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Validated HPLC Method for Simultaneous Estimation of Candesartan and Nifedipine in Hypertension Combination Therapy: Optimization through DoE

Darshil B. Shah[1B]*, Dr. Bhavesh H. Patel[2], Dr. Jignesh S. Shah[3]

1. Research Scholar, K B Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwa Vidyalaya University, Gandhinagar, Gujarat, India.

B. Assistant Professor, L J Institute of Pharmacy, LJ University, Ahmedabad, Gujarat, India.

2. Assistant Professor, K B Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwa Vidyalaya University, Gandhinagar, Gujarat, India.

3. Professor, L J Institute of Pharmacy, LJ University, Ahmedabad, Gujarat, India.

*Corresponding Author Email: darshil.shah@ljinstitutes.edu.in

Introduction: Hypertension poses significant health risks globally, necessitating effective management strategies. Combination therapy, such as Candesartan and Nifedipine, shows promise in improving treatment outcomes. However, analytical challenges in simultaneous estimation underscore the need for advanced techniques.

Methods: A Design of Experiment (DoE) based High-Performance Liquid Chromatography (HPLC) method was developed to optimize chromatographic conditions. Candesartan and Nifedipine samples were analyzed using reversed-phase HPLC with UV-Vis spectrophotometry detection. Statistical analysis and response surface methodology aided in method optimization.

Results and Discussion: Optimal chromatographic conditions were achieved with a composite mobile phase and specific parameters. The Central Composite Design (CCD) facilitated efficient experimentation, yielding robust models for retention time and tailing factor. Validation studies demonstrated specificity, linearity, precision, accuracy, and system suitability of the method.

Conclusion: The developed HPLC method offers accurate and efficient quantification of Candesartan and Nifedipine in combination therapy for hypertension management. Validation studies confirm the method's reliability and accuracy, highlighting its potential for routine analysis and quality control in pharmaceutical formulations.

Key words: Candesartan, Nifedipine, DoE, Validation

Introduction

Hypertension, a widespread health issue impacting millions globally, poses significant risks for cardiovascular diseases and other complications. Although often asymptomatic, untreated hypertension can lead to severe health consequences, including heart disease, stroke, kidney damage, and vision loss. (1, 2) In the realm of hypertension management, ongoing innovations aim to enhance treatment efficacy and patient outcomes. Among these innovations, the combination therapy of Candesartan and Nifedipine has emerged as a promising strategy. (3)

Candesartan, an angiotensin II receptor blocker (ARB), works by selectively blocking angiotensin II type 1 receptors, leading to vasodilation, reduced sodium retention, and decreased peripheral vascular resistance. (4, 5) Nifedipine, a calcium channel blocker (CCB), acts on L-type calcium channels in vascular smooth muscle cells, promoting vasodilation and reducing peripheral vascular resistance. (6-7)

The combination of Candesartan and Nifedipine targets distinct pathways involved in hypertension, resulting in enhanced blood pressure reduction compared to monotherapy. Recent studies, including the DISTINCT trial, have demonstrated the efficacy and safety of nifedipine GITS/candesartan combination therapy, showing its

ability to effectively lower blood pressure and improve side effect profiles across different patient populations. (8-10)

Analytical challenges arise in simultaneously estimating candesartan and nifedipine due to their structural similarities, leading to difficulties in quantification using traditional methods. These challenges underscore the need for advanced analytical techniques to ensure accurate estimation of both medications in combination therapy for hypertension management. (11-12)

To address these challenges, the proposed work aims to develop a Design of Experiment (DoE) based High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of candesartan and nifedipine in a combined dosage form. (13) The study focuses on optimizing chromatographic conditions using DoE principles to achieve accurate and efficient quantification of both medications in a single analysis. Reversed-phase HPLC is utilized for the simultaneous estimation, leveraging its effectiveness in separating compounds with different polarities. The detection method employed is UV-Vis spectrophotometry, a reliable technique for quantifying pharmaceutical compounds. (14-18) By integrating DoE principles with HPLC methodologies, this research aims to establish an optimized analytical approach for the concurrent analysis of candesartan and nifedipine in a combined dosage form.

Materials And Methods

1.2.1 Chemicals

Samples of Candesartan (CAN) and Nifedipine (NIF) active pharmaceutical ingredients (API) were provided as gift samples by Shaimil Laboratories Limited, located in Vadodara, Gujarat. Various HPLC-grade solvents including Methanol, water, and ACN were procured from Thermo Fisher Scientific India Pvt. Ltd. Orthophosphoric acid and phosphate buffer were sourced from Astron Chemicals India. Solutions were prepared using the mobile phase.

1.2.2 Statistical Analysis

The design of experiments (DOE), including the Central Composite Design, data analysis, and desirability function calculations, was conducted using Design-Expert v13.0.12.0 software by Stat Ease. Microsoft Excel 2021 was utilized for computing R-squared (R2), standard deviation (SD), and relative standard deviation (RSD) of validated data.

1.2.3 Preparation of Mobile Phase:

Preparation of buffer: Accurately weighed quantity of 0.85 grams of Potassium dihydrogen phosphate (KH_2PO_4) was transferred in 1000 mL beaker, dissolved in 200 mL HPLC grade water and sonicated for about 10 min and diluted up to the mark with HPLC grade water. It was filtered through 0.45 µm membrane filter. Buffer pH was adjusted to 3.9 using 1% ortho phosphoric acid.

Preparation of 1% ortho phosphoric acid: 1 ml of ortho phosphoric acid was taken and dissolved in 100 ml of water.

For 100 ml of mobile phase, 48 ml of ACN and 52 ml of buffer (48:52) were taken and mixed. Then the mobile phase was degassed for 15 minutes with an ultrasonic bath.

1.2.4 Preparation of Standard stock solution:

The active pharmaceutical ingredients (API) of CAN and NIF were assessed and transferred to the appropriate volumetric flask. Both APIs were liquefied in adequate quantities of mobile phase (48 ACN: 52 Buffer) to produce a 1 mg/ml concentration of each. Solutions for working standards were obtained by diluting standard stock solutions in the mobile phase (4-24 µg/mL for CAN and 5-30 µg/mL for NIF).

1.2.5 Chromatography condition

High-performance liquid chromatography (HPLC) was conducted using a Shimadzu HPLC system (Model LC 2010C HT Liquid Chromatograph) equipped with a dual-plunger pump and UV detection system. LabSolutions software version 5.52 was utilized to operate the chromatographic system and record data. UV spectra were obtained using a UV-1800 Shimadzu UV Spectrophotometer. Separation of compounds was carried out on a Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of a mixture of ACN and 0.05 M potassium dihydrogen phosphate (KH2PO4) buffer in a ratio of 48:52, adjusted to pH 3.88 with 1% orthophosphoric acid, with a flow rate of 1.0 mL/min. Each sample injection involved 20 µL, and detection was performed at a wavelength of 253 nm with a total run time of 10.0 minutes.

1.2.6 Experimental Design and Response Surface Methodology

To optimize the percentage of ACN in the mobile phase, pH, and flow rate for effective separation, a faced central composite design (FCCD) was employed, utilizing a partial factorial design approach. This design included five replicates at extreme levels, incorporating center points and axial points. Derringer's desirability function was utilized to evaluate the coefficient of determination (R2) for the polynomial models, determining the position of the practically optimal condition.

Results and Discussions

Method Development and Optimization:

The RP-HPLC methodology was systematically developed using a Design of Experiment (DOE) approach, exploring various permutations of three pivotal independent variables: pH, acetonitrile (ACN) percentage in the mobile phase, and flow rate. The selection of a wavelength at 253 nm was based on an analysis of the UV spectra overlay for both CAN and NIF, aiming to optimize detector sensitivity and response while minimizing potential signal distortion. Optimal chromatographic separation of the compounds was achieved using a composite mobile phase comprising ACN and 0.05 M potassium dihydrogen phosphate (KH2PO4) buffer in a ratio of 48:52, maintained at a pH of 3.88, and administered at a flow rate of 1 mL/min. Precision and accuracy were enhanced through the application of a central composite design, yielding a robust second-order model for the response variable.

The Central Composite Design (CCD), developed by G.F. Box and K.B. Wilson, was employed to create an efficient experimental plan for a second-order model. This design minimized the number of experiments required while investigating factors such as flow rate, liquid pH, and acetonitrile content. The study focused on retention time and tailing factor in 15 experiments, optimizing conditions by adjusting responses within the effective range of 40% to 50% ACN, a flow rate between 0.8 mL/min and 1.0 mL/min, and a pH between 3.8 and 4.0. Although the mathematical model was complex, it facilitated response estimation and determination of optimal conditions for CAN and NIF analysis.

Table 3 presents the statistical parameters and regression model obtained from ANOVA, indicating the factors affecting retention time and tailing factor for candesartan and nifedipine, respectively. Here P value less than 0.05 indicates that both the models are significant. The P Value for A factor (ACN) is less than 0.05 which indicates that it plays major role in retention time of the drug. The other parameters indicates that model is applicable for routine analysis.

The second equation indicates regarding tailing . In tailing value of C parameter is less than 0.05 which indicates that it affects tailing of the drug.

Table 1: HPLC independent variables for CCD

Factor	Name	Level (-)	Level (0)	Level (+)
A	Flow rate (ml/min)	0.8	0.9	1.0
B	Buffer pH	3.8	3.9	4.0
C	ACN (%v/v)	40	45	50

Table 2: Experimental conditions of HPLC and responses

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A:ACN	B:Flow Rate	C:pH	Retention Time of Candesartan	Tailing of Nifedipine
			ml/min			
8	1	50	1	4	4.04	1.21
2	2	50	0.8	3.6	3.98	1.52

14	3	45	0.9	4.14	5.26	1.38
11	4	45	0.7	3.8	6.33	1.65
12	5	45	1	3.8	4.22	1.53
5	6	40	0.8	4	6.95	1.31
6	7	50	0.8	4	4.62	1.48
15	8	45	0.9	3.8	4.55	1.24
1	9	40	0.8	3.6	8.53	1.44
4	10	50	1	3.6	3.64	1.46
7	11	40	1	4	8.68	1.44
13	12	45	0.9	3.46	4.11	1.92
3	13	40	1	3.6	8.57	1.63
9	14	37	0.9	3.8	9.03	1.52
10	15	53	0.9	3.8	3.12	1.25

Table 3: Statistical Parameter and regression model based on ANOVA

Response	Regression Model	Adjusted R^2	Model P-Value	% C.V.	Adequate Precision
R_t	5.70-1.97A-0.15B+0.11C-0.33AB+0.31AC+0.18BC	0.8631	<0.001	8.20	18.9
T	1.46-0.04A-0.02B-0.11C-0.08AB+0.0038AC-0.03BC	0.9126	<0.001	7.28	12.95

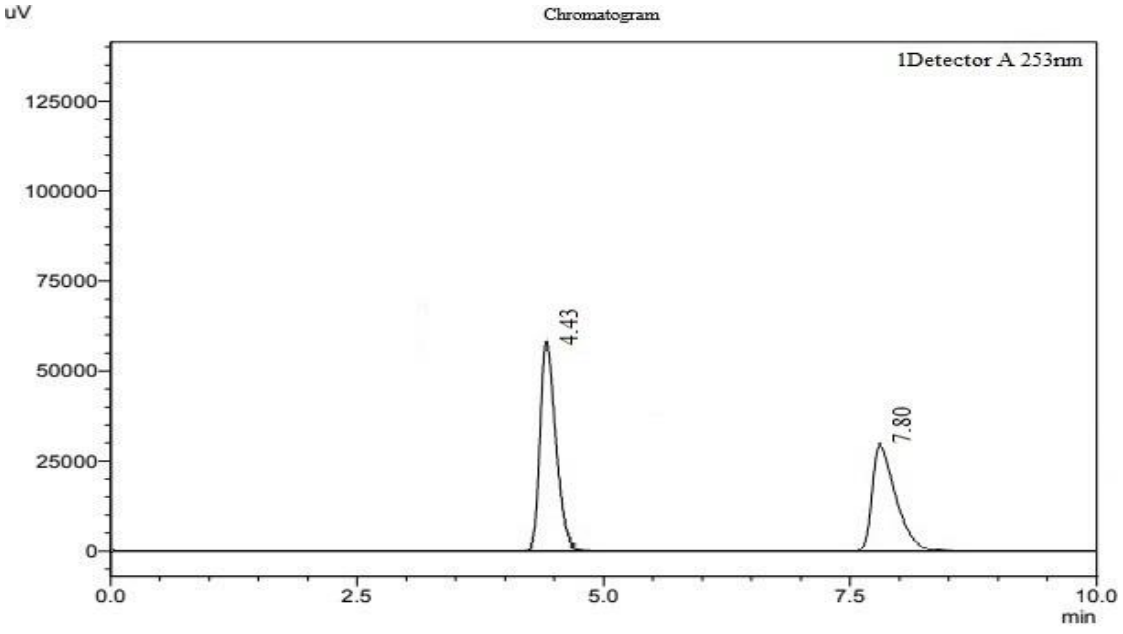


Figure 1: Chromatogram of mixture of CAN and NIF

Validation:

Method validation involves generating documentation that fulfills the requirements of the analytical application and evaluating the developed analytical method. Guidelines from ICH and FDA emphasize the importance of assessing specificity, linearity, precision, accuracy, system suitability, limits of detection and quantification, robustness, and ruggedness to ensure an effective experimental design based on the validated analytical method.

Specificity of the analytical technique was evaluated to confirm that excipients do not affect the retention times of CAN and NIF. An excipient mixture was prepared and injected to determine if it interfered with the retention times of CAN and NIF. No interference was observed in the retention times of CAN and NIF due to excipients.

For Candesartan, as the concentration increased from 4 to 24 mcg/ml, the mean area ranged from 3,128,628 to 18,758,592 µV.s. Correspondingly, the standard deviation (S.D.) of the mean area expanded from 7,583.43 to 93,497.56 µV.s, indicating variability across different concentrations. Despite this variability, the coefficient of variation (% RSD) remained consistently low, ranging from 0.15% to 0.50%, reflecting stable measurements relative to the mean.

Similarly, for Nifedipine, the mean area exhibited a rising trend across concentrations ranging from 5 to 30 mcg/ml, varying from 1,635,051 to 9,841,670 µV.s. With increasing concentrations, the standard deviation (S.D.) of the mean area also increased, spanning from 2,235.31 to 38,048.64 µV.s. However, akin to Candesartan, the coefficient of variation (% RSD) maintained a low range of 0.14% to 0.39%, indicating consistent measurements despite concentration variations.

The repeatability study for Candesartan (CAN) and Nifedipine (NIF) showed consistent results across six replicates. For CAN, at a concentration of 16 mcg/ml, the mean area was 12,437,360 ± 113,956 µV.s with a coefficient of variation (% RSD) of 0.91%. Meanwhile, for NIF, at a concentration of 20 mcg/ml, the mean area was 6,562,515 ± 29,728.78 µV.s, with a coefficient of variation (% RSD) of 0.45%.In the intraday precision study, again based on six replicates, the results varied slightly across concentrations for both CAN and NIF. For CAN, at a concentration of 12 mcg/ml, the mean area was 9,417,368 ± 7,489.03 µV.s, with a coefficient of variation (% RSD) of 0.14%. Meanwhile, for NIF, at a concentration of 15 mcg/ml, the mean area was 4,885,432 ± 4,125.46 µV.s, with a coefficient of variation (% RSD) of 0.57%.In the interday precision study, which also included six replicates, the results showed consistent measurements across different days for both CAN and NIF. For CAN, at a concentration of 12 mcg/ml, the mean area was 9,423,306 ± 9,039.04 µV.s, with a coefficient of variation (% RSD) of 0.79%. Meanwhile, for NIF, at a concentration of 15 mcg/ml, the mean area was 4,890,284 ± 2,458.76 µV.s, with a coefficient of variation (% RSD) of 0.12%. Overall, these findings indicate the reliability and reproducibility of the analytical method for both CAN and NIF.

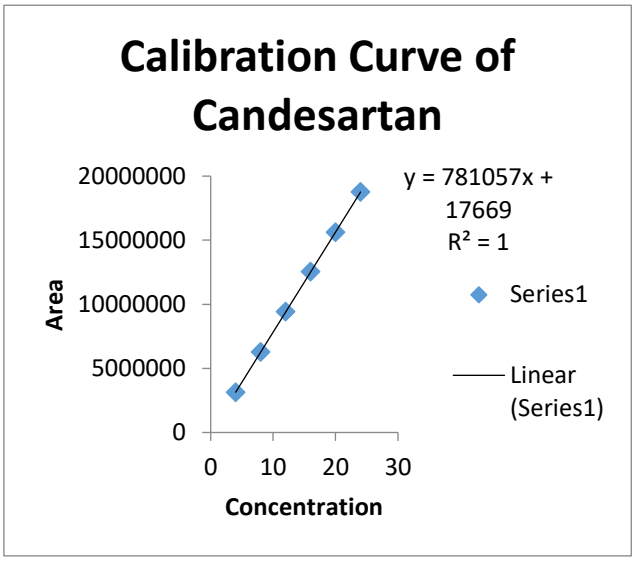
The drug recovery experiments for Candesartan (CAN) and Nifedipine (NIF) yielded consistent results. For CAN, at 50%, 100%, and 150% levels, mean recovery rates ranged from 98.12% to 99.33% with standard deviations between 0.09% and 0.32%. For NIF, recovery rates were between 98.76% and 99.46% with standard deviations ranging from 0.16% to 0.67%. These findings confirm the accuracy of the recovery process for both drugs across various concentration levels.

The tablet formulation assay results for Candesartan (CAN) and Nifedipine (NIF) synthetic mixtures are outlined in Table 4. The label claim concentrations for CAN and NIF were specified as 16 mcg/ml and 20 mcg/ml, respectively. Upon analysis, the measured concentrations in the synthetic mixture were determined to be 15.93 mcg/ml for CAN and 19.94 mcg/ml for NIF. The assay results indicate a high level of concordance with the label claims, with CAN demonstrating an assay percentage of 99.56% ± 0.69% and NIF exhibiting an assay percentage of 99.72% ± 0.82%. These findings suggest that the formulated tablets contain concentrations of CAN and NIF closely aligned with their labeled specifications, affirming the accuracy and reliability of the formulation process.

Table 1: Calibration curve of CAN and NIF

Candesartan			**Nifedipine**		
Conc. (mcg/ml)	Mean Area (µV. s) ±S.D.(n=3)	% RSD	Conc. (mcg/ml)	Mean Area (µV. s) ±S.D.(n=3)	% RSD

4	3128628± 7583.43	0.24	5	1635051± 2235.31	0.14
8	6259229± 26305.62	0.42	10	3263588± 6677.49	0.20
12	9413498±8986.67	0.15	15	4898621± 14463.03	0.29
16	12527640± 33309.26	0.26	20	6564475± 21865.67	0.33
20	15622257± 55413.25	0.35	25	8151046± 36363.57	0.45
24	18758592 ± 93497.56	0.50	30	9841670± 38048.64	0.39



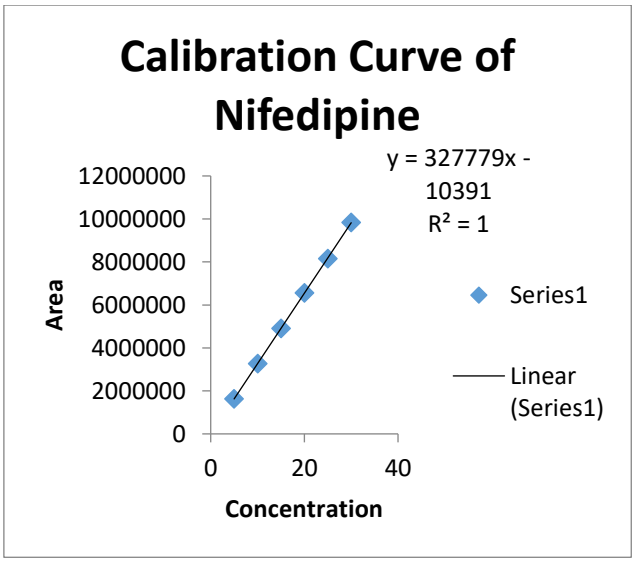


Figure 2(A): Calibration Curve of Curve of CAN
(B): Calibration curve of NIF

Table 2: Precision Study of CAN and NIF

Candesartan			**Nifedipine**		
Repeatability Study of CAN and NIF					
Concentration (mcg/ml)	Mean Area± S.D. (n=6)	% RSD	Concentration (mcg/ml)	Mean Area ± S.D.(n=6)	% RSD

16	12437360 ± 113956	0.91	20	6562515 ± 29728.78	0.45
Intraday precision study of CAN and NIF					
12	9417368±7489.03	0.14	15	4885432±4125.46	0.57
16	12521190±10928.32	0.29	20	6565479±25471.35	0.74
20	156223907±12983.9	0.43	25	8152904 ±29038.46	0.62
Inter day precision study of CAN and NIF					
12	9423306± 9039.04	0.79	15	4890284 ± 2458.76	0.12
16	12529903 ± 23780.02	0.46	20	6567347±10093.36	0.78
20	15638493 ± 22893.10	0.39	25	8139483±22093.44	0.43

Table 3: Accuracy Study of CAN and NIF

Drug	Level	Amount of sample taken (mcg/ml)	Amount of Std. spiked (mcg/ml)	Total Amt. of Drug	Amt. of Std. Recovery Mean	% Recovery
CAN	50	8	4	12	11.92	99.33±0.24
	100	8	8	16	15.68	98.12±0.09
	150	8	12	20	19.74	98.75±0.32
NIF	50	10	5	15	14.92	99.46±0.16
	100	10	10	20	19.78	98.90±0.67
	150	10	15	25	24.69	98.76±0.28

Table 4: Analysis of Pharmaceutical dosage Form

Tablet Formulation	Label claim (mcg/ml)		Amount found (mcg/ml)		% Assay ± S.D (n=3)	
	CAN	NIF	CAN	NIF	CAN	NIF
Synthetic Mixture	16	20	15.93	19.94	99.56 ± 0.69	99.72 ± 0.82

Conclusion:

In summary, the developed HPLC method, guided by a Design of Experiment (DoE) approach, effectively addresses the analytical challenges of simultaneous Candesartan (CAN) and Nifedipine (NIF) estimation in a combined dosage form. Through meticulous optimization using DoE principles, the method ensures accurate and efficient quantification of both medications in a single analysis. Leveraging reversed-phase HPLC coupled

with UV-Vis spectrophotometry detection, it offers a robust approach for concurrent CAN and NIF analysis, capitalizing on their distinct pharmacological properties in hypertension management.Validation studies, adhering to stringent ICH and FDA guidelines, confirm the method's reliability and accuracy. Specificity assessments demonstrate accurate detection of CAN and NIF without excipient interference. Precision studies reveal consistent and reproducible results, indicating the method's reliability for routine analysis. Accuracy studies show high recovery rates across different levels, validating its utility for quantitative analysis of CAN and NIF.Moreover, tablet formulation assay results affirm the method's practical applicability, demonstrating close alignment between measured concentrations and label claims for CAN and NIF. The assay percentages obtained underscore the method's accuracy in quantifying CAN and NIF in pharmaceutical dosage forms, validating its utility in quality control processes. Overall, this research contributes to enhancing hypertension management strategies, benefiting patient care and therapeutic outcomes.

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