Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

# Development and Validation of UV- Spectrophotometric Method for Simultaneous Estimation of Nifedipine and Candesartan Cilexetil in Synthetic Mixture

**Priyanka A. Dhobi.*, Jignasa Modi**
*Parul Institute of Pharmacy and Research, Limda, Waghodiya, Vadodara-391760, Gujarat, INDIA.*

**Received on: 19-03-2015; Revised and Accepted on: 26-03-2015**

## *ABSTRACT*

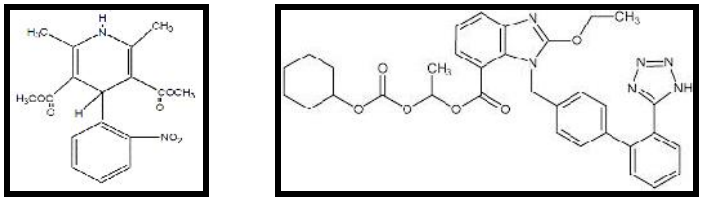
*A simple, accurate and precise UV Spectrophotometric method (Simultaneous Equation Method) developed for the simultaneous estimation of Nifedipine and Candesartan cilexetil in Synthetic mixture. The drugs were determined by Absorptivity values of Nifedipine and Candesartan cilexetil at selected wavelength 235nm and 255nm for NIF and CAN respectively. In this method drugs obey Beer's law using the concentration range 6-21µg/ml and 3.2-11.2µg/ml for NIF and CAN respectively. The correlation coefficient of NIF and CAN was found to be 0.999 and 0.998 respectively. The results of Recovery study for NIF and CAN were found to be within range of 98-102%. Precision study showed that %RSD was within range of acceptance limits (<2%). The method was validated as per ICH Q2 (R1) guideline.*

***Keywords:*** *Nifedipine(NIF), Candesartan cilexetil(CAN), Simultaneous Equation method, UV Spectrophotometry.*

## INTRODUCTION

Nifedipine [1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-3, 5-pyridine dicarboxylic acid dimethyl ester [Fig- a], a dihydropyridine calcium channel antagonist. It is also used for the treatment of vascular disorders such as Raynaud's phenomenon. Particularly nifedipine is used as a second line treatment in hypertension during pregnancy. NIF is official in IP, BP and USP [1-3].Candesartan cilexetil [1-[[(cyclohexyloxy) carbonyl]oxy]ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate [Fig- b] is potent, orally active andselective angiotensin II receptor antagonist, selective for AT1 receptors, with tight binding to and slow dissociation from the receptor. It has no agonist activity. It is rapidly converted to the active substance, Candesartan, by ester hydrolysis during absorption from the gastrointestinal tract. It belongs to benzimidazole class and is used in treatment of Hypertension and angina.

Both drugs are formulated together in the form of tablet dosage form for treatment of hypertension. The chemical structures of both drugs [4-7] were shown in figure 1.



(a) (b)

**Fig. 1: Chemical structure of (a) Nifedipine and (b) Candesartan cilexetil**

From literature survey it reveals that various analytical methods have been reported for estimation of Nifedipine and Candesartan cilexetil individually or in combination with other drugs either as API or in pharmaceutical dosage form. Literature review also reveals that, no analytical methods have been reported for estimation of Nifedipine and Candesartan cilexetil in bulk and synthetic mixture. So the purpose of this work was to develop a simple, precise, accurate and sensitive Simultaneous Equation Method for determination of Nifedipine and Candesartan cilexetil in synthetic mixture.

## MATERIALS AND METHODS

**Instruments:**

The instrument used was double beam UV- visible spectrophotometer (Shimadzu, model 1800, software: UV-Probe 2.34) having two matched quartz cell with 1 cm path length. Sonication of sample solutions was done using ultrasonic cleaner.

**Materials:**

Nifedipine (NIF) drug sample was procured from Osaka Pharmaceuticals, Vadodara and Candesartan cilexetil (CAN) drug sample was gifted by Alembic Pharma, Vadodara.

**Method:**

**Preparation of standard stock solution:**

The stock solution having 1000µg/ml concentration of NIF and CAN were prepared separately by dissolving accurately weighed 100mg of both drugs in 100 ml methanol. Further dilutions of standard stock solutions of both drugs were made with methanol to get the working standard stock solutions of 100µg/ml concentration of NIF and 160µg/ml CAN.

**Method Development (Simultaneous equation method):**

**Selection of Scanning range and Sampling Wavelength:**

The standard stock solution of NIF and CAN were diluted with methanol individually to get the concentration of 10µg/ml for both and was scanned in UV range 200-400 nm. The $\lambda_{max}$ of both the drugs were found to be 235nm and 255nm respectively for NIF and CAN respectively in normal UV spectra shown in figure 2.

***Corresponding author:**
***Priyanka A. Dhobi.***
*Parul Institute of Pharmacy and Research,*
*P.O. Limda, Tal. Waghodiya-391760 Dist. Vadodara,*
*Gujarat, INDIA. Ph: +91-8469837622.*
**E-Mail: priya.dhobi@yahoo.com*

**Development of Simultaneous Equation Method:**

**Preparation of Calibration curve:**

Appropriate volume of aliquots from standard stock solutions of NIF and CAN were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 6, 9, 12, 15, 18and 21µg/ml for NIF and 3.2, 4.8, 6.4, 8.0, 9.6, 11.2µg/ml for CAN respectively. The absorbance vs. concentration was plotted at 235 and 255nm. The straight-line equation was determined.

**Simultaneous Equation Method:**

The method was based on the absorption of drugs (X and Y) at the maximum wavelength. Other quantification analyses of NIF and CAN in Synthetic mixture were performed with the following equations[8,9]:

$$C_X = (A_2ay_1 - A_1ay_2)/\ ax_2ay_1 - ax_1ay_2 \ldots\ldots\ldots\ldots (1)$$
$$C_Y = (A_1ax_2 - A_2ax_1)/\ ax_2ay_1 - ax_1ay_2 \ldots\ldots\ldots\ldots (2)$$

Where $C_X$ and $C_Y$ are the concentrations of X and Y drugs respectively in the diluted sample; $ax_1$ and $ax_2$ are absorptivity of X at $\lambda_1$ and $\lambda_2$; and $ay_1$ and $ay_2$ are absorptivity of Y at $\lambda_1$ and $\lambda_2$. The absorbance of the dilute samples at $\lambda_1$ and $\lambda_2$ are $A_1$ ($A1 = ax_1bC_x + ay_1bc_y$) and $A_2$ ($A_2 = ax_2bC_x + ay_2bc_y$) respectively.

**Method Validation:**

The above proposed method was validated as per the ICH Q2 (R1) guidelines for validation of analytical procedures [10] in order to determine the linearity, Accuracy, Precision, LOD and LOQ.

***Linearity and Range:***

Calibration curve constructed was linear over a selected range of 6-21µg/ml for NIF and 3.2-11.2µg/ml for CAN. The calibration curve of absorbance against concentration plotted was shown in figure 4 and 5. Each concentration was repeated six times. Correlation coefficient and regression line equations for NIF and CAN were calculated and were shown in table no.1.

***Accuracy:***

The accuracy of the developed method was determined by finding out the amount of recovery of Nifedipine and Candesartan cilexetil. For the accuracy standard addition method was used where, as known amount of NIF and CAN were added to the known concentration (7.5µg/ml NIF and 4µg/ml CAN). The amount recovered was found by measuring the absorbance of the solution and was expressed as mean recovery of samples with upper and lower limits of percent relatives of standard deviation. Recovery was done at three different levels i.e. 80%, 100% and 120%, within the linearity range of both the drugs.

***Precision:***

***Repeatability(n=6):***

For the repeatability study, from the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted upto mark with methanol such that it gives the concentration of 15µg/ml and 8µg/ml of NIF and CAN respectively. The absorbance of the solutions was measured at 235nm and 255nm respectively. The procedure was repeated six times and % RSD was calculated and shown in table no. 3.

***Intraday Precision (n=3):***

From the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 12, 15 and 18µg/ml of NIF and 6.4, 8.0, and 9.6µg/ml of CAN. The solutions were analysed three times on the same day and % RSD was calculated and shown in table no. 3.

***Interday Precision (n=3):***

From the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 12, 15 and 18µg/ml of NIF and 6.4, 8.0, and 9.6µg/ml of CAN. The solutions were analysed three times on three different days and % RSD was calculated and were shown in table no. 4.

***Limit of Detection (LOD) and Limit of Quantification (LOQ):***

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can be analysed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ for NIF and CAN were shown in table no. 5.

$$LOD = 3.3 \times (SD/Slope)$$
$$LOQ = 10 \times (SD/Slope)$$

Where,

SD = Standard deviation of the Y- intercepts of the 6 calibration curves.

Slope = Mean slope of the 6 calibration curves.

**Estimation of Nifedipine and Candesartan cilexetil in Synthetic Mixture:**

Combination containing both Nifedipine and Candesartan cilexetil were used for the study. Combine solution equivalent to 60mg of Nifedipine and 32mg of Candesartan cilexetil and transferred in to a 100 ml volumetric flask to bring both drugs in 60:32 ratio and stock solution of this was prepared in methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. This stock solution contains Nifedipine 600µg/ml and Candesartan cilexetil 320µg/ml. Then the appropriate dilution of 6µg/ml (NIF) and 3.2µg/ml (CAN) was made using methanol as solvent. All the determinations were carried out in triplicate. The absorbance of the prepared solutions was measured at 235nm and 255nm and then the concentration of both the drug was calculated using the equation of the straight line representing the calibration curves for Nifedipine and Candesartan cilexetil. The amount of the drug found in Synthetic mixture calculated was shown in table no. 6.

**RESULT**

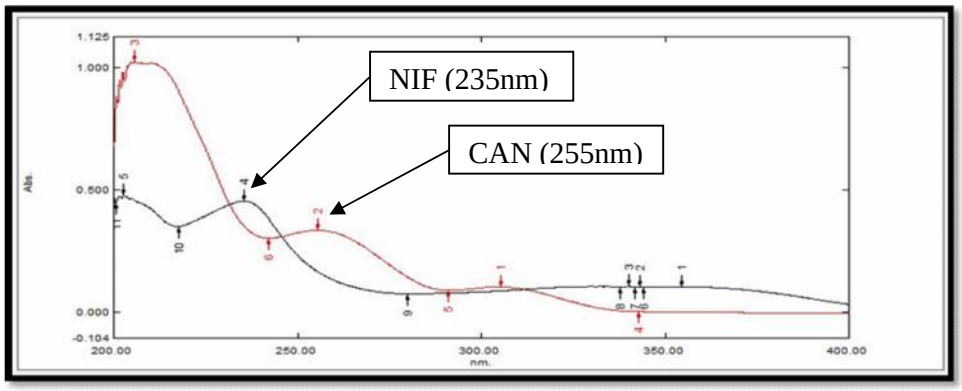



**Fig. 2: Overlay spectra of NIF(10µg/ml) and CAN(10µg/ml) in methanol**

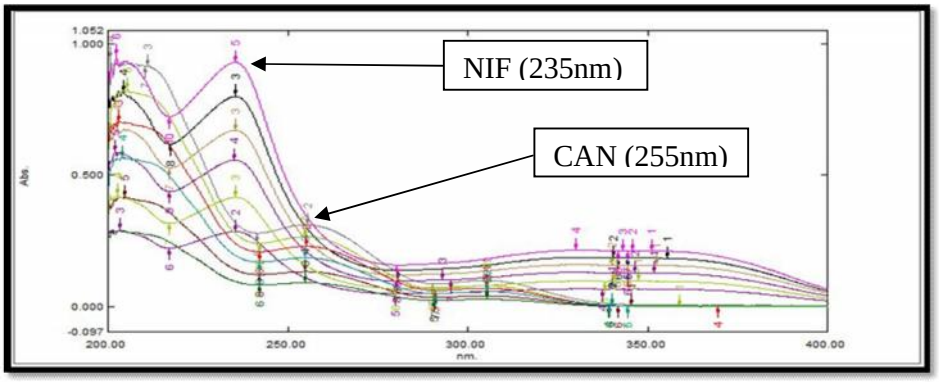



**Fig. 3: Overlain linearity spectra of NIF and CAN in methanol**

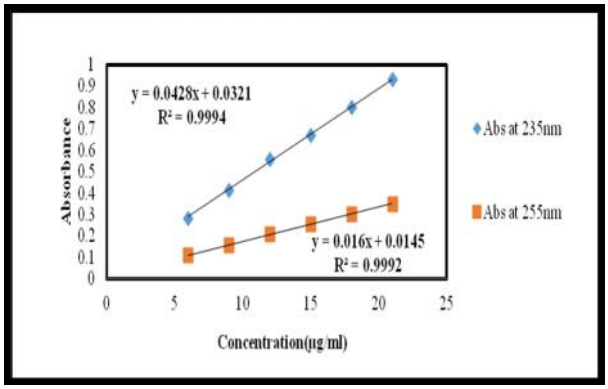



**Fig. 4: Linearity graph of NIF at 235nm and 255nm**

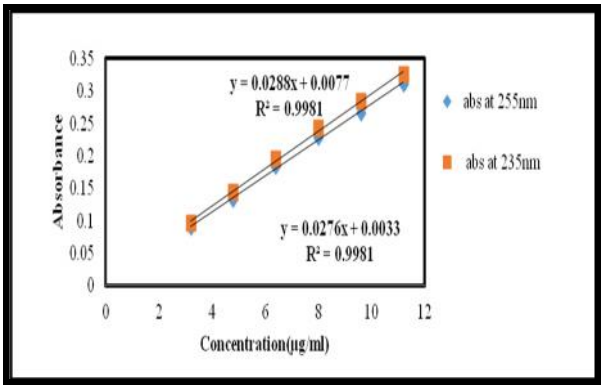



**Fig. 5: Linearity graph of CAN at 235nm and 255nm**

## DISCUSSION

The present paper describes the estimation of NIF and CAN in combination by Simultaneous Equation method. The Beer-Lambert's concentration range was found to be 6-21µg/ml and 3.2-11.2µg/ml for both drug NIF and CAN at 235nm and 255nm respectively. The correlation coefficient was found to be 0.999 for NIF and 0.998 for CAN for proposed method. Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision. The values of LOD and LOQ were 0.186417µg/ml and 0.564901µg/ml for NIF and 0.115876 µg/ml and 0.35114µg/ml for CAN respectively.

**Table No. 1: Optical Characteristics**

| Parameters | Nifedipine | Candesartan cilexetil |
|---|---|---|
| **Beer's law limit (µg/ml)** | 6-21µg/ml | 3.2-11.2µg/ml |
| **Regression equation** | 0.016x + 0.014 | 0.028x + 0.007 |
| **Slope (m)** | 0.0158 | 0.028 |
| **Standard deviation of Intercept (c)** | 0.000894 | 0.000983 |
| **Correlation coefficient ($R^2$)** | 0.999 | 0.998 |

**Table No. 2: Results of Recovery studies**

| Drug | Concentration of STD drug | Recovery level (%) | Amount of drug added(µg/ml) | Amount of drug recovered(µg/ml) | % Mean recovery ±SD |
|---|---|---|---|---|---|
| **NIF** | 7.5 | 80 | 6 | 7.47 | 99.60±1.471 |
| | | 100 | 7.5 | 7.525 | 100.33±1.257 |
| | | 120 | 9 | 7.414 | 98.85±0.211 |
| **CAN** | 4 | 80 | 3.2 | 3.983 | 99.64±1.051 |
| | | 100 | 4 | 3.999 | 99.99±1.440 |
| | | 120 | 4.8 | 4.008 | 100.20±0.572 |

**Table No. 3: Repeatability, Inter-day and Intra-day precision of NIF and CAN**

| Drug | Concentration(µg/ml) | Average ABS±SD | %RSD |
|---|---|---|---|
| **REPEATABILITY(n=6)** | | | |
| **NIF** | 15 | 0.255833 ± 0.001169 | 0.4569 |
| **CAN** | 8 | 0.234833 ± 0.000753 | 0.3205 |
| **INTRADAY PRECISION(n=3)** | | | |
| **NIF** | 12 | 0.211 ± 0.000577 | 0.2731 |
| | 15 | 0.255 ± 0.001528 | 0.5974 |
| | 18 | 0.303 ± 0.001 | 0.3300 |

| | | | |
|---|---|---|---|
| **CAN** | 6.4 | 0.196 ± 0.0010 | 0.5102 |
| | 8.0 | 0.235 ±0.001528 | 0.6490 |
| | 9.6 | 0.286 ±0.001155 | 0.4032 |
| **INTER DAY PRECISION(n=3)** | | | |
| **NIF** | 12 | 0.209 ±0.001528 | 0.7297 |
| | 15 | 0.255 ±0.002 | 0.7843 |
| | 18 | 0.302 ±0.001 | 0.3311 |
| **CAN** | 6.4 | 0.196 ± 0.001 | 0.5120 |
| | 8.0 | 0.234 ± 0.000577 | 0.2460 |
| | 9.6 | 0.285 ± 0.001155 | 0.4046 |

*SD = standard deviation, ABS = Absorbance

**Table No. 4: Analysis of Synthetic Mixture**

| Drugs | Label claim (mg/ml) | Conc. Taken for assay(µg/ml) | Absorbance of sample solution | Concentration found (µg/ml) | % Assay |
|---|---|---|---|---|---|
| **NIF** | 60 | 6 | 0.110 | 5.937 | 98.95% |
| **CAN** | 32 | 3.2 | 0.096 | 3.178 | 99.33% |

**Table No. 5: Limit of detection (LOD) and Limit of Quantification (LOQ)**

| Parameters | Nifedipine | Candesartan cilexetil |
|---|---|---|
| **LOD (µg/ml)** | 0.186417 | 0.115876 |
| **LOQ (µg/ml)** | 0.564901 | 0.35114 |

## CONCLUSION

A simple, accurate and precise UV Spectrophotometric method (Simultaneous equation method) has been developed for the estimation of NIF and CAN in Synthetic mixture. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug at selected wavelength.

## ACKNOWLEDGEMENT

The authors are thankful to Dr. T.Y. Pasha, Principal of Parul Institute of Pharmacy and Research, Vadodara for providing necessary facility for work. The authors are thankful to Osaka Pharmaceuticals and Alembic Pharma, Vadodara for gift sample of Nifedipine and Candesartan cilexetil.

## REFERENCES:

1. The Indian Pharmacopoeia, Vol. III, Ministry of Health and Family welfare, Govt. of India, 6th Edn , Published by Indian Pharmacopoeia Commission, Ghaziabad, **2014**; pp. 193, 522, 2336-2338
2. The British Pharmacopoeia, Vol. II, HMSO Publication, London, **2009**; pp. 1447, 1448
3. The United states Pharmacopoeia, Vol. III, United state Pharmacopoeial convection Inc., Rockville.MD, **2009**; pp. 3083, 3084.
4. Drug bank, "Nifedipine", October-**2014** http://www.drugbank.ni/drugs/DB01115.
5. Drug bank,"Candesartan",October-**2014** http://www.drugbank.ca/drugs/DB00796.
6. Drug profile of candesartan cilexetil, http://www.chemspider.com/Chemical-Structure.2444.
7. An encyclopedia of chemical, drugs and biological, The Merk index, 14th edition, published by Merk research laboratory; 714, 1056.
8. Skoog; et al, Principles of Instrumental Analysis, 6th edition, Belmont, CA: Thomson Brooks/Cole publisher; 169–173.
9. Beckett AH, Stenlake JB, Practical Pharmaceutical chemistry, 4th edition, CBS publishers and distributors; **2001**; 296-300.
10. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use on Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1), **2005**.
11. Sankaa K, Gullapellia R, Patila N, Rao PA and Divana PV. *Scholars Res. Lib.,* **2014**; 6(1): 279-289.
12. Parida P, Mishra SC and Tripathy SP. *Indo Amer. J. Pharma. Res.,* **2014**; 4(5): 2307-2312.
13. Puranik MP, Wadher SJ, Kosarkar AL and Yeole PG. *Int. J. Res. Pharma. Biomed. Sci.,* **2012**; 3(3): 1227-1230.
14. Mathrusri AM, Narendra A and Ravi KK,*J. Drug Deli. Thera.,* **2012**; 2(2): 48-54.

**How to cite this article:**

Priyanka A. Dhobi, Jignasa Modi: Development and Validation of UV- Spectrophotometric Method for Simultaneous Estimation of Nifedipine and Candesartan Cilexetil in Synthetic Mixture, J. Pharm. Res., 2015; 4(3): 112-115.

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Source of support: Nil**