Abstract

Cilnidipine is used for management of hypertension for end-organ protection. A specific, precise, accurate and robust UV-visible spectrophotometric method was developed and validated for stability study of cilnidipine in acidic and alkaline medium. Forced degradation studies have been carried out in alkaline, acidic and oxidative conditions. The measurement of cilnidipine was carried out at 240 nm. Proposed spectrophotometric method was validated for its accuracy, precision, linearity and range, robustness, specificity as per ICH guideline Q2 (R1). 33 full factorial design was implemented for robustness studies where scanning pitch, scanning speed and wavelength were selected as factors and absorbance was taken as the response for experimental runs suggested by the software. The developed method is linear in the range of 2 to 12 µg/ml with correlation coefficient being 0.9980. The % relative standard deviation for inter and intraday precision were below 2%. Developed method was applied for estimation of Cilnidipine in pharmaceutical dosage form and degradation kinetic study of cilnidipine in acidic and alkaline medium using full factorial design. 32 full factorial design was implemented for determination of relationship between critical variables temperature and strength of acid and alkali with critical quality attributes % degradation, rate constant, half-life. Using response surface mathematical model, % degradation, rate constant and half-life of cilnidipine were predicted at different degradation conditions. The both hydrolytic reactions were found to follow zero order degradation kinetics.

Keywords: *- stability indicating, cilnidipine, full factorial design, degradation kinetics, UV-Visible spectrophotometry*

INTRODUCTION

Cilnidipine dilates the afferent arteriole mainly by blocking L-type calcium channels and dilates the efferent arteriole mainly by blocking N-type calcium channels. Therefore, cilnidipine may lower glomerular pressure and have a renal protective effect and used as an antihypertensive[1]. (See figure 1)

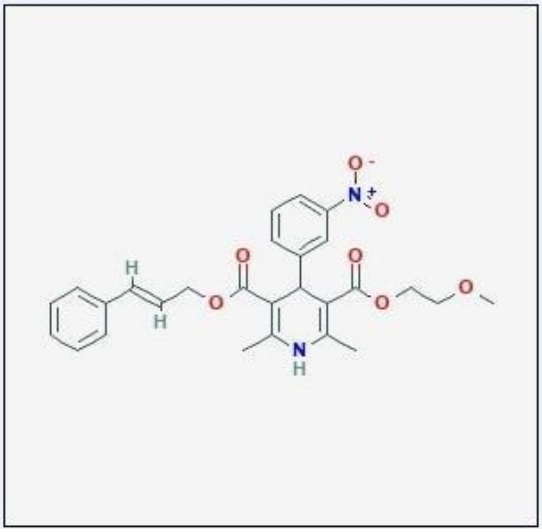

Fig. 1 Chemical Structure of Clindipine

Degradation kinetic studies gives the information regarding the rate of process that generally leads to the inactivation of drug through either decomposition or loss of drug by conversion to a less favorable physical or chemical form. Hence, study of degradation kinetic is important for development of stable formulation, determining optimum storage conditions, selecting the proper container for dispensing and predicting shelf life of drug.

Quality by Design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The concept of QbD applied to analytical method development is known as Analytical Quality by Design (AQbD). A current trend among pharmaceutical industry is to implement AQbD as a part of risk management, and pharmaceutical quality system and pharmaceutical development.[2,3]

An extensive literature review reveals that cilnidipine is official in Japanese Pharmacopoeia and several chromatographic and spectrophotometric methods have been developed for estimation of cilnidipine in its pharmaceutical formulations but no stability indicating spectrophotometric

method is available till date for estimation of cilnidipine in presence of degradation products. Literature review also not described about the degradation kinetic studies using spectrophotometric method in acidic and alkaline medium.[4-24]

UV-Visible spectrophotometric method is quick, less complicated and inexpensive compare to other chromatographic techniques for the stability study of drug molecules and the instrument is present in almost all analytical laboratories which provide almost similar level of accuracy in results as that of other chromatographic techniques.[25]

Therefore it was thought of interest to develop and validate a stability indicating UV-Visible spectrophotometric method for estimation of cilnidipine and expand the study further for degradation kinetic study of cilnidipine in acidic and alkaline conditions using QbD approach.

EXPERIMENTAL INVESTIGATION

Instrumentation

UV-Visible Spectrophotometer: Shimadzu UV-1800, Software: UV Probe Version 2.34, two matched quartz cells with 1 cm light path, RA series REPTECH analytical balance. Design Expert® software, trial version 11.0.4.0

Chemicals and reagents

Cinidipine was procured as a gift sample from Nikshan Pharmaceuticals, Ankleshwar, Methanol was purchased from SD Fine Chemicals Limited, Mumbai, Cilnical 10 (J.B Chemicals, Daman) tablets were purchased from local market.

Preparation of solutions

a) Preparation of standard stock solution of cilnidipine

Accurately weighed 25 mg of cilnidipine was transferred to 25 ml volumetric flask and diluted upto the mark with methanol to get stock solution having strength 1000 µg/ml.

b) Preparation of working standard solution of cilnidipine.

The aliquot of 1 ml from stock solution was transferred to 10 ml volumetric flask and diluted up to the mark with methanol to get solution having strength of 100µg/ml. From the above solution 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with methanol to get final concentration of 10µg/ml.

Preparation of force degraded sample solution

a) Acid degradation

25 mg of drug was transferred to 25 ml volumetric flask and diluted up to the mark with methanol. From above solution 12.5 ml was transferred to 200 ml volumetric flask and 12.5 ml of 1.0 N HCl was added. The solution was heated at 80°C for 5 hours in closed condition. The solution was cooled, transferred and diluted upto mark with methanol in 25 mL volumetric flask. From the above degraded sample, 2 ml solution was transferred to 10 ml volumetric flask and neutralized with 0.5 N NaOH and diluted up to the mark with methanol. From above solution1 ml aliquot was transferred to 10 ml volumetric flask and diluted up to the mark with methanol.

b) Alkaline degradation

25 mg of drug was transferred to 25 mL volumetric flask and diluted up to the mark with methanol. From above solution 12.5 mL was transferred to 200 ml volumetric flask and 12.5 mL of 1.0 N NaOH was added and heated at 80° C for 4 hr. The solution was cooled, transferred and diluted upto mark with methanol in 25 mL volumetric flask. From the above degraded sample, 2 mL solution was transferred to 10 ml volumetric flask and neutralized with 0.5 N HCl and diluted up to the mark with methanol. From above solution, 1 mL aliquot was transferred to 10 mL volumetric flask and diluted up to the mark with methanol.

c) Oxidative degradation

25 mg of drug was transferred to 25 mL volumetric flask and diluted up to the mark with methanol. From the stock solution 12.5 ml was transferred to 200 ml volumetric flask and 12.5 ml of 1% H2O2 was added and heated at 80° C for 2 hours. The solution was cooled, transferred and diluted upto mark with methanol in 25 mL volumetric flask. From the above degraded sample, 2 ml solution was transferred to 10 ml volumetric flask and diluted up to the mark with methanol. 1 ml aliquot from above solution was transferred to 10 ml volumetric flask and diluted up to the mark with methanol.

Selection of wavelength

The working standard solution of cilnidipine having concentration of 10 µg/ml and forced degraded samples were scanned in the range of 200-400 nm keeping methanol as a blank and the wavelength showing the maximum absorbance of cilnidipine without

interference of degradation products was selected as the wavelength for stability study of cilnidipine.

Calibration curve for cilnidipine

Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml from solution having concentration of 100 µg/ml were transferred into 10 ml volumetric flask and diluted up to the mark with methanol to get solutions having strength of 2-12 µg/ml respectively. Absorbance of each solution was measured at detection wavelength for plotting graph of absorbance vs. concentration.

Method validation[26]

For specificity study, spectrum of cilnidipine from tablet dosage form was overlaid with that of standard to confirm purity of cilnidipine. Linearity and range was obtained by repeating the procedure for calibration curve five times. Repeatability of method was determined by performing seven replicates analysis of the same working solution (10µg/ml). For intraday precision, procedure for calibration curve was repeated three times in three replicates on same day for intra-day precision. For inter-day precision, procedure for calibration curve was repeated three times in three replicates on three consecutive days. Accuracy of the method was performed by spiking 80, 100, and 120% of the standard cilnidipine with preanalysed sample of cilnidipine in three replicates. Robustness study was carried out using 3^3 full factorial design where scanning speed (low, medium, high), wavelength (238 nm, 240 nm, 242 nm), and scanning pitch (0.05, 0.1, 0.2) were chosen as factors and working standard solution was analysed according to experimental runs suggested by the software. LOD and LOQ for the method was determined by using equation as specified in ICH Q2 (R1) guidelines which are as follows

$$LOD = \frac{3.3 \times deviation\ of\ y - intercepts\ of\ regression\ lines}{slope\ of\ the\ calibration\ curve}$$

$$LOQ = \frac{10 \times deviation\ of\ y - intercepts\ of\ regression\ lines}{slope\ of\ the\ calibration\ curve}$$

Assay of marketed formulation

Twenty tablets were weighed and finely powdered. The quantity of the powder equivalent to 10 mg of cilnidipine was transferred to 10mL volumetric flask. The flask was filled to about 80 % with methanol, sonicated for 10 min and diluted to mark with methanol. The solution was filtered through Whatman filter no.41. From the above solution, 1 mL was transferred into the 10mL volume flask

and diluted to mark with methanol. Aliquot of 0.6 mL of previous solution was further diluted upto 10 mL with methanol. Absorbance of resulting solution was measured at 240 nm using UV-Visible spectrophotometer using methanol as blank. The amount of cilnidipine present in marketed formulation was determined by fitting absorbance values into the equation representing calibration curve of cilnidipine.

Degradation kinetic study of cilnidipine in acidic and alkaline medium

Degradation kinetic study was carried out in acidic (HCl) and alkaline (NaOH) medium. 3^2 full factorial design was applied where three levels of temperature (30, 40, 50°C) and strength of HCl and NaOH (0.01, 0.05, 0.1 N) for acid and alkaline degradation kinetic studies of cilnidipine using design expert software (trial version) and concentrations were found out using regression line equation. The degradation rate constant, % Degradation and half-life were studied to study the effect of temperature and strength of HCl and NaOH on stability of cilnidipine. Nine experimental runs were performed in laboratory and measured responses were placed in software against respective experimental runs for ANOVA and response surface analysis.

Procedure for experimental run suggested by design expert software*:*

a) Acid degradation

From stock solution, 12.5 ml was withdrawn and transferred in series six volumetric flasks of 200 mL, 12.5 ml of 0.01 N HCl was added in each flask and allowed to degrade at 40°C. For zero minute reading, solution was transferred, cooled and diluted upto mark with methanol in 25 mL volumetric flask. From the above sample solution, 2 ml aliquot was transferred, neutralized and diluted up to 10 ml with methanol. Aliquot of 1mL of resulting solution was diluted upto mark with methanol, absorbance measured at 240 nm and concentration determined using regression line equation of calibration curve data. At interval of 30 min one degraded sample was withdrawn and treated same as described for zero minute reading up to 2 hr and 30 min. Same procedure was repeated for all suggested conditions as shown in table 1. The order of hydrolytic reactions was determined by graphical method and rate constant, half-life and % degradation were calculated.

b) Alkaline degradation

From stock solution 12.5 ml was withdrawn and transferred to 200 ml volumetric flask, 12.5 ml of 0.01 N NaOH was added and allowed to degrade at 40°C. For zero minute reading, solution was transferred, cooled and diluted upto mark with methanol in 25 mL volumetric flask. From the degraded sample solution, 2 ml aliquot was withdrawn, neutralized and diluted to 10 ml with methanol. Aliquot of 1mL of resulting solution was diluted upto mark with methanol, absorbance measured at 240 nm and concentration determined using regression line equation of calibration curve data. At interval of 30 min one degraded sample was withdrawn and treated same as described for zero minute reading up to 2 hr and 30 min. Same procedure was repeated for all suggested conditions as shown in table 1. The order of hydrolytic reactions was determined by graphical method and rate constant, half-life and % degradation were calculated.

Table 1. Degradation kinetic conditions for acid and alkaline hydrolysis

Temperature	Concentration of HCl / NaOH	Time points for collection of samples (min)
30±2°C	0.01 N	0, 30, 60, 90, 120, 150
	0.05 N	0, 30, 60, 90, 120, 150
	0.1 N	0, 30, 60, 90, 120, 150
40±2°C	0.01 N	0, 30, 60, 90, 120, 150
	0.05 N	0, 30, 60, 90, 120, 150
	0.1 N	0, 30, 60, 90, 120, 150
50±2°C	0.01 N	0, 30, 60, 90, 120, 150
	0.05 N	0, 30, 60, 90, 120, 150
	0.1 N	0, 30, 60, 90, 120, 150

RESULT AND DISCUSSION

Selection of wavelength for detection:

Working standard solution of cilnidipine and forced degraded samples of all three conditions were scanned in range of 200-400 nm using methanol as blank. Spectrum of standard cilnidipine was overlaid with that of all forced degraded samples (see figure 2) to determine wavelength for interference free quantitation of cilnidipine in presence of degradation products formed in all three stress conditions. All three forced degraded samples showed negligible absorbance of cilnidipine in range of 200-400 nm that indicated cilnidipine was completely degraded (see table 2) and degradation products formed in all three different conditions did not show significant absorbance in same range. If focused on spectrum of standard cilnidipine that showed maximum absorbance at 240 nm hence it was selected as wavelength for interference free quantitation in stability study of cilnidipine in different stress conditions as suggested in regulatory guidelines.

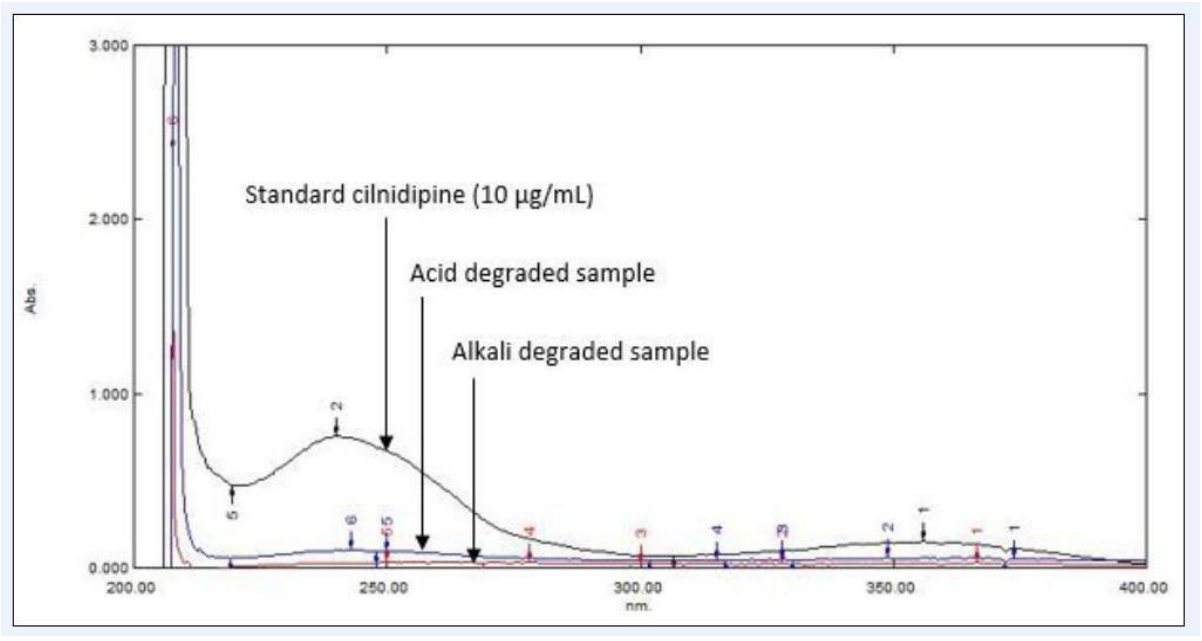

Fig. 2

Table 2. Results for force degradation studies

Force degradation condition	Condition	Initial concentration do cilnidipine (µg/ml)	Final concentration of cilnidipine (µg/ml)	% Degradation
Acidic hydrolysis (1.0N HCl)	5 hrs heating, 80˚C	10	-	100%
Alkaline hydrolysis (1.0N NaOH)	4 hrs heating, 80˚C	10	-	100%
1.0 % H2O2	2 hrs heating, 80˚C	10	-	100%

Solution stability study:

For to check stability of working standard solution, initial absorbance of working standard solution was measure at 270 nm and compared with absorbance of the same after 24 hours. The results showed negligible change in absorbance and spectrum of cilnidipine with respect to time duration of 24 hours that indicated working standard solution is stable in methanol upto 24 hours.

Preparation of calibration curve:

After selection of wavelength for quantitation of cilnidipine, absorbance of different standard solutions of cilnidipine in concentration range of 2-12µg/mL were measure at 240 nm to check linearity of absorbance with respect to concentration to plot calibration curve of absorbance verses concentration of cilnidipine. Calibration curve was found linear with respect to concentration of cilnidipine in range of 2-12 µg/ml.

Method validation

For specificity study of developed method, spectrum of degraded samples and sample from tablet dosage form were overlaid with that of standard cilnidipine. The absorbances of standard and sample cilnidipine were found to be 0.784 ± 0.002. The spectrum of sample cilnidipine showed good co-relation with spectrum of cilnidipine and spectrum of degraded samples had negligible absorbance in

range of 200-400 nm that indicated degradation products and excipients of formulation did not interfere in quantitation of cilnidipine. Hence developed method has applicability in estimation of cilnidipine in tablet dosage forms and stability study in deferent stress conditions. The developed method was found to be linear in the concentration range of 2-12 µg/ml with correlation coefficient of 0.9980. % RSD of repeatability were found to be 0.14%. % RSD of inter-day and intra-day precision study were found to be 0.57 - 1.87 % and 0.41- 1.11% respectively. LOD and LOQ were found to be 0.02µg/ml and 0.06µg/ml respectively. % RSD for all precision study were found less than 2% that implies developed method is precise. % recovery data was in the range of 98.50 - 99 % indicates method is accurate. The factors selected for robustness study were found to be insignificant as the values of "Prob > F" are greater than 0.050 which indicates that no significant changes on absorbance was observed in given ranges of scanning speed, scanning pitch, and wavelength (see table 3.) hence method is robust. Summary of validation parameter is depicted in table 4.

Table 3. ANOVA table for robustness study

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.559E-006	4	6.397E-007	2.11	0.1004	Not Significant
A-wavelength	6.050E-007	1	6.050E-007	2.00	0.1664	
B-scanning pitch	1.800E-007	1	1.800E-007	0.60	0.4458	
C- scanning speed	1.774E-006	2	8.869E-007	2.93	0.0669	
Residual	1.028E-005	34	3.025E-007			

Table 4. Summary of validation parameters

Validation parameters	Results
Linearity range	2 - 12 µg/ml
Regression coefficient (R^2)	0.9980
% Recovery	98.50 - 99%
Precision (%RSD[a])	
Repeatability (n=7)	0.14
Intra-day precision (n=3)	0.41-1.11
Inter-day precision (n=3)	0.57 – 1.87
Limit of Detection (LOD)	0.02 µg/ml
Limit of Quantitation (LOQ)	0.06 µg/ml
Specificity	Specific

Assay of marketed formulation

The developed method was applied for estimation of cilnidipine in marketed formulation. The percentage of cilnidipine in marketed formulation was calculated from calibration curve and % assay was found to be 99.17% as shown in table 5.

Degradation kinetic study of cilnidipine in acidic medium and alkaline medium

3^2 full factorial design was applied for response surface analysis to determine relationship of strength of HCl and NaOH and temperature with critical responses rate constant, half-life and % degradation of cilnidipine. All suggested experimental runs by design expert software were performed in laboratory measured responses were entered in the design expert software (see table 6 and 7) for ANOVA and response surface analysis.

Table 5. Assay of marketed formulation

Amount of drug in tablet power (mg)	Average absorbances (n=3)	Amount of drug found in powder (mg)	Assay (%)
10	0.7938 ± 0.005	9.917	9.17%

Table 6. Experimental runs and responses for degradation kinetic study of cilnidipine in acidic media

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Std.	Temperature (˚C)	Strength of HCl (N)	Rate constant ($K\times10^{-8}$ molar/min)	Half-life (minute)	% Degradation
1	30	0.1	3.05	339.793	18.404
2	40	0.1	3.29	315.253	23.79
3	50	0.1	5.0	207.687	36.111
4	30	0.05	1.99	520.054	14.421
5	40	0.05	2.38	435.649	13.36
6	50	0.05	2.52	410.81	18.256
7	30	0.01	0.59	1784.58	6.958
8	40	0.01	0.96	1077.78	4.202
9	50	0.01	1.34	782.034	9.59

Table 7. Experimental runs and responses for degradation kinetic study of cilnidipine in alkaline media

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Std.	**Temperature (˚C)**	**Strength of NaOH (N)**	**Rate constant ($K\times10^{-8}$ molar/min)**	**Half-life (minute)**	**% Degradation**
1	30	0.1	2.36	407.4	18.404
2	40	0.1	2.99	330	22.725
3	50	0.1	3.13	299.4	25.007
4	30	0.05	1.68	634.8	11.813
5	40	0.05	1.89	561	13.36
6	50	0.05	2.17	477.6	15.686
7	30	0.01	0.53	407.4	4.202
8	40	0.01	1.25	842.4	6.958
9	50	0.01	1.36	740.4	10.126

From data of ANOVA table, main effect of temperature and strength of HCl were found significant for rate constant, half–life and % degradation of cilnidipine in acidic medium. Interaction between two factors was significantly affects responses % degradation, half-life and rate constant. The response surface analysis showed quadratic model for % degradation and half-life and linear for rate constant. The following mathematical models were used for prediction of responses at different stress condition in acid degradation. (See table 8,9,10 and figure 3, 4, 5)

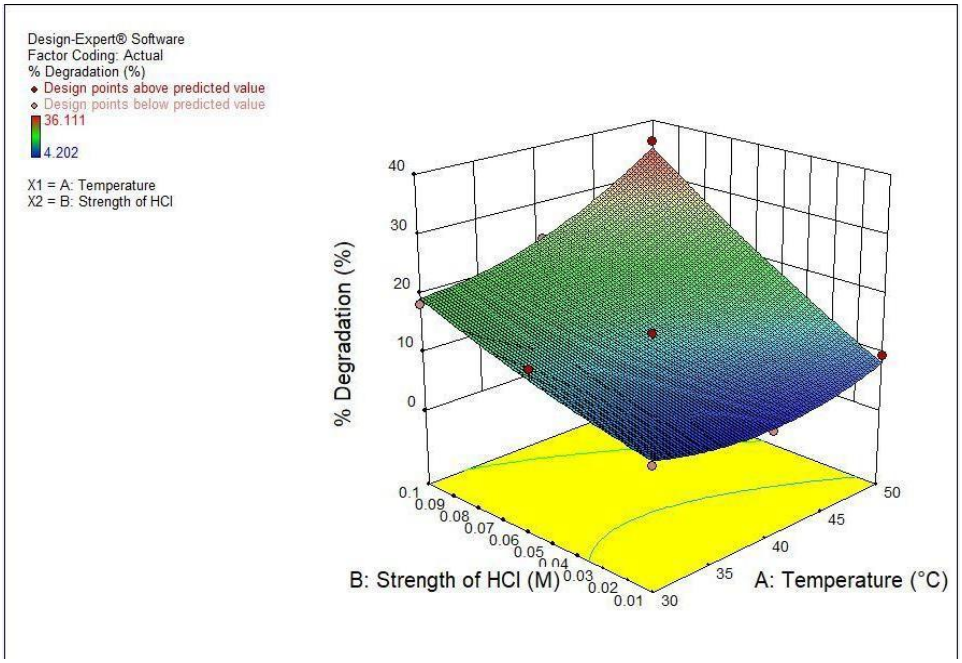

Figure. 3

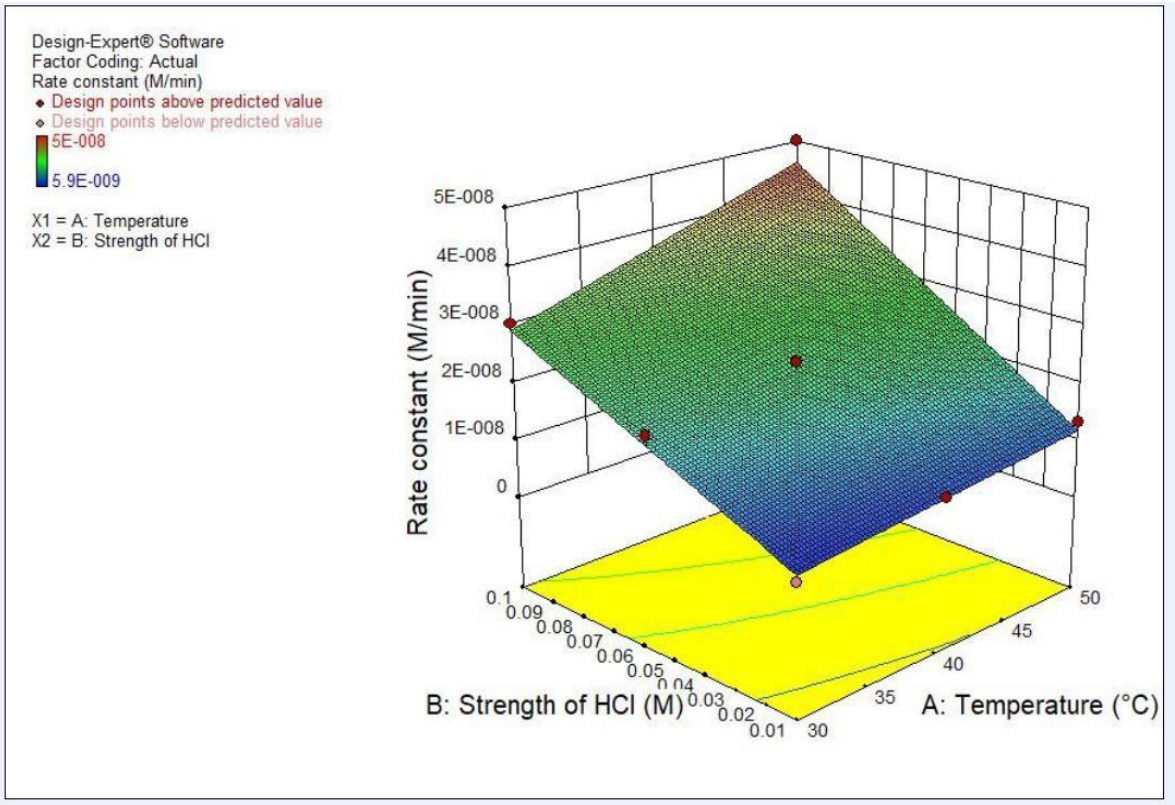

Figure. 4

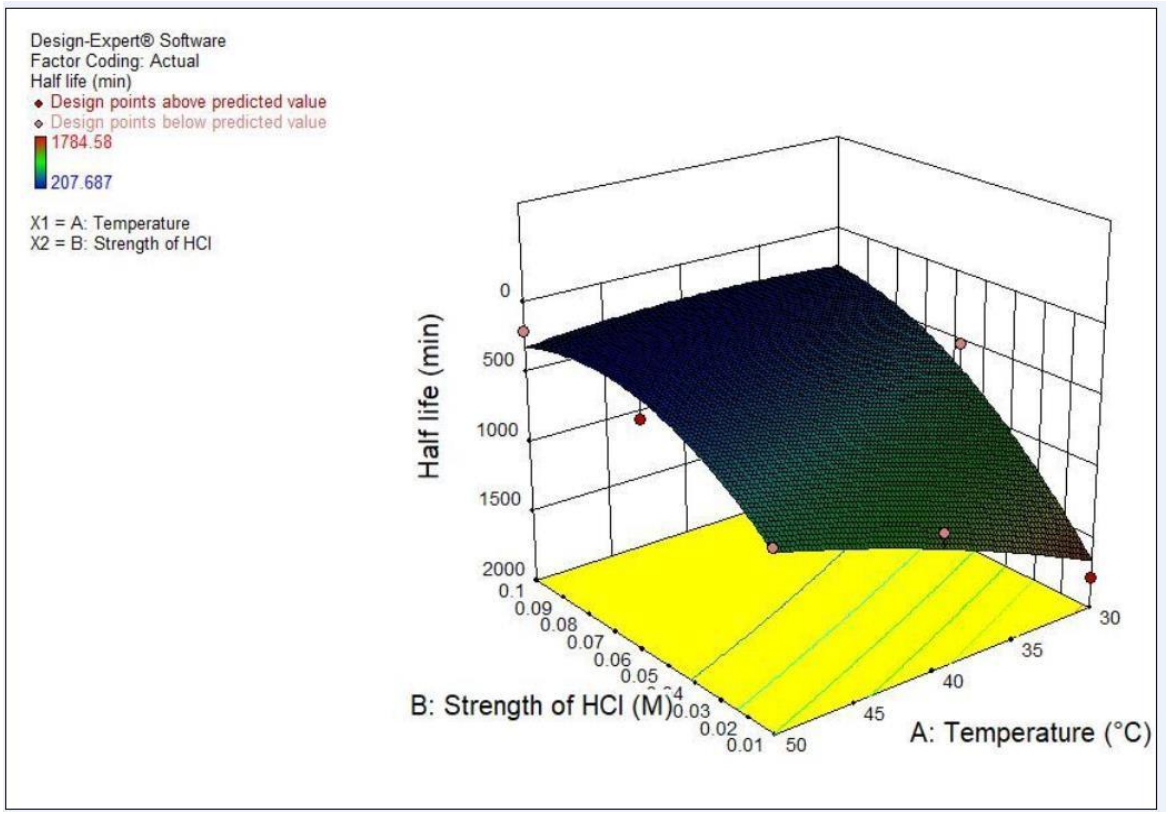

Figure. 5

% degradation = +59.12411 – 2.74611 * Temperature – 177.13116 * strength + 8.37500 * Temperature * Strength + 0.033605 * Temperature^2 + 502.70754 * Strength^2

Rate constant = -5.10399E - 010 + 1.71667E-010 * Temperature + 4.62963E-008 * Strength + 6.66667E-009 * Temperature * Strength

Half-life = +4195.49181 – 91.32424 * Temperature – 45185.82320 * Strength + 483.58000 * Temperature * Strength + 0.54995 * Temperature^2 + 1.41274E+0.005 * Strength^2

From data of ANOVA table main effect of temperature and strength of NaOH were found significant for rate constant, half–life and %degradation of cilnidipine in alkaline medium. Interaction between two factors was found significant for half-life.

The response surface analysis showed linear model for rate constant and half-life, linear and quadratic for % degradation. The following mathematical models were used for prediction of responses at different stress condition in acid degradation.(see table 8,9,10 and figure 6,7,8)

Rate constant = -0.56419 + 0.034833 * Temperature +19.77778 * Strength of alkali

% Degradation = -4.04919 + 0.24850 * Temperature + 91.42648 * Strength of alkali + 0.37722 * Temperature * Strength of alkali + 5.10345E-005 * Temperature^2 + 541.77948 * Strength of alkali^2

Half-life = +56.31573 – 0.93006 * Temperature – 539.77778 * Strength of alkali + 9.72222 * Temperature * Strength of alkali

Table 8. ANOVA table for rate constant

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
Alkaline	Model	5.48	2	2.74	194.27	<0.0001	Significant
	A-Temperature	0.73	1	0.73	51.61	<0.0001	
	B-Strength of NaOH	4.75	1	4.75	336.92	<0.0001	
	Residual	0.14	10	0.014			

Acidic	Model	1.400E-015	3	4.666E-016	68.96	<0.0001	Significant
	A-Temperature	1.739E-016	1	1.739E-016	25.69	0.0007	
	B-Strength of HCl	1.190E-015	1	1.190E-015	175.86	<0.0001	
	AB	3.600E-017	1	3.600E-017	5.32	0.0465	
	Residual	6.090E-017	9	6.767E-018			

Table 9. ANOVA table for Half-life

	Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Alkaline	Model	446.96	3	148.99	13.86	0.0010	Significant
	A-Temperature	93.77	1	93.77	8.72	0.0161	
	B-Strength of NaOH	276.62	1	276.62	25.73	0.0007	
	AB	76.56	1	76.56	7.12	0.0257	
	Residual	96.76	9	10.75			
Acidic	Model	2.050E+006	5	4.100E+005	31.14	0.0001	
	A-Temperature	2.579E+005	1	2.579E+00519.59	19.59	0.0031	
	B-Strength of HCl	1.290E+006	1	1.290E+006	97.94	<0.0001	
	AB	1.894E+005	1	1.894E+005	14.39	0.0068	
	A^2	8353.12	1	8353.12	0.63	0.4519	
	B^2	2.260E+005	1	2.260E+005	17.17	0.0043	
	Residual	92170.83	7	13167.26			

Table 10. ANOVA table for % degradation

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
Alkaline	Model	384.10	5	76.82	189.16	<0.0001	Significant
	A-Temperature	44.83	1	44.83	110.38	<0.0001	
	B-Strength of NaOH	335.25	1	335.25	825.55	<0.0001	
	AB	0.12	1	0.12	0.28	0.6170	
	A^2	7.193E-005	1	7.193E-005	1.771E-004	0.9898	
	B^2	3.32	1	3.32	8.19	0.0243	
	Residual	2.84	7	0.41			
Acidic	Model	754.56	5	150.91	75.84	<0.0001	Significant
	A-Temperature	97.40	1	97.40	48.95	0.0002	
	B-Strength of NaOH	552.10	1	552.10	277.46	<0.0001	
	AB	56.81	1	56.81	28.55	0.0011	
	A^2	31.19	1	31.19	15.67	0.0055	
	B^2	2.86	1	2.86	1.44	0.2694	
	Residual	13.93	7	1.99			

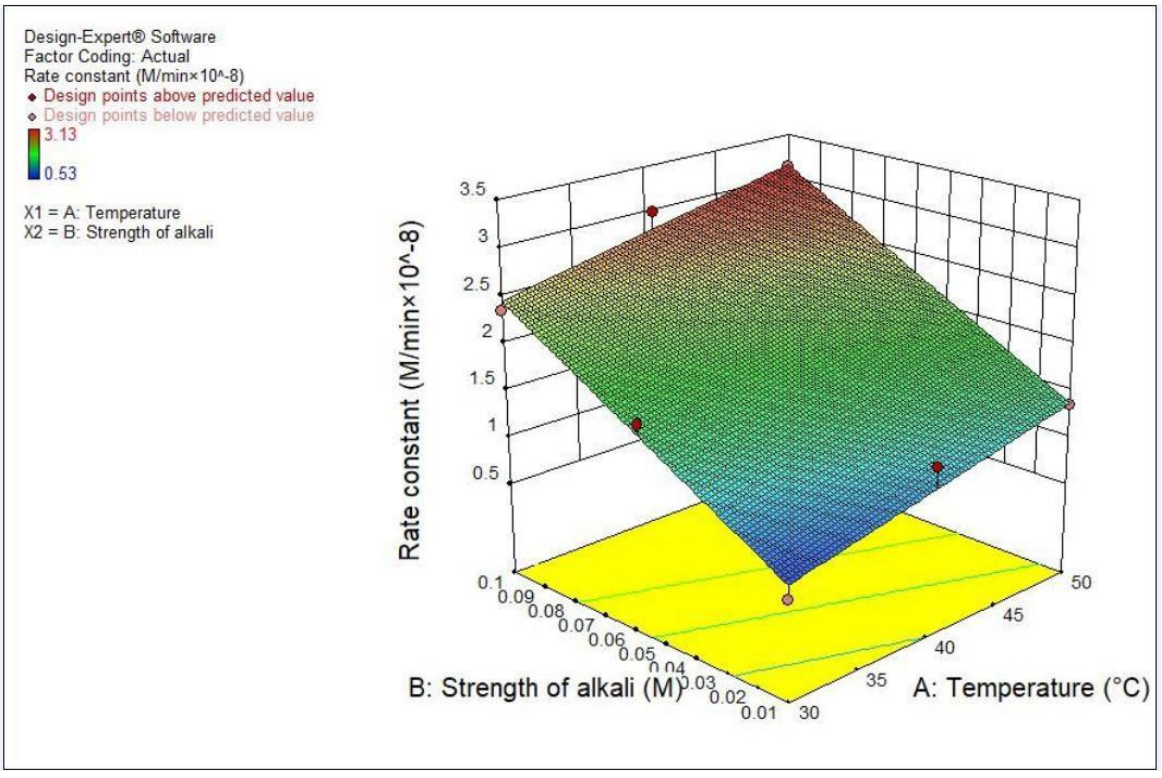

Figure. 6

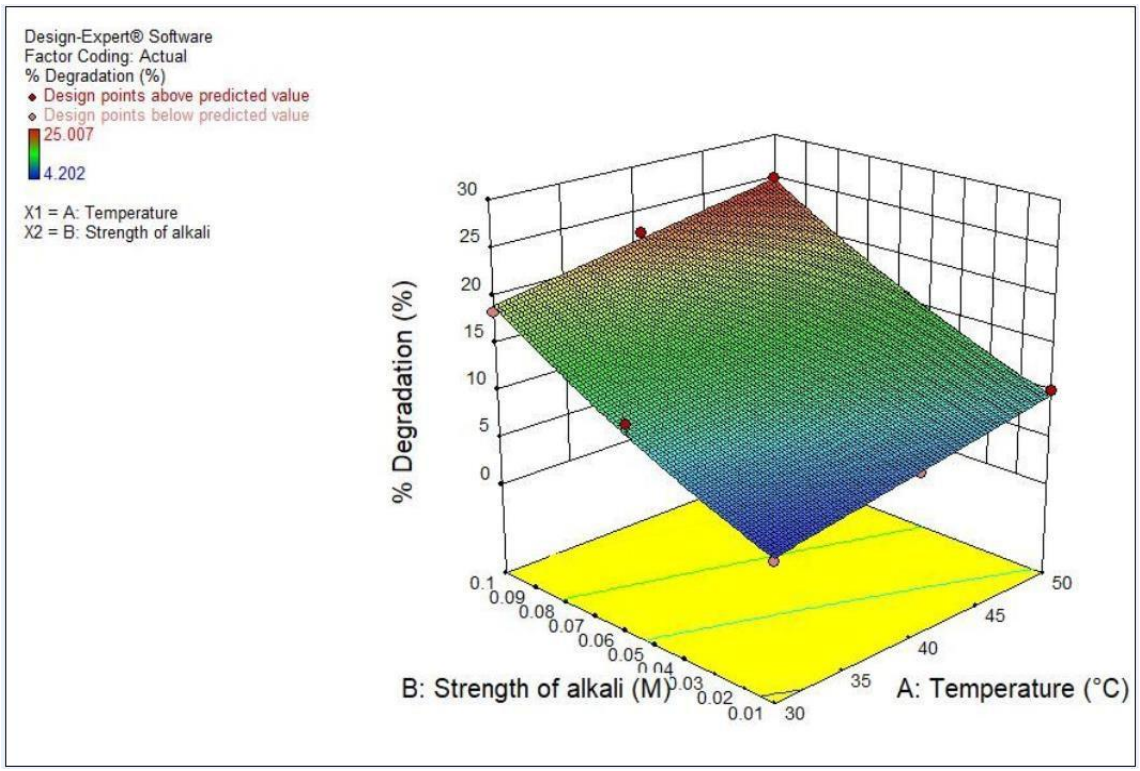

Figure. 7

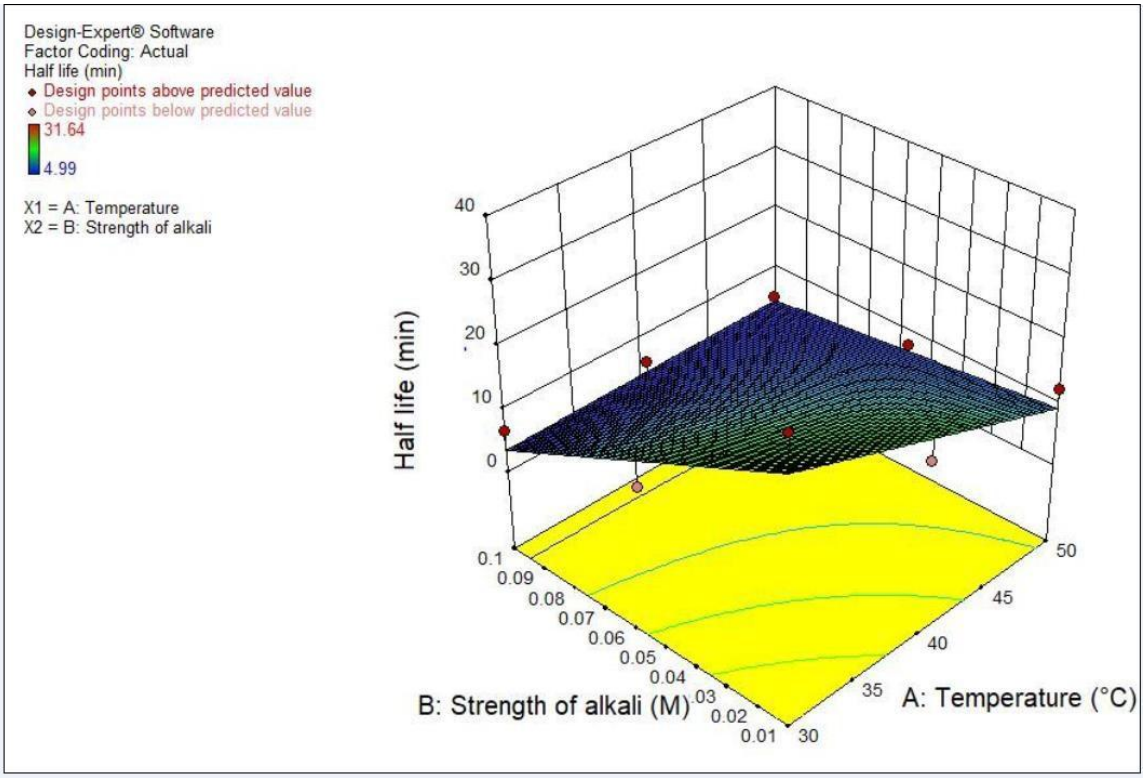

Figure. 8

The predicted R^2 is in reasonable agreement with the adjusted R^2 of all three response surface model for both acid and alkaline degradation that implies predicted responses would be in good agreement with experimental values of all three responses and can be used to navigate design space.

The plot of C v/s Time was found linear for all degradation conditions in both acidic and alkaline mediums which indicates degradation of cilnidipine follows zero order kinetic in acidic and alkaline medium.(see figure 9 and 10) As the temperature and strength of NaOH and HCl increases the rate constant and %degradation increases and the half-life decreases.

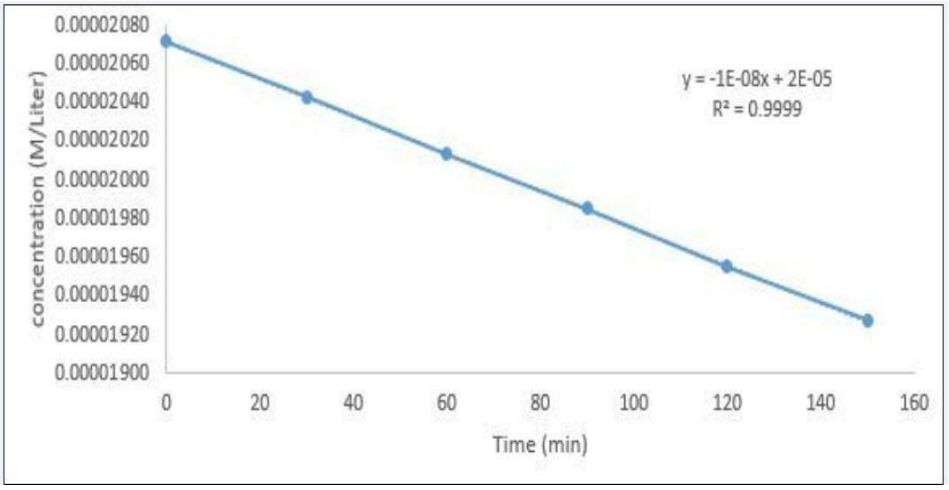

Figure. 9

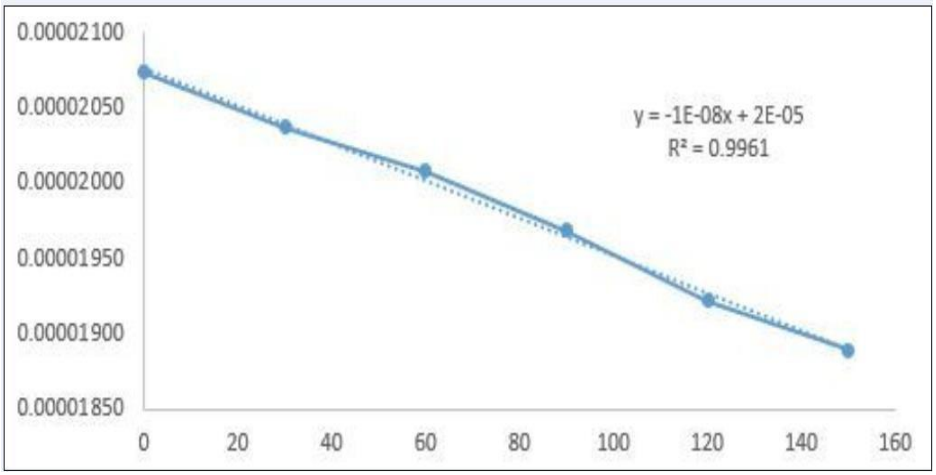

Figure. 10

CONCLUSION

Stability indicating UV-visible spectrophotometric method has been developed for interference free quantitation of cilnidipine in presence of degradation products formed in acidic, alkaline and oxidative stress conditions. As per literature review chromatographic methods were only reported for stability study of cilnidipine in different stress conditions. The developed method offers several advantages for economical, quick, eco-friendly and accurate stability indicating quantitation of cilnidipine over published chromatography method for the same. The developed method was found stability indicating, linear, specific, precise, accurate and sensitive as per ICH guideline. The robustness study using 3^3 full factorial design proved the selected factors (wavelength, scanning speed, scanning pitch) have insignificant affect on absorbance of cilnidipine. The developed analytical method was applied for analysis of marketed tablet formulation and results were in agreement with labeled claim of cilnidipine. 3^2 full factorial response surface model was applied for degradation kinetic study of cilnidipine in different stress conditions like acidic and alkaline. In all degradation conditions cilnidipine follows zero order degradation kinetics.

ACKNOWLEDGEMENT

The authors are thankful to Nikshan Pharmaceuticals, Ankleshwar for providing the gift sample of cilnidipine and Principal of Maliba Pharmacy College for providing the resources for the work.

REFERENCES

I. Tripathi KD. Essentials of Medical Pharmacology.7th ed. New Delhi: Jaypee Brothers Medical Publishers; 2013. pp. 505-508

II. Anderson MJ, Whitcomb PJ. DOE Simplified Practical Tools for Effective Experimental. 3rd ed. CRC Press Taylor and Frances Group, pp. 39 – 69

III. Lewis GA, Mathieu D, Phan-Tan-Luu R. Pharmaceutical Experimental Design. Marcel Dekker, 1999

IV. The Japanese Pharmacopoeia, The Ministry of Health, Labour and Welfare. Seventeenth Edition, 2016; pp. 704 - 705

V. Safi MM. Spectrophotometric Method for the Estimation of Cilnidipine in Bulk and

Pharmaceutical Dosage Forms. Oriental Journal of Chemistry. 2013;29(1):131 – 134

VI. Sidhdhapara M, Patel B, Parmar A. Development and Validation of Spectrophotometric Method for Simultaneous Determination of Cilnidipine and Olmesartan Medoximil in Tablet Dosage Form. International Journal of Pharmacy and Biological Sciences. 2014;4(2):97 – 101

VII. Desai Dhwani et al. Method Development and Validation of Cilnidipine and Metoprolol Succinate in Combined Dosage Form. Pharmaceutical methods. 2016; 7(1): 28-34

VIII. Yuusuke Konno et al. Vasodilatory Effect of Cilnidipine, an L-type and N-type Calcium Channel Blocker, on Rat Kidney Glomerular Arterioles. International heart journal. 2008;49(6):723-732

IX. Ahmed Manzoor et al. RP-HPLC Method Development and Validation for Simultaneous Estimation of Cilnidipine and Olmesartan Medoxomil in Combined Tablet Dosage Form. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;4(1):785-795

X. Dr. Madhukar et al. Analytical Method Development and Validation for The Determination of Cilnidipine Tablet Dosage Form by RP-HPLC Techniques. Journal of Pharma Research.

XI. Sunitha N. et al. Method Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Olmesartan and Cilnidipine in Bulk and Formulations. International Journal of Pharmaceutical Research & Allied Sciences. 2015;4(3):127-135

XII. Ruhina T. et al. A Novel RP HPLC Method for Development and Validation of Cilnidipine in Bulk and Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Technology & Innovation. 2017;5(24):72-80

XIII. Shah DM, Doshi BD. Development and Validation of HPTLC Method for Simultaneous Estimation of Nebivolol Hydrochloride and Cilnidipine in Combined Pharmaceutical Tablet Dosage Form. International Journal of Pharma Research & Review, June 2016;5(6):1-7

XIV. Dedhiya PP. et al. Development and Validation of HPTLC Method for Simultaneous Estimation of Olmesartan Medoxomiland Cilnidipine in Their Combined Pharmaceutical Dosage Forms. Journal of Pharmacy and Applied Sciences. 2015;2(2):20-27

XV. Minase A. et al. Developement and Validation of Analytical Method for Simultaneous Estimation of Cilnidipine and Olmesartan Medoxomilin Bulk and Tablet Dosage Form by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(7):508-11

XVI. Jain P. et al. Development and Validation of Spectrophotometric and RP-HPLC Method for Estimation of Olmesartan Medoxomilin Tablet Dosage Form. International Journal of Pharma and Bio Sciences. 2010;1(2):1-7

XVII. Avula S, Naveen K, Ramana V. Validated RP-HPLC method for the estimation of olmesartan informulation. An International Journal of Advances in Pharmaceutical Sciences. 2011;2(3):251-256

XVIII. Lee H, Seo J, Lee H, Jeong S, Cho Y, Lee K. Development of a liquid chromatography/ negative ion electrospray tandem mass spectrometry assay for the determination of cilnidipine in humanplasma and its application to a bioequivalence study. Journal of Chromatography. 2008;862(1):246-51

XIX. Vahora S, Mehta F, Chhalotiya U, Shah D. Dual wavelength spectrophotometric method forestimation of cilnidipine and telmisartan in their combined dosage form. Research & Reviews: Journal of Pharmaceutical Analysis.2014;3(2):22 -29

Science Research. 2010;2(4):307-312

XX. Chaudhari P, Bhalerao A. Method validation for spectrophotometric estimation of cilnidipine. Journal of Chem Tech Research. 2012;1(4):1186-1188

XXI. Parambi D. et al.. A validated HPTLC determination of an angiotensin receptor blocker olmesartan medoxomil from tablet dosage form. International Journal of Pharmaceutical Sciences Review and Research. 2010;4(3):36-39

XXII. Raja B, Rao L. Development and validation of aRP-HPLC method for simultaneous estimation of olmesartan and hydrochlorothiazide in combined tablet dosage form. International Journal of Research in Pharmacy and Chemistry. 2011;1(3):714-717

XXIII. Chabukswar A, Kuchekara B, Jagdalea S, Mehetre D, More A, Lokhandeb P. Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Olmesartan Medoxomil and Amlodipine Besylate in Tablet Dosage Form. Archives of Applied

XXIV. O'Neil MJ. The Merck Index: - An Encyclopedia of Chemicals, Drugs and Biological. 14th ed. Whitehouse Station, NJ, USA: Merck and Co. Inc; 2006. pp.2275, 6839

XXV. Skoog DA, Holler FJ, Crouch SR. Principle of Instrumental Analysis. 6thed. Thomson Higher Education, USA, pp. 336 – 362

XXVI. ICH Harmonized Tripartite Guideline Validation of Analytical Procedures: Text and Methodology, Q2(R1). 2005, 1-17