World Journal of Pharmaceutical and Life Sciences

Impact Factor: 8.21 www.wjpls.org Coden USA: WJPLA7

COMPARATIVE STABILITY STUDY OF NANO FORMULATION CONTAINING CARVEDILOL AND BERBERINE

Shubham Singh[1]*, Pradeep Kumar Yadav[2], A. K. Singhai[3]

[1]Student- School of Pharmacy, LNCT University, Bhopal (M.P.)
[2]Associate Professor - School of Pharmacy, LNCT University, Bhopal (M.P.)
[3]Director- School of Pharmacy, LNCT University, Bhopal (M.P.)
Department of Pharmaceutical Chemistry, School of Pharmacy, LNCT University, Bhopal Madhya Pradesh, 462042.

***Corresponding Author: Shubham Singh**

Student- School of Pharmacy, LNCT University, Bhopal (M.P.)

DOI: https://doi.org/10.5281/zenodo.20134339

How to cite this Article: Shubham Singh[1]*, Pradeep Kumar Yadav[2], A.K. Singhai[3] (2026). Comparative Stability Study Of Nano Formulation Containing Carvedilol And Berberine. World Journal of Pharmaceutical and Life Sciences, 12(5), 370–380.

Article Received on 21/03/2026 **Article Revised on 11/04/2026** **Article Published on 01/05/2026**

ABSTRACT

The present study provides a detailed comparative evaluation of the stability of Nano formulations containing Carvedilol and Berberine, with emphasis on quantitative performance under different storage conditions. The optimized Nano formulations exhibited a mean particle size of 145.2 ± 2.6 nm for carvedilol-loaded nanoparticles and 152.8 ± 3.1 nm for berberine-loaded nanoparticles, while the co-loaded Nano formulation showed a slightly increased size of 160.4 ± 2.9 nm, indicating uniform Nano scale distribution. The zeta potential values were found to be −28.6 ± 1.4 mV (carvedilol), −30.2 ± 1.6 mV (berberine), and −32.5 ± 1.3 mV (combined), suggesting strong electrostatic stabilization and reduced aggregation tendency. Entrapment efficiency was significantly higher in the co-loaded system (85.7 ± 2.0%) compared to individual formulations (78.5 ± 2.2% for carvedilol and 81.3 ± 2.5% for berberine), reflecting improved drug incorporation within the Nano carrier matrix. Stability studies were carried out over a period of 90 days under both intermediate (25°C ± 2°C/60% RH) and accelerated (40°C ± 2°C/75% RH) conditions. The results indicated that all formulations maintained good stability; however, the co-loaded Nano formulation demonstrated superior performance. Particle size showed only a marginal increase of 3.2% in the combined system, compared to 4.8% and 4.5% in carvedilol and berberine formulations, respectively. Drug content remained above 95% in all cases, with the co-loaded formulation retaining 97.2% of its initial drug concentration, whereas individual formulations retained 95.5% (carvedilol) and 95.9% (berberine). Furthermore, the percentage drug degradation was lowest in the combined system (2.8%) compared to 4.5% and 4.1% observed for carvedilol and berberine Nano formulations, respectively. These findings suggest that the co-encapsulation of carvedilol and berberine within a single Nano formulation not only enhances entrapment efficiency but also improves resistance to environmental stress factors such as temperature and humidity. The improved stability can be attributed to the protective effect of the Nano carrier system and possible intermolecular interactions between the drugs, which reduce degradation pathways. Overall, the study confirms that co-loaded Nano formulations offer a more stable and efficient drug delivery system, potentially leading to enhanced therapeutic efficacy, improved patient compliance, and extended shelf life compared to conventional and single-drug Nano formulations.

KEYWORDS: Particle size showed only a marginal increase of 3.2% in the combined system, compared to 4.8% and 4.5% in carvedilol and berberine formulations, respectively.

1. INTRODUCTION

Nanoparticles are colloidal carrier systems generally ranging in size from 10 to 1000 nm, in which the drug may be dissolved, entrapped, encapsulated, or adsorbed within a polymeric matrix. Due to their extremely small particle size and large surface area, nanoparticles exhibit improved interaction with biological membranes, leading to enhanced absorption and therapeutic efficiency **(Salatin *et al.*, 2015).** Polymeric nanoparticles, particularly those prepared using biodegradable

polymers, are widely preferred because of their biocompatibility, non-toxicity, and sustained release characteristics. Among the various polymers available, poly lactic-co-glycolic acid (PLGA) is one of the most extensively used polymers in nanoparticle formulation **(Ansary *et al.*, 2014).** PLGA is approved for pharmaceutical use because it degrades into lactic acid and glycolic acid, which are naturally metabolized in the body. PLGA nanoparticles can protect drugs from degradation, improve drug encapsulation, prolong release, and enhance therapeutic outcomes **(Sun *et al.*, 2024).**

Carvedilol is a non-selective beta-adrenergic blocking agent widely used in the treatment of hypertension, congestive heart failure, myocardial infarction, and other cardiovascular disorders **(Gupta *et al.*, 2023).** In addition to its beta-blocking activity, Carvedilol possesses antioxidant and vasodilatory properties, which contribute to its cardioprotective effects. However, Carvedilol belongs to Biopharmaceutical Classification System (BCS) Class II drugs and exhibits poor aqueous solubility and low oral bioavailability due to extensive first-pass metabolism **(Hafez *et al.*, 2024).** These drawbacks limit its therapeutic efficiency and require higher doses to achieve the desired effect. Incorporation of Carvedilol into nanoparticle systems has therefore been investigated as an effective strategy to improve its solubility, dissolution rate, absorption, and sustained delivery **(Fernandes *et al.*, 2018).**

Berberine is a naturally occurring isoquinoline alkaloid isolated from several medicinal plants including *Berberis aristata*, *Coptis chinensis*, and *Hydrastis canadensis*. Berberine has attracted significant attention because of its broad range of pharmacological activities such as antidiabetic, antihyperlipidemic, antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects **(An *et al.*, 2022).** It has been widely studied for the management of metabolic disorders, particularly diabetes mellitus and hyperlipidemia. Despite its remarkable therapeutic potential, Berberine suffers from poor bioavailability due to low gastrointestinal absorption, poor permeability, and rapid metabolism **(Ai *et al.*, 2021).** These limitations reduce its clinical effectiveness when administered through conventional dosage forms. Nanoformulation approaches have therefore been explored to improve the pharmacokinetic behavior and therapeutic efficacy of Berberine **(Jawed Khan *et al.*, 2023).**

Combination therapy involving Carvedilol and Berberine may provide synergistic therapeutic benefits in the management of cardiovascular and metabolic disorders. Carvedilol helps reduce blood pressure and cardiac workload, while Berberine contributes through glucose-lowering, antioxidant, and lipid-regulating mechanisms **(Zhang *et al.*, 2016).** A nanoparticle formulation containing both drugs may improve their stability, enhance bioavailability, provide sustained drug release, and reduce dose-related side effects. Such combined nanoformulations may therefore represent a promising strategy for improving therapeutic performance and patient compliance **(Xie *et al.*, 2021).**

The present study was therefore designed to perform a comparative stability study of nanoparticle formulations containing Carvedilol and Berberine prepared using PLGA as the polymeric carrier and PVA as the stabilizing agent.

2. MATERIAL AND METHODS

2.1 Chemicals

Berberine and Carvedilol was procured from Sigma-Aldrich, USA. Ethanol and DMSO was received from Rankem. Methanol was acquired from Merck, India. PLGA (Poly(lactic-co-glycolic acid)) was procured from Evonik Industries, Germany while, Polyvinyl Alcohol (PVA) was procured from Loba Chemie, India.

2.2 Preliminary study

2.2.1 Organoleptic study

In the initial phases of preformulation studies, the characterization of a standard pure drug begins with an organoleptic evaluation. The appearance and color of the substance was ascertained by evaluating its texture, and important information about its odor profile was obtained by evaluating its smell. **(A B D Selvam, 2010).**

2.2.2 Solubility study

Solubility is defined as the maximum amount of a drug that can dissolve in a specific solvent at a controlled temperature to form a homogeneous solution. According to the United States Pharmacopeia (USP), solubility is classified based on the volume of solvent required to dissolve 1 g of solute, ranging from very soluble to practically insoluble. The equilibrium solubility of the drug was determined using the shake-flask method, in which an excess quantity of drug was added to selected solvents to obtain a saturated solution. After equilibration, the samples were visually examined to assess the solubility behavior of the drug in different solvents. **(Edit Baka *et al.*, 2008).**

2.2.3 Melting point determination

As an essential part of the physical characterization of the drug, the melting point was determined . The capillary tube method was used to find the drug's melting point. **(Fei Mao *et al.*, 2016).**

2.2.4 pH determination

Carvedilol and Berberine were dissolved in distilled water in order to measure the pH of the pure drugs samples for the preformulating investigation. To make sure the systems reached equilibrium, the solution were stirred and sonicated for a predetermined amount of time. To guarantee measurement accuracy, the pH meter was calibrated using standard buffer solutions with pH values of 7.0 and 10.0 prior to analysis. Once the reading stabilized at room temperature, the glass electrode was

submerged in sample, and the pH value was noted **(Hammer *et al.*, 2014).**

2.3 Identification of pure drug

2.3.1 Fourier transform Infrared spectroscopy (FTIR)

The structural characterization and identification of the pure drug samples were performed using Fourier Transform Infrared (FTIR) spectroscopy. Initially, the analysis was conducted utilizing a Perkin Spectrum BX spectrophotometer. Sample preparation involved a standardized potassium bromide (KBr) pellet technique, where approximately 2% of the finely powdered drug was mixed with dried KBr to produce a 50 mg pellet. The infrared spectra were recorded across a scanning range of 400 to 4000 cm^{-1}. The resulting spectral data provided comprehensive insights into the molecular vibrations associated with specific functional groups. This analysis served to verify the structural integrity of the compounds and confirmed the identity of the drug samples through the comparison of characteristic absorption bands **(Bansal, R. *et al.*, 2021).**

2.4 Simultaneous estimation method development by UV spectroscopy

Standard stock solutions of Carvedilol and Berberine were prepared separately by dissolving accurately weighed 10 mg quantities of each drug in 10 mL of acetonitrile to obtain concentrations of 1000 µg/mL. From these primary stock solutions, 1 mL aliquots were further diluted to 10 mL with acetonitrile to prepare sub-stock solutions of 100 µg/mL. Appropriate dilutions of the sub-stock solutions were then made to obtain working standard solutions in the concentration range of 4–12 µg/mL for Berberine and 5–25 µg/mL for Carvedilol. These solutions were used for linearity studies and determination of absorptivity constants for the simultaneous equation method. The working standard solutions were individually scanned in the UV-visible region using a double-beam SHIMADZU UV-1700 Pharma Spec spectrophotometer to determine the wavelength of maximum absorbance (λmax) of both drugs.

2.5 Simultaneous estimation (Shubhangi Bhalerao et al., 2024)

The simultaneous equation method, also known as the Vierordt method, was used for the simultaneous estimation of Carvedilol and Berberine in a mixture, as both drugs absorb at each other's λmax. In this method, the absorptivity values of Carvedilol (ax1 and ax2) and Berberine (ay1 and ay2) were determined at two selected wavelengths, λ1 and λ2. The absorbances of the mixture at these wavelengths were recorded as A1 and A2. Based on the principle of additive absorbance, two simultaneous equations were formed.

$A1 = (ax1 \times Cx) + (ay1 \times Cy)$ and

$A2 = (ax2 \times Cx) + (ay2 \times Cy)$,

where Cx and Cy represent the concentrations of Carvedilol and Berberine, respectively. By solving these equations using the derived mathematical expressions, the concentrations of both drugs in the sample were accurately determined.

2.6 Method validation by UV Spectrophotometry

Method validation is the process of verifying that the analytical technique used for a particular test is appropriate for its intended use.

2.6.1 Linearity

The linearity of an analytical procedure is the ability (within a given range) to obtain test results, which are directly proportional to the concentration of an analytes in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analytes in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Linearity was established over a concentration range of 5-25µg/ml for Carvedilol, while the studied range for Berberine was 4-12µg/ml **(Moosavi and Ghassabian 2018).**

2.6.2 Precision

The precision of analytical procedure expresses closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions **(Vidushi *et al.*, 2017).** It may be considered at three levels: it is expressed as standard deviation or coefficient of variation.

%RSD = (standard deviation / mean) x100

- **Repeatability:** This parameter was evaluated by assessing the precision under identical operating conditions over a short interval of time, utilizing six replicates (n=6).
- **Intermediate Precision:** This expressed the variations occurring within the same day across three distinct time intervals; since sampling was performed in triplicate at each interval, a total of nine samples were analyzed (n=9).
- **Reproducibility:** This accounted for within-laboratory variations across three different days. Sampling was conducted in triplicate each day, resulting in a total of nine replicates (n=9).

2.6.3 Robustness

The robustness of an analytical procedure is a measured of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was assessed by having two different analysts perform the sampling in triplicate within the same laboratory **(Collins *et al.*, 2017).**

2.6.4 Ruggedness

The Ruggedness of an analytical procedure is the reproducibility of test result obtained by the analysis of

the same sample under variety of condition, such as different laboratories, different analyst, and different assay temperature. In this study Ruggedness was established by analyzing samples in triplicates at two various temperatures **(Karageorgou and Samanidou 2014).**

2.7 Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.
Detection Limit (DL) = 3.3*(σ/S)
Where σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

2.7.1 Limit of quantification

The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Quantization Limit (QL) = 10*(σ/S)
Where, σ= the standard deviation of the response
S= the slope of the calibration curve

2.8 Formulation of nanoparticle

Nanoparticles of Berberine, Carvedilol, and their combined formulation were prepared by the solvent evaporation method using PLGA as the polymer. Accurately weighed amounts of Berberine, Carvedilol, or both drugs in a 1:1 ratio, along with 300 mg of PLGA, were dissolved in methanol to form the organic phase. An aqueous phase containing 0.3% w/v polyvinyl alcohol (PVA) was prepared separately as a stabilizer. The organic phase was added dropwise into the aqueous phase under continuous stirring to form an oil-in-water emulsion, followed by probe sonication for 10 minutes to reduce particle size and obtain a stable nanoemulsion. The mixture was further stirred at room temperature for solvent evaporation, resulting in nanoparticle formation. The nanoparticles were collected by centrifugation, washed with distilled water to remove unentrapped drug and excess stabilizer, and finally dried and stored in a desiccator for further evaluation. (Muthu *et al.*, 2009).

Table 1: Ingredients used in nanoparticle formulation.

Name of Ingredient	Formulation I (Berberine)	Formulation II (Carvedilol)	Formulation III (Combined)
Berberine	100 mg	—	50 mg
Carvedilol	—	100 mg	50 mg
PLGA	300 mg	300 mg	300 mg
Polyvinyl Alcohol (PVA)	0.3%	0.3%	0.3%
Methanol (Solvent)	10 mL	10 mL	10 mL
Sonication Time	10 min	10 min	10 min
Distilled Water	q.s	q.s	q.s

2.9 Evaluation parameter of Nanoparticle formulation

2.9.1 Organoleptic properties

The organoleptic properties of the prepared nanoparticle formulations were evaluated by visual and sensory inspection. The formulations were examined for color, odor, clarity, and overall homogeneity **(Bilous and Kovalevska, 2019).**

2.9.2 Particle size

Particle size is a critical parameter for the characterization of nanoparticles, as it can influence drug release, stability, and bioavailability. The particle size of the prepared nanoparticles was determined using a Malvern Zetasizer (Malvern Instruments) **(Akbari *et al.*, 2011)**

2.9.3 Zeta potential

Zeta potential is an important parameter used to assess the surface charge and stability of nanoparticles. The zeta potential of the prepared nanoparticles was measured using a Malvern Zetasizer (Malvern Instruments) **(Tantra *et al.*, 2010).**

2.9.4 Entrapment efficiency

Entrapment efficiency (EE%) was determined to evaluate the amount of drug successfully encapsulated within the nanoparticles. An accurately weighed quantity of nanoparticles was dispersed in a suitable solvent and centrifuged at 10,000 rpm for 30 minutes to separate the free drug from the entrapped drug. The supernatant containing the unencapsulated drug was collected, and the drug concentration was measured using a UV–visible spectrophotometer at the characteristic wavelength of the drug. High entrapment efficiency indicates effective drug loading and successful nanoparticle formulation. The entrapment efficiency was calculated using the following formula: **(Song *et al.*, 2008).**
Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc.*100

2.9.5 Scanning Electron Microscopic (SEM)

Scanning Electron Microscopy (SEM) was used to evaluate the surface morphology, shape, and size of the prepared nanoparticles. For analysis, a small quantity of lyophilized nanoparticles was mounted on a metallic stub and coated with a thin gold layer using a sputter coater to improve conductivity. The sample was then examined under a scanning electron microscope at suitable

magnification to observe particle morphology and surface characteristics. SEM analysis helped confirm the spherical shape, surface smoothness, and nanoscale size of the nanoparticles, supporting the particle size results obtained from Zetasizer analysis **(Mohan and Renjanadevi, 2016).**

2.10 Stability Studies (Combined Nanoparticle Formulation)

The optimized combined Berberine and Carvedilol nanoparticle formulation was subjected to accelerated stability studies by storing it at 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/70 ± 5% RH for three months in a stability chamber. The formulation was evaluated at intervals of 0, 30, 60, and 90 days for parameters such as particle size, polydispersity index, and entrapment efficiency. The study was conducted according to ICH guidelines, and the results obtained at each interval were compared with the initial values to identify any significant changes. The findings confirmed the physical and chemical stability of the combined nanoparticle formulation and demonstrated its suitability for short-term storage **(González et al., 2022).**

3. RESULT AND DISCUSSION

3.1 Preliminary study.

3.1.1 Organoleptic properties of Berberine and Carvedilol.

Table 2: Organoleptic Properties of Berberine and Carvedilol.

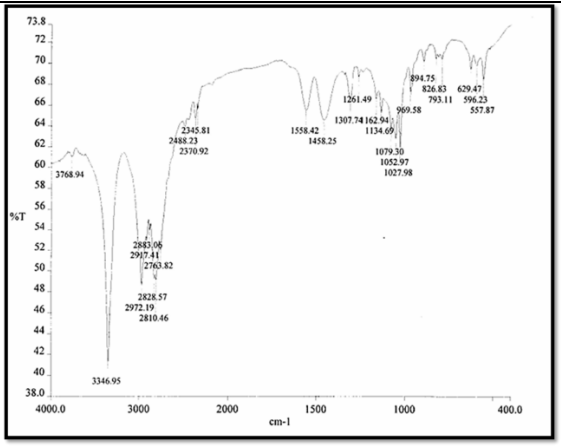
Organoleptic Properties	Berberine	Carvedilol
Colour	Yellowish	White
Odour	Odourless	Odourless
Physical appearance	Crystalline Powder	crystalline powder
State	Solid	Solid

3.1.2 Melting point and pH of Berberine and Carvedilol.

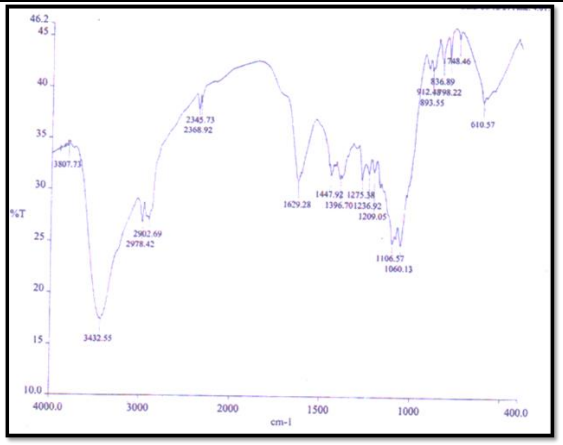
Table 3: Melting point and pH of Berberine and Carvedilol.

Drug	Reference (pH)	Observed (pH)	Reference (Melting point)	Observed (Melting point)
Berberine	4-7pH	6.3 pH	145- 146 ºC	144 ºC
Carvedilol	7-8pH	7.5 pH	114- 119 ºC	115 ºC

3.2 Identification of Carvedilol and Berberine standard compound by FTIR



Graph 1: FTIR of pure Carvedilol drug



Graph 2: FTIR of pure Berberine drug

Table 4: FTIR Interpretation of Carvedilol.

Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
3400-3300 (cm^{-1})	3346.95	N-H stretching	aliphatic primary amine
3000-2840 (cm^{-1})	2972.19	C-H stretching	Alkane
2600-2550 (cm^{-1})	2488.23	S-H stretching	Thiol
1550-1500 (cm^{-1})	1458.25	N-O stretching	Nitro compound
1275-1200 (cm^{-1})	1261.49	C-O stretching	Alkyl aryl ether
1070-1030 (cm^{-1})	1052.97	S=O stretching	Sulfoxide

Table 5: FTIR Interpretation of Berberine.

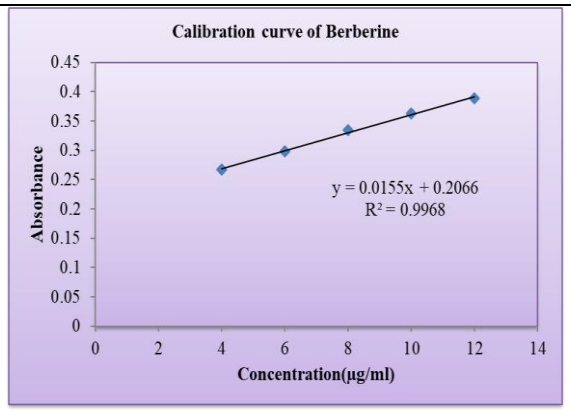
Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
3500- 3400 (cm^{-1})	3432.55	N-H Stretching	Primary amine
3000-2840 (cm^{-1})	2902.69	C-H Stretching	Alkane

2600-2550 (cm^{-1})	2368.92	S-H Stretching	Thiol
1648-1638 (cm^{-1})	1629.28	C=C Stretching	Alkene
1410-1380 (cm^{-1})	1396.70	S=O Stretching	sulfonyl chloride
1070-1030 (cm^{-1})	1060.13	S=O Stretching	Sulfoxide

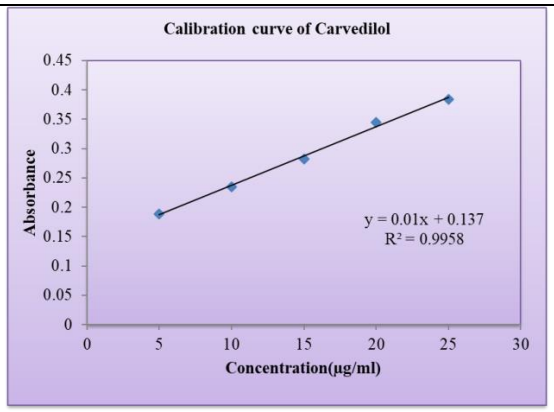
3.3 Simultaneous equation method

Table 6: Simultaneous estimation of Berberine and Carvedilol.

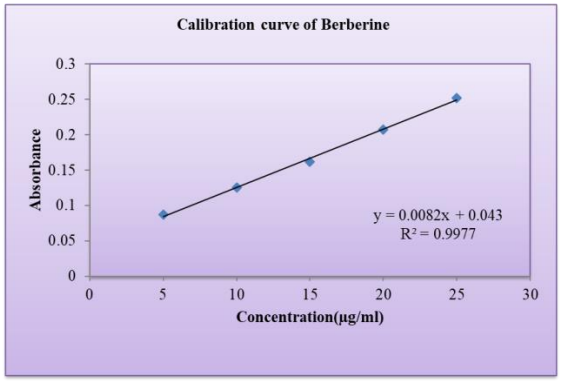
Conc.(µg/ml)		Absorbance			
Berberine	Carvedilol	Berberine		Carvedilol	
		231.0nm	242.0nm	231.0nm	242.0nm
4	**5**	0.267	0.126	0.087	0.189
6	**10**	0.298	0.158	0.125	0.235
8	**15**	0.334	0.203	0.162	0.282
10	**20**	0.363	0.236	0.207	0.345
12	**25**	0.389	0.265	0.252	0.384
Conc.(µg/ml)		**Absorptivity**			
Berberine	**Carvedilol**	**Berberine**		**Carvedilol**	
		231.0nm	**242.0nm**	**231.0nm**	**242.0nm**
4	**5**	0.066	0.031	0.017	0.037
6	**10**	0.049	0.026	0.012	0.023
8	**15**	0.041	0.025	0.010	0.018
10	**20**	0.036	0.023	0.010	0.017
12	**25**	0.032	0.022	0.010	0.010



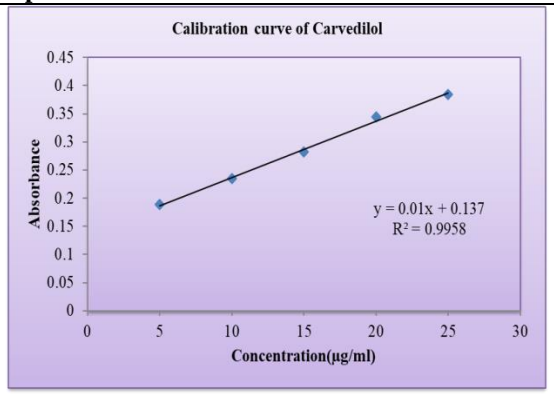
Graph 3: Calibration curve of Berberine at 231.0nm



Graph 4: Calibration curve of Berberine at 242.0nm



Graph 5: Calibration curve of Carvedilol at 231.0nm



Graph 5: Calibration curve of Carvedilol at 242.0nm

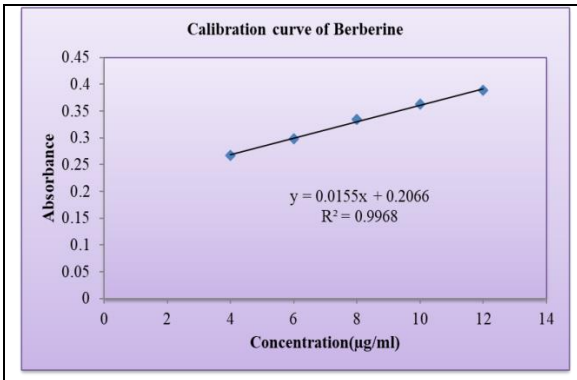
3.4 Method validation

3.4.1 Linearity and range

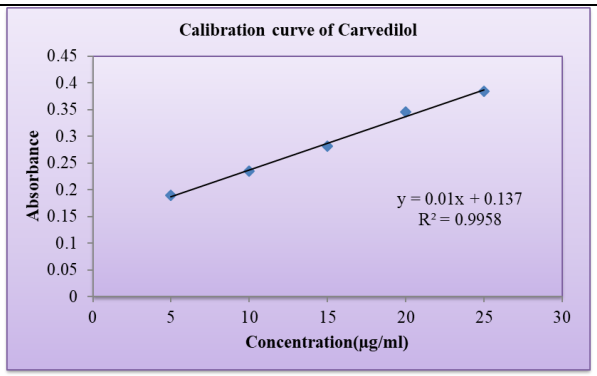
Table 7: Calibration data of Berberine at 231.0nm and Carvedilol at 242.0nm.

Conc. (µg/ml)	Mean Abs	Abs1	Abs2	Abs3	Conc. (µg/ml)	Mean Abs	Abs1	Abs2	Abs3
	Berberine at 231.0nm					Carvedilol at 242.0nm			
4	0.267	0.268	0.262	0.272	**5**	0.189	0.193	0.183	0.192

6	0.298	0.304	0.297	0.293	**10**	0.235	0.235	0.234	0.239
8	0.334	0.337	0.332	0.334	**15**	0.282	0.286	0.282	0.279
10	0.363	0.362	0.368	0.359	**20**	0.345	0.347	0.342	0.348
12	0.389	0.395	0.387	0.385	**25**	0.384	0.388	0.379	0.385
Mean	0.330	0.333	0.329	0.328	**Mean**	0.188	0.093	0.083	0.092
SD	0.002				**SD**	0.003			
%RSD	0.757				**%RSD**	1.065			



Graph 6: Calibration curve of Berberine at 231.0nm



Graph 7: Calibration curve of Carvedilol at 242.0nm

3.4.2 Precision study

3.4.2.1 Repeatability

Table 8: Repeatability of Berberine at 231.0nm and Carvedilol at 242.0nm.

Concentration (µg/ml)	Absorbance of Berberine	Concentration (µg/ml)	Absorbance of Carvedilol
6	0.298	10	0.234
6	0.294	10	0.233
6	0.297	10	0.233
6	0.294	10	0.236
6	0.296	10	0.235
6	0.298	10	0.236
Mean	0.296	**Mean**	0.234
SD	0.001	**SD**	0.001
% RSD	0.619	**% RSD**	0.587

3.4.2.2 Intraday Precision.

Table 10: Result of Intraday precision of Berberine at 231.0nm and Carvedilol at 242.0nm.

Concentration (µg/ml)	Absorbance (Morning)	Absorbance (Afternoon)	Absorbance (Evening)	Concentration (µg/ml)	Absorbance (Morning)	Absorbance (Afternoon)	Absorbance (Evening)
	Berberine at 231.0nm				Carvedilol at 242.0nm		
6	0.302	0.292	0.297	10	0.238	0.236	0.233
6	0.298	0.294	0.298	10	0.234	0.237	0.232
6	0.305	0.295	0.302	10	0.236	0.238	0.234
Mean	0.301	0.293	0.299	**Mean**	0.236	0.237	0.233
SD	0.003	0.001	0.002	**SD**	0.002	0.001	0.001
%RSD	1.164	0.520	0.884	**%RSD**	0.847	0.421	0.429
AVG % R.S.D	0.856			**AVG % R.S.D**	0.566		

3.4.2.3 Interday Precision

Table 11: Result of Interday Precision of Berberine at 231.0nm and Carvedilol at 242.0nm.

Concentration (µg/ml)	Day 1 Absorbance	Day 2 Absorbance	Day 3 Absorbance	Concentration (µg/ml)	Day 1 Absorbance	Day 2 Absorbance	Day 3 Absorbance
	Berberine at 231.0nm				Carvedilol at 242.0nm		
6	0.297	0.298	0.306	10	0.235	0.236	0.239
6	0.299	0.298	0.303	10	0.236	0.239	0.238
6	0.302	0.304	0.298	10	0.233	0.237	0.242

Mean	0.299	0.3	0.302	**Mean**	0.234	0.237	0.239
SD	0.002	0.003	0.004	**SD**	0.001	0.001	0.002
%RSD	0.840	1.154	1.336	**%RSD**	0.650	0.643	0.868
AVG % R.S.D	1.110			**AVG % R.S.D**	0.721		

3.4.2.4 Ruggedness

Table 12: Result of ruggedness of Berberine at 231.0nm and Carvedilol at 242.0nm.

Concentration (µg/ml)	Analyst-1	Analyst-2	Analyst-1	Analyst-2
	Absorbance	Absorbance	Absorbance	Absorbance
	Berberine at 231.0nm		Carvedilol at 242.0nm	
6	0.292	0.298	0.243	0.238
6	0.294	0.302	0.242	0.233
6	0.289	0.296	0.239	0.235
Mean	0.293	0.298	0.241	0.235
SD	0.004	0.003	0.002	0.002
% RSD	1.377	1.022	0.862	1.069

3.4.2.5 Robustness

Table 13: Results showing robustness of Berberine at 231.0nm and Carvedilol at 242.0nm.

Concentration (µg/ml)	Absorbance at 8^0C	Absorbance at 45^0C	Concentration (µg/ml)	Absorbance at 8^0C	Absorbance at 45^0C
	Berberine at 231.0nm			Carvedilol at 242.0nm	
6	0.289	0.297	10	0.232	0.239
6	0.287	0.299	10	0.234	0.236
6	0.288	0.306	10	0.230	0.238
Mean	0.288	0.300	**Mean**	0.232	0.237
SD	0.001	0.004	**SD**	0.002	0.001
% RSD	0.347	1.571	**% RSD**	0.862	0.642

3.5 LOD and LOQ of Berberine and Carvedilol

Table 14: Results showing LOD and LOQ of Berberine and Carvedilol.

Drug name	Wavelength	LOQ (µg/ml)	LOD (µg/ml)
Berberine	231.0nm	1.334	0.439
Carvedilol	242.0nm	3.00	0.99

Table 15: Optical Characteristics and Validation Study of Drugs.

Parameters	Berberine	Carvedilol
Wavelength λ max nm	231.0nm	242.0nm
Beer's law limit µg/ml	4-12	5-25
Correlation coefficient (R^2)	0.996	0.995
Slope	0.015	0.01
Intercept	0.206	0.137
SD	0.002	0.003
% RSD	0.757	1.065
Precision		
Repeatability (% RSD)	0.619	0.587
Intraday (% RSD)	0.856	0.566
Interday (% RSD)	1.110	0.721
Ruggedness		
Analyst 1 (% RSD)	1.377	0.862
Analyst 2 (% RSD)	1.022	1.069
Robustness		
Temp. 8^0C (% RSD)	0.347	0.862
Temp. 45^0C (% RSD)	1.571	0.642
LOQ (µg/ml)	1.334	3
LOD (µg/ml)	0.439	0.99

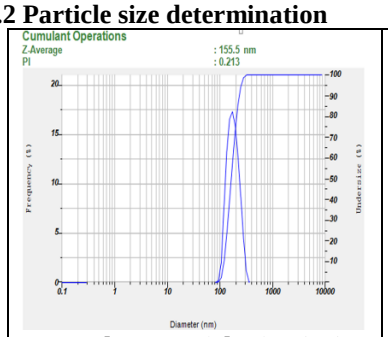
3.6 Characterization Parameters of nanoparticle formulation

3.6.1 Organoleptic properties.

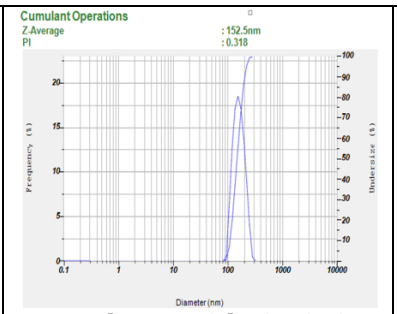
Table 16: Organoleptic properties.

Parameters	Formulation I (Berberine)	Formulation II (Carvedilol)	Formulation III (Combined)
Appearance	Stable nanosuspension	Stable nanosuspension	Stable nanosuspension
Colour	Pale yellow	White to off-white	Pale yellowish-white
Homogeneity	Uniform dispersion	Uniform dispersion	Uniform dispersion

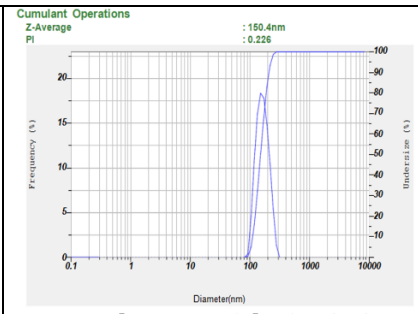
3.6.2 Particle size determination



Graph 8: Particle size (F1)



Graph 9: Particle size (F2)

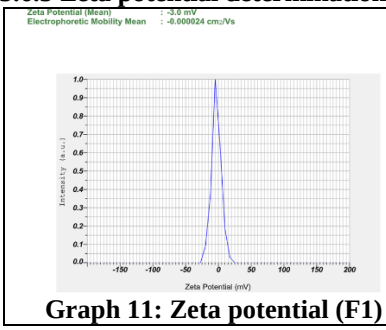


Graph 10: Particle size (F3)

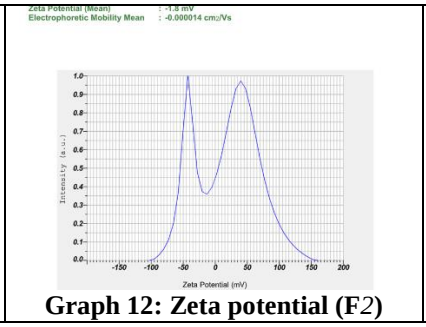
Table 17: Result of Particle size of Nanoparticle formulations.

Formulations	Particle size (nm)
Nanoparticle (F1)	155.5nm
Nanoparticle (F2)	152.5 nm
Nanoparticle (F3)	**150.4 nm**

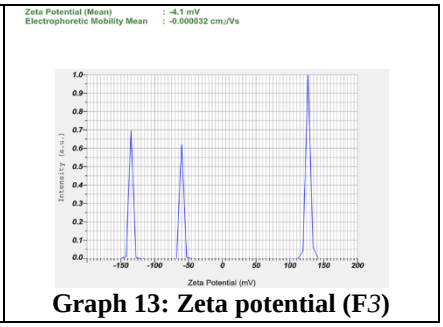
3.6.3 Zeta potential determination.



Graph 11: Zeta potential (F1)



Graph 12: Zeta potential (F*2*)



Graph 13: Zeta potential (F*3*)

Table 18: Result of Particle size, Zeta potential and Entrapment efficiency of Nanoparticle formulations.

Formulations	Particle size (nm)	Zeta potential	Entrapment Efficiency (%)
Nanoparticle (F1)	155.5nm	-3.0 mV	79.67
Nanoparticle (F2)	152.5 nm	-1.8 mV	83.30
Nanoparticle (F3)	**150.4 nm**	**-4.1 mV**	**89.67**

3.6.4 Scanning electron microscopy characterization of F3 Nanoparticle formulation

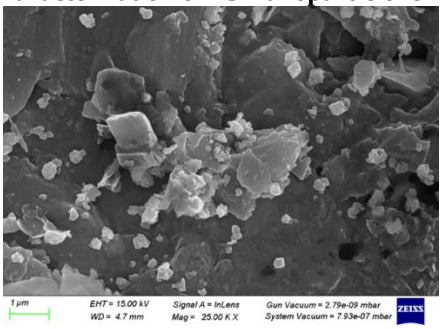


Figure 1: SEM.

3.7 Stability study of combined Nanoparticle formulation.

Table 19: Stability Study of Optimized combined Nanoparticle Formulation.

Time (Days)	25 ± 2°C / 60 ± 5% RH		40 ± 2°C / 75 ± 5% RH	
	Particle size (nm)	Entrapment efficiency (%)	Particle size (nm)	Entrapment efficiency (%)
0	150.4	89.67	150.4	89.67
30	151.2	89.10	152.0	88.85
60	152.6	88.45	154.1	87.90
90	153.8	87.80	156.0	86.95

DISCUSSION

The present study demonstrated the successful development and evaluation of Berberine and Carvedilol-loaded PLGA nanoparticles using the solvent evaporation method. Organoleptic evaluation confirmed the purity and identity of both drugs, while solubility studies revealed their greater affinity toward polar organic solvents such as methanol and DMSO, supporting their suitability for nanoparticle formulation. Melting point, pH, and FTIR studies further confirmed the authenticity, compatibility, and structural integrity of the drugs. The FTIR spectra showed the characteristic functional groups of both Berberine and Carvedilol, indicating the absence of significant chemical interaction or degradation during analysis. The developed UV spectrophotometric simultaneous equation method proved to be simple, precise, accurate, and reliable for the estimation of Berberine and Carvedilol in combined formulations. Both drugs obeyed Beer–Lambert's law within the selected concentration ranges and exhibited excellent linearity with high correlation coefficients. Validation parameters including repeatability, precision, ruggedness, robustness, LOD, and LOQ were all within acceptable limits, confirming the suitability of the method for routine quantitative analysis.

Nanoparticle characterization studies demonstrated successful formation of nanosized particles with good entrapment efficiency and acceptable stability. Among all formulations, F3 showed the most desirable characteristics, including the smallest particle size, comparatively better zeta potential, and highest entrapment efficiency. The improved performance of F3 may be attributed to better drug–polymer interaction and effective stabilization by PLGA and PVA. SEM analysis further confirmed the formation of discrete nanoparticles with predominantly spherical morphology and acceptable surface characteristics, supporting efficient encapsulation of the drugs within the polymer matrix.

Stability studies revealed that the optimized combined nanoparticle formulation remained physically stable under both long-term and accelerated storage conditions for 90 days, with only minimal variations in particle size and entrapment efficiency. These findings indicate that the developed Berberine and Carvedilol-loaded PLGA nanoparticles possess good physicochemical stability and may serve as a promising nano drug delivery system for improved therapeutic performance.

4. CONCLUSION

The study successfully developed a stable Nano formulation of Carvedilol and Berberine with improved physicochemical properties. The optimized formulation (F3) showed the best performance in terms of particle size, entrapment efficiency, and stability. The validated UV method proved to be accurate, precise, and reliable for simultaneous estimation. Stability studies confirmed minimal changes under different conditions, indicating good formulation stability. Overall, the combined Nano formulation is a promising approach for enhancing drug stability and therapeutic effectiveness.

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