IMPLEMENTATION OF QBD APPROACH TO THE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CILNIDIPINE AND NEBIVOLOL IN TABLET DOSAGE FORM

Prasanna Ku. Pradhan[1]*, T. Y. Pasha[2], N. M. Mehta[1], Anil Bhandari[1]

Jodhpur National University, Jodhpur[1]
Baroda College of Pharmacy, Vadodara[2]

AUTHOR FOR CORRESPONDENCE :
Prasanna Ku. Pradhan
prasanna.k.pradhan@gmail.com
Mob. No.: 8141865430

ABSTRACT

A Second Derivative UV Spectroscopic method was developed for the analysis of Cilnidipine (CIL) and Nebivolol HCl (NEB) in its combined dosage according to Quality by design (QbD) to ensure predictable quality with desired and predetermined specifications. As per QbD approach variable parameters were identified designed into Ishikawa diagram. Screenings of critical parameters were done by observation as well as Principal Component Analysis. Screened critical parameters for the method were solvent methanol, sample preparation tablet and matrix, wavelength: 232.6 nm for NEB and 263.8 nm for CIL, slit width: 1.0, scan speed medium, sampling interval: 0.2 and amplification factor 500. With the above set of critical parameters validation was performed as per ICH Q2 (R1) guidelines. Method was found to be accurate, precise and hence can be useful for routine analysis of CIL and NEB in tablet dosage form simultaneously.

Keywords : Quality by design, Second Derivative UV Spectroscopic method, Cilnidipine, Nebivolol HCl, Validation.

INTRODUCTION

Quality by Design approach looks into the quality of analytical process during the development stage itself. It states that quality should be built into the process design rather than testing into final results of analytical process [1]. QbD is defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management" [2]. In alignment with the approach proposed in the draft FDA guidance for process validation, a three-stage approach [3] can be applied to method validation.

Stage 1. Method Design.

Define method requirements and conditions and identify critical parameters.

Stage 2. Method Qualification.

Confirm that the method is capable of meeting its design intent.

Stage 3. Continued Method Verification.

Gain ongoing assurance to ensure that the method remains in a state of control during routine use.

A critical function of Stage 1 is the design of an Analytical Target Profile (ATP) for the method. To design the ATP, it is necessary to determine the characteristics that will indicate the methods performance. In this case these selected from the performance characteristics described in ICH Q2 as per the traditional approach [4]. Instead of being applied in a tick box manner, they are investigated by a risk assessment exercise as described in ICH Q9 [5] in combination with carefully designed development studies to identify the critical method and sources of variation [6]. Variables are then investigated by robustness and ruggedness experiments to understand the functional relationship between method input variables and each of the method performance characteristics and the results are compared to the desired outcome defined in the ATP. From this, one can identify a set of operational method controls. Also, having evaluated the critical method parameters and gained a better understanding of the method through structured experimentation, a control strategy can be built into the method to ensure a consistent performance throughout its life cycle [7]. A key advantage of the QbD approach for all of the above situations is the flexibility to perform a qualification against the specific ATP defined for the intended use of the method [8].

Applying the principles of QbD to analytical methods could result in more robust methods which produce consistent, reliable, and quality data throughout the life cycle and in turn will lead to less method incidents when used in the routine environment. This would mean less time spent on investigations and ultimately save time and money.

Cilnidipine (CIL) 1,4- Dihydro- 2,6- dimethyl- 4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2- methoxy ethyl (2E)-3-phenyl-propenyl ester is a novel and unique dihydropyridine calcium channel blocker that possesses a slow-onset, long-lasting vasodilating effect. Nebivolol HCl is, α, α' [Imino bis (methylene) bis [6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol]; α, α'- (imino-dimethylene) bis [6-fluoro-2-chromanmethanol], is a β1-Blockers (Anti-Hypertensive). Nebivolol is the racemate (dl-nebivolol) of the enantiomers l-nebivolol and d-nebivolol. It is a competitive and highly selective beta-1 receptor antagonist with mild vasodilating properties, possibly due to an interaction with the L-arginine/nitric oxide pathway.

Literature survey reveals various method for analysis of NEB [9, 10, 11, 12, 13] and CIL [14,15,16,17,18,19,20,21] but no method reported for simultaneous estimation of this combination in tablet dosage form.

IMPLEMENTATION OF QbD APPROACH

According to ICH Q8 (R2) guidelines, an experimental work was planned and QbD approach was implemented as follows.

METHOD DESIGN.

The method design stage includes establishing the method performance requirements, developing a method that will meet these requirements and then performing appropriate studies to understand the critical method variables that must be controlled to assure the method is robust and rugged.

METHOD PERFORMANCE REQUIREMENTS

Utilizing a QbD approach, it is essential at this stage that sufficient thought be given to the intended use of the

method and that the objectives or performance requirements of the method be fully documented. This represents the Analytical Target Profile (ATP) for the method. In this case ATP is the estimation of CIL and NEB in tablet dosage form using spectrophotometric method.

METHOD DEVELOPMENT

Once the ATP has been defined, an appropriate technique and method conditions must be selected in order to meet the requirements of the ATP.

METHOD UNDERSTANDING

Based on an assessment of risk (i.e., the method complexity and the potential for robustness and ruggedness issues) one can perform an exercise focused on understanding the method to better understand what impact key input variables might have on the method's performance characteristics. From this, one can identify a set of operational method controls.

RISK ASSESSMENT

Experiments can be run to understand the functional relationship between method input variables and each of the method performance characteristics. Knowledge accumulated during the development and initial use of the method provides input into a risk assessment (using tools such as Fishbone diagram and FMEA) which may be used to determine which variables need studying and which require controls.

DESIGN OF EXPERIMENTS

Robustness experiments are typically performed on parametric variables using Design of Experiments (DoE) to ensure that maximum understanding is gained while minimizing the total number of experiments. Depending on the type of method, surrogate measures of characteristics such as accuracy or precision may be evaluated.

METHOD DESIGN OUTPUT

A set of method conditions will have been developed and defined which are expected to meet the ATP. Those conditions will have been optimized based on understanding of their impact on method performance. QbD-based treatment of the robustness of an analytical method requires the assessment of all parameters (factors) which most strongly influence selectivity (results) alone and in combination. The experimental verification of many factors simultaneously is impractical and associated with extreme technical difficulties and expense. Some authors, have employed statistical studies, such as Plackett-Burman or fractional factorial designs and risk-based approaches to overcome the challenge and reduce the experimental Work load. Other procedures include running automated robustness experiments. The present paper, however, employs statistical analysis that is principal component analysis which exhibits factor extraction of variable parameters to evaluate robustness.

MATERIALS AND METHODS

Chemicals and Reagents

Both the APIs CIL and NEB were procured form Divi Lab., Vadodara. All chemicals and reagents used were of analytical grade and purchased from Merck chemicals, India. Formulation marketed by Eris Pharma Pvt. Ltd., India as the brand name Lnbeta 5 was procured from local vendor.

Instrumentation

Spectrophotometric measurements were carried out using a double beam UV visible spectrophotometer of Shimadzu UV-1700 (Japan) enabled with UV Probe 2.21software, and 10mm path length with 1 cm quartz cells.

SPECTROPHOTOMETRIC CONDITIONS

For the selection of analytical wavelength, standard solution of both the drugs and their synthetic mixture was scanned in the spectrum mode from 400nm to 200nm. Obtained spectrum was derivatized to second order for finding out ZCP. (Figure 1).

Preparation of Stock Standard and Working Solution

For the method, methanol was used as a solvent. Stock solution was prepared by transferring 10 mg of both in 10mL volumetric flask separately and dissolving in methanol to obtain 1000 μg/mL concentration. Working solution (100 μg/mL) was used for initial spectral scan in spectrophotometric method and further dilutions for linearity were prepared from stock solution.

Linearity Studies

From the working stock standard solution (100 μg/mL) suitable aliquots were pipetted and were diluted to 10 mL to obtain the concentration of 1,2,3,4 and 5 μg/ml of NEB and 2,4,6,8 and 10 μg/ml of CIL.

Estimation of Tablet Dosage Form

Twenty tablets were weighed and average weight was noted. Then the tablets were triturated and powder equivalent to average weight was weighed and transferred to 10mL volumetric flask and diluted with 6mL methanol. It was shaken for five minutes and this solution was filtered immediately through Whatman filter paper No. 41. Volume was adjusted up to the mark with methanol and diluted suitably to obtain concentration 3 and 6 mcg/ml of NEB and CIL respectively.

Determination of variable parameters

According to QbD approach, the first step is to selection of variable parameters for the respective method. Thus, the variable parameters for both the spectrophotometric methods were designed as Ishikawa diagram (Figure 2).

For all the variable parameters as stated in Ishikawa diagram, the absorbances were recorded over the concentration range as per method. Working solution 3 μg/mL of NEB, 6 μg/mL of CIL and mixture containing both were scanned from 400 to 200nm. All ZCP wavelengths were used as variable parameters. The solubility was studied in various solvents including distilled water, 0.1 N NaOH, 0.1 N HCl, and methanol. The sharpness of spectra was compared for selection of critical parameter. Scan speed was varied as fast, medium, slow, and very slow over the range 400– 200 nm, while slit width and sampling interval were varied in particular ranges of 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0nm and 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 nm, respectively. For the optimization of 2nd order derivative method, scaling factor and delta lambda were varied as 2, 3, 4, and 5 and 2, 4, 8, and 16, respectively. Amplitudes of linear concentrations were noted and evaluated.

Extraction of Critical Parameter

From the evaluated variable parameters, critical parameters were extracted by two ways, observation and Principal Component Analysis of PLS Solo software. By comparing the spectral shape, sharpness, and absorbances of linearity and range, few parameters were selected as critical parameters.

Validation

By using extracted critical parameters method validation was performed according to ICH Q2 (R1) guidelines. The parameters studied were linearity, precision, accuracy, LOD, LOQ, and robustness.

Precision

The reproducibility of all these three methods was determined by repeating the above methods at different time intervals (morning, afternoon, and evening) on the same day (Intraday precision) and on three consecutive days (interday precision).The intraday and interday variation for the estimation was carried out at three

different concentration levels of 1, 3 and 5 μg/mL for NEB and 2.6 and 10 μg/mL for CIL (Table 3).

Accuracy

The accuracy of the method was performed by calculating % recovery of standard by the standard addition method. Known amounts of standard solutions of both drugs were added at 80%, 100% and 120% levels to preanalyzed test sample solutions of 2 μg/mL of NEB and 3 μg/mL CIL. At each level of the amount three determinations were performed. The amount of was estimated by applying obtained absorbance values to regression equation.

Specificity

The interference of excipients was evaluated by adding 20 μg/mL of microcrystalline cellulose (MCC), corn starch to sample solution separately and absorbance was evaluated.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using following equation.

$LOD = 3.3 \times \sigma / S$, $LOQ = 10 \times \sigma / S$

where σ is the standard deviation of intercept and S is the slope of the calibration curve.

Robustness

Robustness of the method was assessed by making variations in solvent (methanol).

RESULTS AND DISCUSSION

Implementation of QbD approach was carried out by studying variable parameters in the analytical method development. Critical parameters were extracted by observation of results as well as performing principal component analysis. Also, each method was validated according to ICH Q2 (R1) guidelines.

A second order derivative spectrophotometric method has been developed and validated for the determination of both the drugs in pharmaceutical formulation. QbD approach was carried out by varying 31 parameters and extracted critical parameters by using principal component analysis and by observation. The extracted critical parameters are summarized in (Table 1).

Statistical data for calibration curve shows NEB and CIL followed the linearity range 1-5 g/mL and 2- 10 g/mL respectively (Table 2). The inter-day and intra-day analysis showing % RSD less than 2 indicating methods precision (Table 3). The % recovery for NEB and CIL was found to be 101.86% and 100.28% (Table 4). There was no interference of excipients and no statistical difference between different conditions showing that the method was specific and robust. The amount of drug estimated in pharmaceutical formulation by proposed method was in good agreement with the label claim (Table 5).

CONCLUSION

By observing validation parameter and assay results, it can be concluded that developed second order derivative spectrophotometric method is simple and rapid, accurate and precise and can be useful method for routine analysis of CIL and NEB in its combined dosage form. Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process.

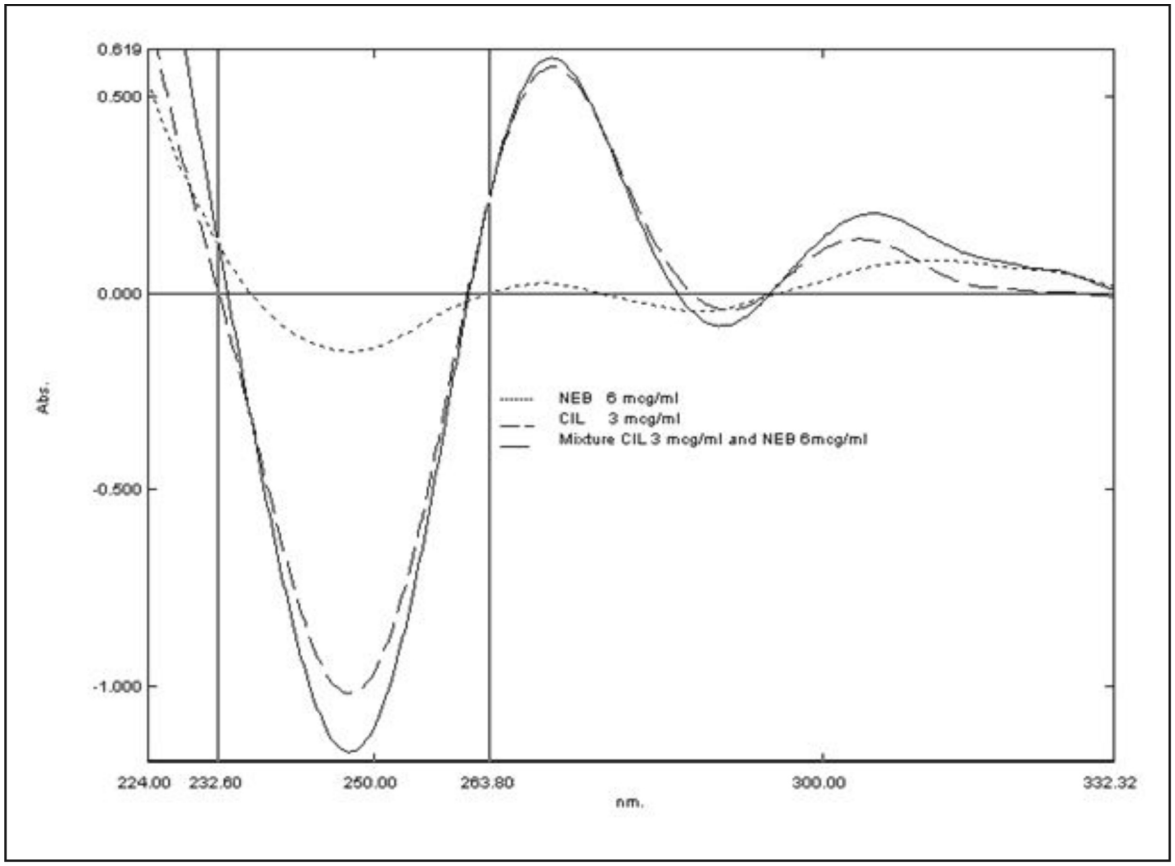

Figure 1. Overlain spectra of CIL (3 mcg/ml), NEB (6 mcg/ml) and MIX

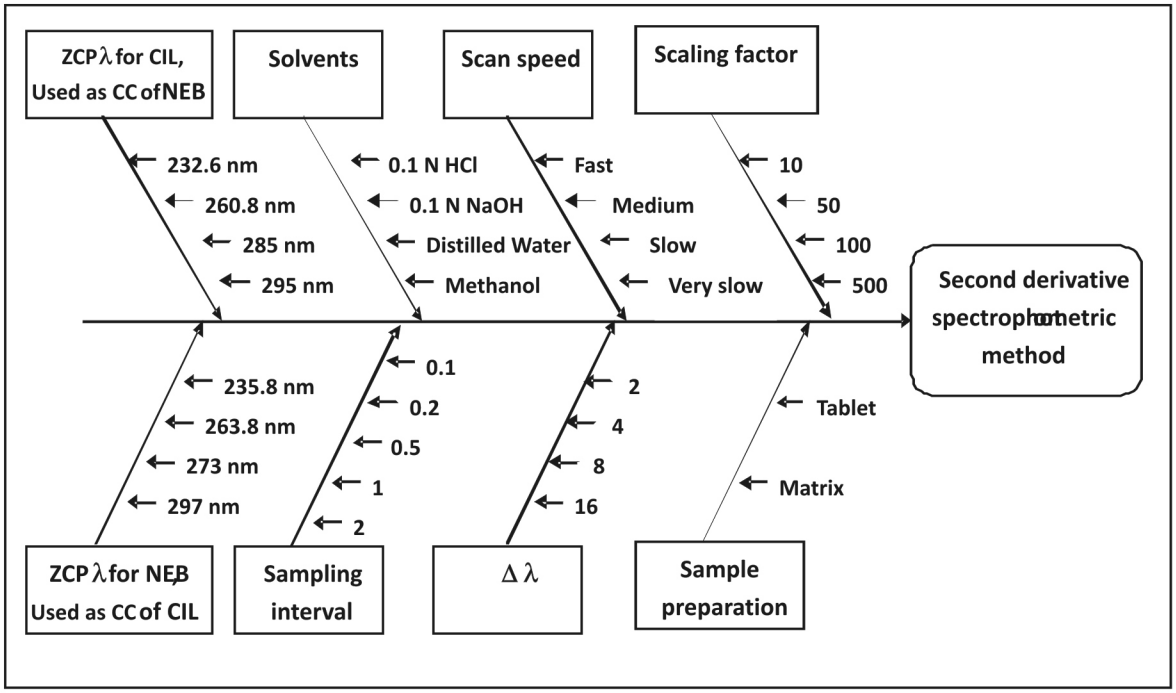

Figure 2. Ishikawa diagram for spectrophotometric method

Table 1. Critical parameters extracted

By observation		By principal component analysis	
Parameter	**Extracted result**	**Parameter**	**Extracted result**
Solvent	Methanol	Wavelength	232.6 for NE B 263.8 for CIL
		Scan speed	Medium
Sample preparation	Tablet	Scaling factor	500
		Slit width	1.0
	Matrix	Sampling interval	0.2
		Delta lambda	16

Table 2. Statistical data for calibration curve

Parameters	**CIL**	**NEB**
Wavelength (nm)	263.8	232.6
Linearity Range (µg/ml)	2 - 10	1 - 5
Regression Equation	y=0.0486x+0.0314	y=0.0392x+0.0117
Correlation Coefficient (r2)	0.9995	0.9965
LOD	0.452341	0.367053
LOQ	1.370732	1.112281

Table 3. Precision Data

Drug	**Concentration (µg/mL)**	**Intraday (% RSD) (n = 3)**	**Interday (% RSD) (n = 3)**
CIL	2	1.59	2.07
	6	1.03	1.29
	10	0.62	0.74
	Mean	1.08	1.37
NEB	1	1.41	1.41
	3	1.33	1.83
	5	1.22	1.56
	Mean	1.33	1.60

Table 4. Accuracy Data

Level	Concentration taken (µg/mL)		Concentration found (µg/mL)		% Recovery		Mean	
	CIL	NEB	CIL	NEB	CIL	NEB	CIL	NEB
80%	2.4	1.6	2.37	1.66	98.89	103.54		
100%	3	2	3.05	2.04	101.78	102.17	100.28	102.32
120%	3.6	2.4	3.61	2.43	100.19	101.25		

Table 5. Result of analysis of commercial tablet by proposed method

Tablet	Label Claim (mg/ Tab)		% label claim estimated	
	CIL	NEB	CIL	NEB
Batch I	10	5	100.53	100.05
Batch II	10	5	101.28	100.68

[1] ICH Topic Q8 (R2), "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '09), Pharmaceutical Development, 2009.

[2] A.B. Godfrey and R. S. Kenett, "Joseph M. Juran, a perspective on past contributions and future impact,"Quality and Reliability Engineering International, vol. 23, no. 6, pp. 653–663, 2007.

[3] P. Nethercote, P. Borman, T. Bennett et al., QbD for Better Method Validation & Transfer, Pharmaceutical Manufacturing, 2010.

[4] ICH Topic Q2 (R1), "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '94), Validation of Analytical Procedures, 1994.

[5] ICH Topic Q9, "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '94), Quality RiskManagement, 2005.

[6] P. Borman, P. Nethercote, M. Chatfield et al., The Application of Quality by Design to Analytical Methods, PharmTech, 2007.

[7] L. X. Yu, Pharmaceutical quality by design: product and process development, understanding, and control, Pharmaceutical Research, 25, (4), 2008, 781–791.

[8] M. Pohl, M. Schweitzer, G. Hansen et al., Implications and opportunities of applying the principles of QbD to analytical measurements, Pharmaceutical Technology Europe, 22(2), 2010, 29–36,.

[9] A. A. Shirkhedkar; P. M. Bugdane; S. J. Surana, J. Chromatographic Science, Stability-Indicating TLC-Densitometric Determination of Nebivolol Hydrochloride in Bulk and Pharmaceutical Dosage Form 2010, 48, 109-113

[10] Niraj Vyas, Sangita Panchal, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Nebivolol and Indapamide in Pharmaceutical Dosage Form, Asian J. Pharm. Ana. 2014; Vol. 4: Issue 3, Pg98-102

[11] A. L. Rao; K. R. Rajeswari; G.G. Sankar. Spectrophotometric Methods for the Determination of Selected Drugs in Pharmaceutical Formulations, J. Chem. Pharm. Res., 2010, 2(1), 280-282.

[12] D. A. Shah; K. K. Bhatt; R. S. Mehta; S. L. Baldania; T. R. Gandhi. Stability Indicating RP-HPLC Estimation of Nebivolol Hydrochloride in Pharmaceutical Formulations, Indian J. Pharm. Sci. 2008, 70(5), 591–595

[13] L. J. Patel; B. N. Suhagia; P. B. Shah. RP-HPLC and HPTLC Methods for the Estimation of Nebivolol hydrochloride in Tablet Dosage form. Indian J. Pharm. Sci. 2007, 69(4): 594–596.

[14] Mohd IS and Medidi S, Simultaneous estimation of Telmisartan and Cilnidipine in bulk and in tablet formulation using RP-HPLC. International Journal of Advances in Pharmaceutical Sciences, 5, 2014, 2142-2148.

[15] Prajakta P and Santosh VG, Simultaneous RP-HPLC estimation of Cilnidipine and telmisartan in combined tablet dosage form. Pelagia Research Library Der Chemica Sinica, 4, 2013, 6-10.

[16] Amit SM, Manjusha ND and Sanjay DS, Developement and validation of analytical method for simultaneous estimation of Cilnidipine and olmesartan medoxomil in bulk and tablet dosage form by RPHPLC. International Journal of Pharmacy and Pharmaceutical Science. 2014; 6.

[17] Tushar KK, Darshil BS and Dilip GM, Development and validation of q-absorbance ratio Spectrophotometric method for simultaneous estimation of Cilnidipine and metoprolol succinate in bulk and combined dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2014, 6.

[18] Vaghela S, Jagdish K, Pinak P and Nehal S, Devlopment and validation of high performance thin layer chromatographic Method for Cilnidipine and metoprolol succinate in their combined Pharmaceutical dosage form. International Journal of Research in Pharmaceutical and Nano Sciences. 2014, 3, 61 - 72.

[19] Mohammed MS, Spectrophotometric Method for the Estimation of Cilnidipine in Bulk and Pharmaceutical Dosage forms. Oriental Journal of Chemistry. 2013, 29, 131-134.

[20] Pankaj PC and Bhalerao V, Method validation for spectrophotometric estimation of Cilnidipin. International Journal of Pharmacy and Pharmaceutical Sciences. 2012, 4.

[21] Haripriya M and Neethu A, "Development and validation of UV spectrophotometric method for the simultaneous estimation of Cilnidipine and telmisartan in tablet dosage form utilizing simultaneous equation and absorbance ratio method". Research article pharmaceutical sciences. Jan-Mar 2013;3:343 348