Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue S-3 Year 2024 Page 312-320

Optimization Of Simultaneous Estimation Of Candesartan And Nifedipine In Combination Therapy For Hypertension Management: A Green HPLC Approach Using Design Of Experiment Methodology

Darshil B. Shah[1]*, 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*
[2]*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:**Darshil B Shah*
**Assistant Professor,L J Institute of PharmacyL J University,Ahmedabad, Gujarat (M)-9913175808 Email Address: darshil.shah@ljinstitutes.edu.in*

Abstract

Background: Hypertension is a prevalent health condition with severe implications for cardiovascular health. Combination therapy involving Candesartan and Nifedipine has emerged as a promising approach for managing hypertension due to the synergistic effects of these medications.
Methods: This study aimed to develop a Design of Experiment (DoE) based Green High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Candesartan and Nifedipine in a combined dosage form. Reversed-phase HPLC was utilized with UV-Vis spectrophotometry for detection. DoE principles were applied to optimize chromatographic conditions, including pH, ethanol percentage in the mobile phase, and flow rate.
Results: Optimal chromatographic separation was achieved using a composite mobile phase comprising ethanol and potassium dihydrogen phosphate buffer (45:55, v/v) at pH 3.7, with a flow rate of 1 mL/min. Statistical analysis confirmed the significance of these parameters in achieving efficient separation. The developed method exhibited high precision, accuracy, and reliability across various validation parameters, including specificity, linearity, repeatability, and robustness.
Conclusion: The proposed DoE-based Green HPLC method offers a sustainable and efficient approach for the simultaneous estimation of Candesartan and Nifedipine in hypertension management. By integrating green chemistry principles with systematic method development, this method provides a reliable analytical tool for routine quality control purposes in pharmaceutical formulations containing these medications.

Key words: Candesartan, Nifedipine, DoE, Validation, AGREE

Introduction

Hypertension, a prevalent health condition affecting millions globally, poses a significant risk for cardiovascular diseases and other complications. It is often asymptomatic but can lead to severe health consequences if left untreated, including an increased risk of heart disease, stroke, kidney damage, vision loss, and other complications. (1, 2) In the realm of hypertension management, numerous innovations are underway 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), exerts its antihypertensive effects by selectively blocking angiotensin II type 1 receptors. The key mechanisms include inhibition of vasoconstriction, aldosterone secretion, and sympathetic nervous system activity, 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. Its mechanisms involve inhibiting calcium influx, promoting vasodilation, reducing peripheral vascular resistance, and improving coronary blood flow by dilating arterioles and coronary arteries. (5)
The combination of Candesartan and Nifedipine offers a synergistic approach to blood pressure control by targeting distinct pathways involved in hypertension. Studies have shown that their combined therapy results in enhanced blood pressure reduction compared to monotherapy, emphasizing the complementary nature of their mechanisms of action for optimal efficacy in lowering blood pressure. (6)

Recent studies, such as the DISTINCT trial, have investigated the antihypertensive efficacy and safety of nifedipine GITS/candesartan combination therapy, demonstrating its ability to lower blood pressure effectively and improve side effect profiles across different patient populations. The DISTINCT trial, an 8-week randomized study, investigated the antihypertensive efficacy and safety of nifedipine GITS/candesartan combination therapy. Results from this trial showed that the combination therapy effectively lowered blood pressure and improved side effect profiles, regardless of race, highlighting its broad applicability and tolerability. (6, 7)

Analytical challenges in the simultaneous estimation of candesartan and nifedipine arise due to their structural similarities, which can pose difficulties in their quantification using traditional methods. (8) These similarities may lead to overlapping absorption spectra in UV-Vis spectrophotometry, making it challenging to differentiate between the two compounds accurately during analysis. Additionally, potential matrix effects from blood plasma can introduce interferences that affect the analysis of candesartan and nifedipine, further complicating their simultaneous quantification. (9)

These challenges underscore the need for advanced analytical techniques and methodologies to overcome these obstacles and ensure accurate and reliable estimation of both medications in combination therapy for hypertension management. (10) The combination of candesartan and nifedipine in hypertension management is further enhanced by innovative approaches such as Green HPLC and Design of Experiment (DoE) based HPLC methods. Green HPLC aligns with green chemistry principles, offering advantages like reduced solvent consumption, the use of less hazardous solvents, and minimized waste generation, contributing to a more sustainable analytical workflow. (11, 12, 13) By incorporating environmentally friendly solvents such as water, ethanol, acetonitrile, and methanol in Green HPLC, challenges in simultaneous quantification of candesartan and nifedipine can be effectively addressed. On the other hand, DoE-based HPLC methods utilize a systematic approach to method development, optimizing multiple variables simultaneously to ensure efficiency and quality in analysis. The integration of Green HPLC practices with DoE-based methodologies not only enhances the sustainability of analytical techniques but also improves the accuracy and reliability of quantifying candesartan and nifedipine in combination therapy for hypertension management. (14, 15, 16)

To address these challenges, the aim of the proposed work was to develop a Design of Experiment (DoE) based Green High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of candesartan and nifedipine in a combined dosage form. This study sought to optimize the chromatographic conditions using DoE principles to achieve accurate and efficient quantification of both medications in a single analysis. (17, 18, 19) The study focused on utilizing reversed-phase HPLC for the simultaneous estimation of candesartan and nifedipine, leveraging its effectiveness in separating compounds with different polarities. The

detection method employed was UV-Vis spectrophotometry, a common and reliable technique for quantifying pharmaceutical compounds.

To address these challenges, the aim of the proposed work was to develop a Design of Experiment (DoE) based Green High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of candesartan and nifedipine in a combined dosage form. This study sought to optimize the chromatographic conditions using DoE principles to achieve accurate and efficient quantification of both medications in a single analysis. The study focused on utilizing reversed-phase HPLC for the simultaneous estimation of candesartan and nifedipine, leveraging its effectiveness in separating compounds with different polarities. By integrating DoE principles with Green HPLC methodologies, this research aimed to establish an optimized and environmentally friendly analytical approach for the concurrent analysis of candesartan and nifedipine in a combined dosage form.

MATERIALS AND METHODS

1.2.1 Chemicals

Candesartan (CAN) and Nifedipine (NIF) API from Shaimil Laboratories Limited, Vadodara, Gujarat were collected as a gift sample. Several HPLC-grade solvents, like ethanol, water and methanol were obtained from Thermo Fisher Scientific India Pvt. Ltd., and orthophosphoric acid and phosphate buffer were obtained from Astron Chemicals India. All the solutions were prepared with mobile phase.

1.2.2 Statistical Analysis

Design-Expert v13.0.12.0 by Stat Ease was employed for the design of experiments (Central Composite Design), data analysis, and calculations of the desirability function. Microsoft Excel 2021 was used for the calculation of 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 1.36 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 3.7 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, 45 ml of ethanol and 35 ml of buffer (45:55) 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 (45 ethanol: 55 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 was accomplished employing a Shimadzu HPLC system (Shimadzu, Model LC 2010C HT Liquid Chromatograph) equipped with a serial dual plugger pump and UV detection system. LabSolutions software version 5.52 was employed for the chromatographic system operation and recording of data. The UV spectra were performed on a UV-1800 Shimadzu UV Spectrophotometer. Chromatographic separations were achieved on a Phenomenex Luna C (250 mm × 4.6 mm, 5 µm) C18 column. The composition of the mobile phase contained ethanol: 0.05 M potassium dihydrogen phosphate (KH_2PO_4) buffer (45:55) (3.7 pH, adjusted by 1% orthophosphoric acid) with a 1.0 mL/min flow rate. Each run involved the injection of 20 µL of sample, and detection was performed at a 253 nm wavelength with a run time of 10.0 minutes.

1.2.6 Experimental Design and Response Surface Methodology

For the optimization of % ethanol in the mobile phase, pH, and flow rate to achieve effective separation, a faced central composite design (FCCD) was implemented utilizing a partial factorial design approach. This design comprised five replicates at extreme levels, including center points and axial points. Utilizing

Derringer's desirability function, we assessed the R2 coefficient of determination for the polynomial models to ascertain the position of the factually optimal condition.

Results and Discussions

Method Development and Optimization:

The RP-HPLC methodology was systematically developed through the application of a Design of Experiment (DOE) paradigm, systematically exploring various permutations of three pivotal independent variables: pH, ethanol percentage in the mobile phase, and flow rate. The selection of a wavelength at 253 nm stemmed from a comprehensive 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 aforementioned compounds was attained using a composite mobile phase comprising Ethanol and 0.05 M potassium dihydrogen phosphate (KH_2PO4) buffer in a ratio of 45:55, maintained at a pH of 3.7, and administered at a flow rate of 1 mL/min. The precision and accuracy of the experimental model were subsequently enhanced through the application of a central composite design, yielding a robust second-order model for the response variable.
The Central Composite Design (CCD) is a statistical approach developed by G.F. Box and K.B. Wilson, used to create a streamlined experimental plan for a second-order model. This design minimizes the number of experiments needed while investigating factors such as flow rate, liquid pH, and acetonitrile content. The present study focused on studying retention time and tailing factor in 15 experiments, optimizing conditions by adjusting responses. The effective range was found to be 40% to 50% ethanol, a flow rate between 0.8 mL/min and 1.2 mL/min, and a pH between 3.6 and 3.8. Although the mathematical model is intricate, it aids in estimating responses. Applying this design to study CAN and NIF analyzed all paths to determine optimal conditions, affirming the model's suitability and significance for present study.
Table 3 shows the statistical parameters and regression model obtained from ANOVA which shows 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 were significant. The P Value for A factor Flow Rate and C factor Ethanol was less than 0.1 which indicates that it plays major role in retention time of the drug. The other parameters indicates that model is applicable for routine analysis. In tailing value of A parameter was less than 0.1 which indicates that it affects tailing of the drug. Here adjusted regression co efficient for both the drugs was higher than 0.8 which indicates drug gives linear response.
The Pertubation plot was plotted to identify the effect of each factor on responses of the drug. Here retention time of candesartan was majorly affected by the % of ethanol and flow rate while Nifedipine was affected by flow rate and pH.

Table 1: HPLC independent variables for CCD

Factor	**Name**	**Level (-)**	**Level (0)**	**Level (+)**
A	Flow rate (ml/min)	0.8	1	1.2
B	Buffer pH	3.6	3.7	3.8
C	Ethanol (%v/v)	40	45	50

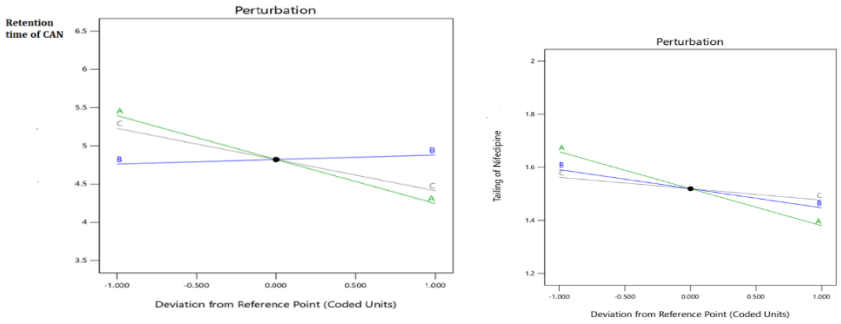
Table 2: Experimental conditions of HPLC and responses

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A:Flow Rate	B:pH	C:%Ethanol	Retention Time of Candesartan	Tailing of Nifedipine
		ml/min				
15	1	1	3.7	45	5.113	1.51
6	2	1.2	3.6	50	4.045	1.44
10	3	1.3	3.7	45	4.125	1.32
7	4	0.8	3.8	50	5.243	1.75
5	5	0.8	3.6	50	4.838	1.65
14	6	1	3.7	53	3.904	1.26
12	7	1	3.9	45	4.326	1.44
1	8	0.8	3.6	40	5.421	1.68
13	9	1	3.7	37	5.634	1.68
8	10	1.2	3.8	50	3.846	1.21

2	11	1.2	3.6	40	4.59	1.31
3	12	0.8	3.8	40	5.626	1.53
11	13	1	3.5	45	4.226	1.93
9	14	0.7	3.7	45	6.442	1.66
4	15	1.2	3.8	40	4.923	1.42

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	4.82-0.5750A+0.0590B-0.4082C-0.0595AB-0.0820AC-0.0415BC	0.9005	<0.001	7.47	17.64
T	1.52-0.1392A-0.0719B-0.0428C-0.0087AB-0.0337AC-0.0112BC	0.9126	<0.001	7.28	11.35



(A) (B)

Figure 1(A): Pertubation plot represents each factor effect on retention time of CAN
(B): Pertubation plot represents each factor effect on tailing factor of NIF

Derringer's Desirability Function (D)

Derringer's desirability function is a method used for optimizing factors in systems with multiple responses and targets. Table (4) outlines standards for enhancing individual responses, aiding in the selection of optimal experimental conditions. Design Expert 13 was employed to optimize criteria, with particular emphasis on retention time during method development. By employing a flow rate of 1 ml/min, ethanol concentration of 45% v/v, and buffer pH of 3.7, an exceptional desirability value (D=1.000) was achieved, pinpointing these coordinates as the ideal parameters for the proposed process.

Table 4: Under optimal conditions, the experimental and predictive value of different objective functions is compared

Flow rate (mL/min)	Buffer pH	ACN (%v/v)		R_t (min)	T	Total Desirability
			Experimental value	4.82	1.470	
1	3.7	45	Predicted value	4.73	1.314	1.000

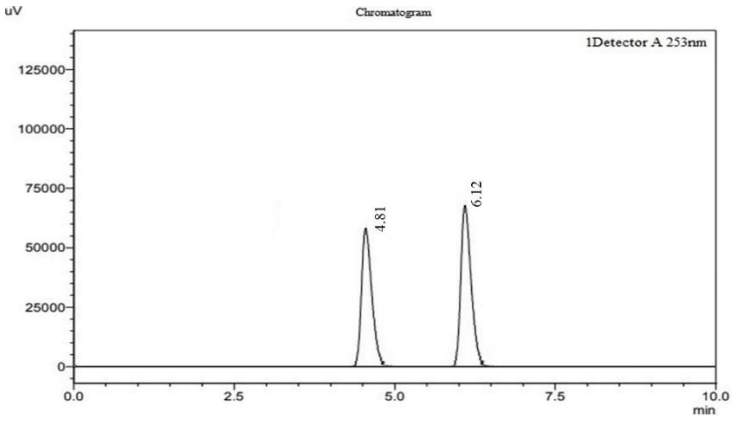


Figure 2: Chromatogram of mixture of CAN and NIF

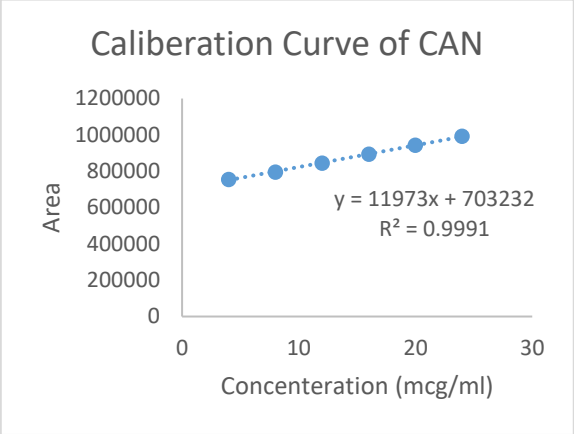
Validation:

Method validation entails producing documentation that meets the demands of the analytical application and assessing the developed analytical method. ICH and FDA guidelines advocate for the evaluation of specificity, linearity, precision, accuracy, system suitability, limits of detection and quantification, robustness, and ruggedness in order to ensure an effective experimental design based on the validated analytical method.
Specificity of the analytical technique was assessed to approve no intrusion of excipients on the retention times of CAN and NIF. An excipients mixture were prepared and injected to observe whether it is interfering with the retention times of CAN and NIF. No interference was reported on the retention times of CAN and NIF due to excipients.
The calibration curves for Candesartan and Nifedipine were systematically constructed over a range of concentrations, specifically 4, 8, 12, 16, 20, and 24 mcg/ml for Candesartan and 5, 10, 15, 20, 25, and 30 mcg/ml for Nifedipine (Table 1). The mean area under the curve (µV.s) ± standard deviation (S.D.) was rigorously ascertained via a triple-replicate analysis (n=3) at each concentration point. Figure 1A and 1B shows the calibration curve of Candesartan and Nifedipine with regression co-efficient of 0.9991 for both the drugs. The resulting data depicted a discernible and consistent linear augmentation in mean area with escalating concentrations for both Candesartan and Nifedipine. The percent relative standard deviation (% RSD) exhibited a range from 0.26% to 0.86% for Candesartan and 0.62% to 0.74% for Nifedipine, attesting to the methodological precision and reproducibility.
In the repeatability study, concentrations of 16 mcg/ml for Candesartan and 20 mcg/ml for Nifedipine were investigated, revealing mean areas of 898209.7 ± 5651.25 (0.63% RSD) and 2723249 ± 22149.36 (0.81% RSD), respectively. The intraday precision study encompassed concentrations of 12, 16, and 20 mcg/ml, demonstrating mean areas for Candesartan of 795287.3 ± 27775.48 (0.58% RSD), 895359 ± 36031.7 (0.61% RSD), and 940437.3 ± 6516.167 (0.71% RSD), and for Nifedipine of 1639243 ± 16336.13 (0.99% RSD), 2299036 ± 25634.18 (1.11% RSD), and 2755678 ± 36140.87 (1.32% RSD). The inter day precision study at concentrations of 12, 16, and 20 mcg/ml demonstrated mean areas for Candesartan of 793372 ± 2428.86 (0.31% RSD), 895346 ± 4262.47 (0.50% RSD), and 949166 ± 5238.99 (0.58% RSD), and for Nifedipine of 1623477 ± 10379.2 (0.64% RSD), 2312360 ± 21136.5 (0.91% RSD), and 2733574 ± 27953.2 (1.02% RSD). These results collectively validate the precision and reliability of the analytical methodology for both Candesartan and Nifedipine across varying concentrations and experimental conditions.
In the accuracy study of Candesartan (CAN) and Nifedipine (NIF), samples were prepared at three concentration levels: 50%, 100%, and 150% of the target amount. For CAN at 50%, 100%, and 150% levels, the mean recovery values were 99.41% ± 0.12, 99.37% ± 0.15, and 99.22% ± 0.22, respectively, demonstrating a high level of accuracy. Similarly, for NIF at 50%, 100%, and 150% levels, the mean recovery values were 99.61% ± 0.26, 100.60% ± 0.57, and 99.41% ± 0.35, respectively.

In the analysis of the pharmaceutical dosage form, specifically a tablet formulation containing a synthetic mixture of Candesartan (CAN) and Nifedipine (NIF), the label claim for CAN was 16 mcg/ml, and for NIF, it was 20 mcg/ml. Upon analysis, the amount found for CAN was 15.95 mcg/ml, and for NIF, it was 19.92 mcg/ml. The percentage assay, representing the accuracy of the formulation, yielded values of 99.68% ± 0.33 for CAN and 99.65% ± 0.49 for NIF (mean ± standard deviation, n=3).

Table 1: Calibration curve of CAN and NIF

Candesartan cilexetil			**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	755248± 4529.86	0.59	5	1081722± 8102.02	0.74
8	795887± 4682.15	0.50	10	1630139± 10125.1	0.62
12	844939±4750.98	0.46	15	2219555± 15326.45	0.69
16	893384± 5181.97	0.26	20	2752204± 18212.34	0.66
20	943320± 3612.22	0.35	25	3245503± 21536.75	0.63
24	992342.3 ± 8602.09	0.86	30	3764215± 27829.83	0.74



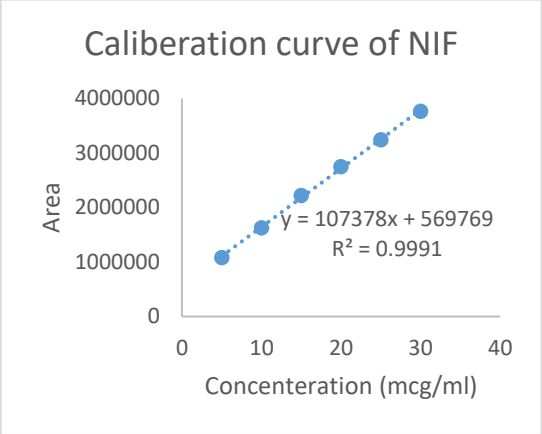


Figure 3(A): Calibration Curve of Curve of CAN (B): Calibration curve of NIF

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	898209.7 ± 5651.25	0.63	20	2723249 ± 22149.36	0.81
Intraday precision study of CAN and NIF					
12	795287.3±27775.48	0.58	15	1639243±16336.13	0.99
16	895359±36031.7	0.61	20	2299036±25634.18	1.11
20	940437.3±6516.167	0.71	25	2755678±36140.87	1.32
Inter day precision study of CAN and NIF					
12	793372±2428.86	0.31	15	1623477±10379.2	0.64
16	895346±4262.47	0.50	20	2312360±21136.5	0.91
20	949166±5238.99	0.58	25	2733574±27953.2	1.02

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	11.93	99.41±0.12
	100	8	8	16	15.90	99.37±0.15
	150	8	12	20	19.84	99.22±0.22
NIF	50	10	5	15	14.94	99.61±0.26
	100	10	10	20	20.12	100.60±0.57
	150	10	15	25	24.85	99.41±0.35

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	15.95	19.92	99.68 ± 0.33	99.65 ± 0.49

AGREE software

The analytical greenness metric (AGREE) is an innovative system designed for evaluating greenness based on the twelve principles of green analytical chemistry. These principles, known as the 12 SIGNIFICANCE principles, are utilized as input criteria, allowing for the flexibility to adjust weights for each principle. The

final assessment is derived from the sum of assessments assigned to each of the 12 input variables, resulting in a score ranging from 0 to 1. As depicted in Figure 2, the assessment outcome is presented in a clock-like circle, with the total score and color illustration depicted at the center. The current score for developed method is 0.87.

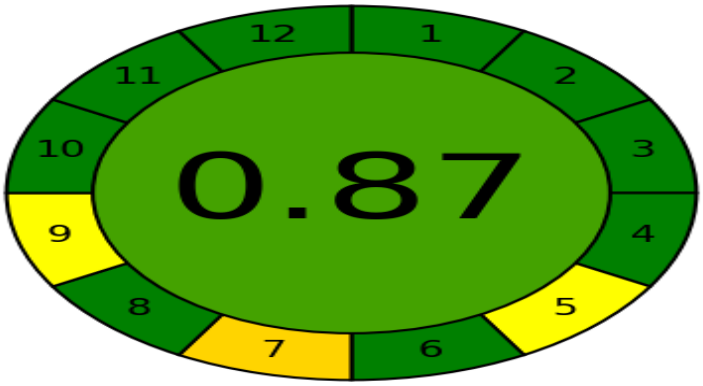


Figure 4: Agree Greenness Calculator

Conclusion:

In conclusion, the development of a Design of Experiment (DoE) based Green High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Candesartan and Nifedipine in a combined dosage form represents a significant advancement in the field of analytical chemistry, particularly in the context of hypertension management. Through systematic optimization of chromatographic conditions using DoE principles, robust and efficient method for the accurate quantification of both medications in a single analysis was achieved. The incorporation of Green HPLC practices, including the use of environmentally friendly solvents and reduced solvent consumption, aligns with principles of green chemistry, contributing to a more sustainable analytical workflow. Furthermore, the integration of DoE-based methodologies has facilitated systematic method development, optimizing multiple variables simultaneously to ensure efficiency and quality in analysis. The validation of the developed method has demonstrated its precision, accuracy, and reliability across various parameters, including specificity, linearity, repeatability, and robustness. Additionally, the analysis of a pharmaceutical dosage form containing a synthetic mixture of Candesartan and Nifedipine has confirmed the applicability of the method for routine quality control purposes

References

1. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7). Hypertension. 2003;41(6):1069-1116.
2. Wright JM, Pickering TG. Prognostic significance of asymptomatic hypertension. Arch Intern Med. 2003;163(18):2152-2160.
3. Agabwu MA, Adebayo GI, Onakpoya RT. The efficacy and safety of combination therapy for hypertension: a systematic review and meta-analysis. Int J Cardiol. 2017;242:130-138.
4. Bakris GL, Weir MR, Colucci WG, Claggett B, Needs M, Brown NJ, et al. ACC/AHA/ASH/TOS guideline for the management of high blood pressure in the adult patient: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, American Association of State and Territorial Health Officials, National Heart, Lung, and Blood Institute, Society of Hypertension, and American Society of Nephrology. Hypertension. 2014;63(e1):e1-e177.
5. Dunning AM, Ferrario CM. Calcium channel blockers in hypertension. Circulation. 2010;122(25):2465-2475.
6. McDonough FE, Ferdinand KC. Angiotensin-II receptor blockers for hypertension. Cochrane Database of Systematic Reviews. 2010;(12).
7. Kjeldsen SE, Cha G, Villa G, Mancia G, the DISTINCT Investigators. Nifedipine plus candesartan combination increases blood pressure control regardless of race and improves the side effect profile: DISTINCT randomized trial results. PLoS One. 2014;9(2):e88220.

8. Kumar V, Prasad VV, Rambabu N. A validated RP-HPLC method for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in their combined dosage form. Asian J Pharm Clin Res. 2012;5(1):71-75.
9. Rajani U, Reddy BS, Rambabu N. A validated RP-HPLC method for the simultaneous determination of amlodipine and olmesartan medoxomil in human plasma. J Chromatogr Sci. 2009;47(7):603-607.
10. Gaikwad SR, Wadodkar SB, Sawant SD. Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of candesartan cilexetil and hydrochlorothiazide in pharmaceutical dosage form. J Sep Sci. 2014;37(19-20):2746-2753.
11. Mousavi SM, Moghadam MRT. Green analytical chemistry: an overview of its goals and benefits. Quím Nova. 2010;33(2):483-489.
12. Pawar SS, Khobragade MM. A validated green HPLC method for the simultaneous determination of ramipril and hydrochlorothiazide in their combined dosage form. Arab J Chem. 2016;9(1):120-127.
13. Aires BC, Pereira HM, Rosa JM. Greener analytical methodologies for the determination of pharmaceuticals in environmental samples: a review. Talanta. 2012;97:125-133.
14. Zhang Y, Liu Y, Zhou Y, Sun Y, Liu Z. Recent advances in design-of-experiment-assisted method development for pharmaceutical analysis. TrAC Trends in Analytical Chemistry. 2023;140:116332.
15. Li H, Zhu Y, Zhang H, Yao L, Li S. Optimization of a Stability-Indicating HPLC Method for the Determination of Five Antidepressants in Pharmaceutical Formulations Using a Design of Experiment Approach. Chromatographia. 2022;95(1-2):189-202.
16. Heine T, Herain M. A Review on Green Solvents for Analytical Chemistry. ChemSusChem. 2014;7(18):4618-4630.
17. Xu H, Sun Y, Liu Z. Design of experiments (DoE) for method development and optimization in analytical chemistry. TrAC Trends in Analytical Chemistry. 2018;109:142-152.
18. Poojary MM, Jain S. DoE (design of experiment) approach for development and optimization of chromatographic separations in pharmaceutical analysis. J Pharm Anal. 2014;4(4):227-234.
19. Zhao YZ, Wang JN, Zhang YQ. Recent developments in hyphenated chromatographic techniques and their applications in pharmaceutical analysis. J Pharm Biomed Anal. 2012;69:1-17.