

DEVELOPMENT AND STATISTICAL OPTIMIZATION USING FACE-CENTERED CENTRAL COMPOSITE DESIGN WITH VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR QUANTIFICATION OF AN ELLAGIC ACID NANOSUSPENSION

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Abstract

Ellagic acid (EA) is a naturally occurring polyphenolic compound with potent antioxidant, anti-inflammatory, and anticancer activities. However, its poor aqueous solubility and low oral bioavailability limit its therapeutic potential. The present study aimed to develop and optimize a PVP-K30-coated Ellagic Acid nanosuspension using the antisolvent precipitation technique, characterize the optimized formulation, and develop and validate a simple UV spectrophotometric method for its quantitative estimation. Ellagic Acid nanosuspensions were prepared by antisolvent precipitation followed by ultrasonication. A Face-Centered Central Composite Design (FC-CCD) was employed to optimize the concentrations of PVP-K30 and Xanthan Gum, with particle size and viscosity as the critical quality attributes. The optimized formulation was characterized by FTIR, DSC, and SEM. Entrapment efficiency, drug loading, and UV spectrophotometric method validation were performed according to ICH Q2(R2) guidelines. The optimized nanosuspension exhibited a particle size of 552.7 nm, viscosity of 15.3 cP, entrapment efficiency of $90.85 \pm 0.56\%$, and drug loading of $31.24 \pm 0.39\%$. UV spectrophotometric analysis showed that Ellagic Acid exhibited a maximum absorbance (λ_{max}) at 277 nm in the DMSO:Ethanol (50:50, v/v) solvent system. FTIR and DSC analyses confirmed drug–excipient compatibility and reduced crystallinity of Ellagic Acid, while SEM revealed nearly spherical particles with uniform morphology. The developed UV spectrophotometric method was linear over the concentration range of 2–32 $\mu\text{g/mL}$ ($R^2 = 0.9931$) and demonstrated acceptable accuracy, precision, robustness, specificity, sensitivity, and reproducibility. The optimized Ellagic Acid nanosuspension exhibited desirable physicochemical characteristics and efficient drug incorporation. The validated UV spectrophotometric method provides a simple, rapid, economical, and reliable analytical tool for the routine quality control and quantitative estimation of Ellagic Acid nanosuspension formulations.

Keywords: Ellagic Acid, Nanosuspension, Antisolvent Precipitation, UV Spectrophotometric Method Validation, Physicochemical Characterization.

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Introduction

Ellagic acid (EA), a naturally occurring polyphenol, is well known for its wide therapeutic activities include potent antioxidant, anti-inflammatory and anti-neoplastic activities[1]. However, the practical implementation of EA as an active therapeutic drug is substantially impeded by its undesirable physicochemical qualities despite its extremely promising pharmacologic attributes[2]. First, EA undergoes significant first-pass metabolism in the liver and gut and has an extremely poor water solubility ($\sim 9.7 \mu\text{g/mL}$). These limitations result in extremely poor oral bioavailability, limiting its therapeutic efficacy and preventing its development towards broad clinical application[3]. The overcoming of these intrinsic physicochemical barriers is important for fully realizing

the therapeutic efficacy of EA. Formulation scientists should prioritize the development of innovative drug delivery systems that enhance the dissolution rate, solubility, and overall bioavailability of EA.

Nanoparticle-based formulations have proven to be highly effective and adaptable for solving the solubility challenges of hydrophobic drugs like EA[1,2]. Nanosuspensions-carrier-free colloidal dispersions of pure drug particles stabilized with surfactants or specific polymers—present a promising strategy to improve both the apparent Dissolution and solubility rate. This improvement is primarily due to the dramatic increase in the effective surface area of the active pharmaceutical ingredient[1]. These nanosuspensions are usually

prepared by liquid antisolvent precipitation (LASP) method because to their simplicity, cost effectiveness and low energy need as compared to the traditional top-down mechanical attrition methods[3]. This grassroots methodology involves completely dissolving the pharmaceutical compound in an organic solvent, followed by precipitation through the rapid addition of an antisolvent, typically an aqueous solution containing polymeric stabilizers[4]. In antisolvent precipitation, the selection of appropriate stabilizing excipients such as Polyvinylpyrrolidone K30 (PVP-K30) and suspending agents like Xanthan gum is crucial for preventing particle development and agglomeration via steric and electrostatic stabilization.

The pharmaceutical formulations and methods were traditionally optimized using an empirical "One-Factor-At-A-Time" (OFAT) approach, which is time consuming, resource intensive, and ignores the interaction effects between the independent factors[5]. These empirical trial-and-error approaches have been more or less replaced by the modern pharmaceutical development by the The Quality by Design (QbD) framework aims to ensure predetermined product quality by systematically analyzing how formulation and process variables impact the critical quality attributes (CQAs) of the final product. Within QbD, the Design of Experiments (DoE)-especially the Face-Centered Central Composite Design (FC-CCD)-serves as a valuable statistical tool for optimizing formulation parameters[3]. FC-CCD allows a precise definition of the experimental design space and the development of highly predictive mathematical models with few experimental trials[3,4]. The physical stability and the biopharmaceutical efficacy of the formulation which are directly dependent on the reduced particle size and ideal viscosity in the production of nanosuspension can be optimized statistically by finding the optimal concentration of critical stabilizers like PVP-K30 and Xanthan gum.

Besides the optimization of formulations, a methodical, precise and targeted analytical approach is needed for the reliable measurement of the active component in the nanocarrier matrix. Nowadays, many advanced chromatographic methods have been developed for the quantification of EA in diverse matrices, however these approaches are expensive and require complicated sample preparation procedures and large amounts of organic solvents[6,7]. Therefore, there is great need for the development of a simple, inexpensive, and rapid UV-Vis spectrophotometric method for routine in-process quality evaluation, encapsulation efficiency determination, and in vitro dissolution study of EA nanosuspensions[5,8]. Therefore, the developed method needs to be validated as per International Council for Harmonisation (ICH) Q2(R2) guidelines to demonstrate its reliability, reproducibility and applicability for its intended pharmaceutical use[9,10]. The parameters for method validation (linearity, accuracy, precision, robustness, limits of detection (LOD), limits of quantification (LOQ) and specificity) should be thoroughly assessed to ensure that the formulation excipients do not hinder the quantitative assessment of the drug.

Although a large amount of research has been conducted on different nanosuspensions and many scientific papers have demonstrated the therapeutic benefits of EA, there is still a lack of literature on the systematic approach to improve the formulation of EA and its measurement. The literature rarely offers a combined approach of meticulous statistical optimization of PVP-K30 coated EA nanosuspensions through an FC-CCD mathematical approach, alongside the concurrent development of a well-validated matrix-specific analytical method. Also, detailed physical compatibility and crystallinity of EA with PVP-K30 by co-precipitation should be done using FTIR, DSC and SEM for complete elucidation of the stabilization mechanisms of this formulation[11].

Hence, the systematic development of methods and the improvement of the formulations are the main focus of this manuscript. The main objectives of the present work were to prepare an effective PVP-K30 coated EA nanosuspension by antisolvent precipitation followed by ultrasonication, statistically optimize the formulation variables (PVP-K30 and Xanthan gum concentrations) using Face-Centered Central Composite Design to minimize the particle size and optimize the viscosity, characterize the optimized nanosuspension for structural morphology and physical compatibility by FTIR, DSC and SEM, and develop and validate a rapid, specific and robust UV spectrophotometric method for routine estimation of EA in the novel nano-matrix as per the ICH Q2(R2) guidelines.

Materials And Methods

Two matched 1 cm quartz cells were employed in a UV-Visible double beam spectrophotometer (UV-1800, Shimadzu, Japan). Every chemical and reagent used was of the analytical reagent (AR) grade. Yarrow Chem Products (Mumbai, India) provided ellagic acid, while HiMedia Laboratories Pvt. Ltd. (Mumbai, India) provided polyvinylpyrrolidone K30 (PVP-K30). The analytical-grade ethanol and dimethyl sulfoxide (DMSO) used in the spectrophotometric analysis were acquired from Merck Specialities Pvt. Ltd. (Mumbai, India).

Standard Stock Solution

A precisely weighed 10 mg of Ellagic Acid was placed in a 10 mL volumetric flask. Around 7 mL of DMSO:Ethanol (50:50, v/v) was added, and the mixture was sonicated for 10 minutes to achieve complete dissolution. The flask was then filled to the mark with the same solvent mixture to prepare a primary stock solution of 1000 µg/mL Ellagic Acid. From this primary stock, 1 mL was transferred into another 10 mL volumetric flask and diluted to the mark with DMSO:Ethanol (50:50, v/v), yielding a secondary stock solution of 100 µg/mL Ellagic Acid[12,13].

Calibration Curve

From the 100 µg/mL secondary stock solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1.6, and 3.2 mL were extracted and put into different 10 mL volumetric flasks. After that, DMSO:Ethanol (50:50, v/v) was added to each flask until it was full, yielding final concentrations of 2, 4, 6, 8, 16, and 32 µg/mL, respectively.[12,13,14]

Drug-Excipient Compatibility Studies by Fourier Transform Infrared (FTIR) Spectroscopy

Drug-excipient compatibility studies were performed using FTIR to evaluate the possible interactions between Ellagic Acid and Polyvinylpyrrolidone K30 (PVP-K30). FTIR spectra of pure Ellagic Acid, PVP-K30, the physical mixture (1:1, w/w), and the optimized Ellagic Acid nanosuspension were recorded using an FTIR spectrophotometer[15].

Differential Scanning Calorimetry (DSC)

The thermal behavior and compatibility of Ellagic Acid with PVP-K30 were estimated using Differential Scanning Calorimetry (DSC) (DSC-60 Plus, Shimadzu, Kyoto, Japan). About 5–10 mg each of pure Ellagic Acid, PVP-K30, their physical mixture (1:1, w/w), and the optimized Ellagic Acid nanosuspension were weighed and sealed in standard aluminum pans; an empty sealed pan served as the reference. Samples were heated from 30°C to 300°C at 10°C/min under a nitrogen flow of 40 mL/min. The resulting thermograms were compared to detect

changes in the melting endotherm of Ellagic Acid and to identify any potential physicochemical interactions or changes in its crystalline nature after nanosuspension formulation[15,16].

Preparation of PVP K30-Coated Ellagic Acid Nanosuspension

PVP K30-coated ellagic acid nanosuspension was prepared using the anti-solvent precipitation method followed by ultrasonication. First, the organic phase was made by dissolving ellagic acid (EA) in ethanol, while the aqueous phase (anti-solvent) was prepared by dissolving PVP K30 in distilled water. The organic phase was added dropwise to the aqueous phase under continuous magnetic stirring to induce rapid precipitation of ellagic acid nanoparticles. Stirring was continued until a uniform suspension formed. This suspension was then probe-sonicated to break up aggregates and further reduce particle size, resulting in a stable, uniformly dispersed PVP K30-coated ellagic acid nanosuspension suitable for characterization and biological evaluation[17].

PVP-K30 coated Ellagic Acid Nanosuspension: Preparation method

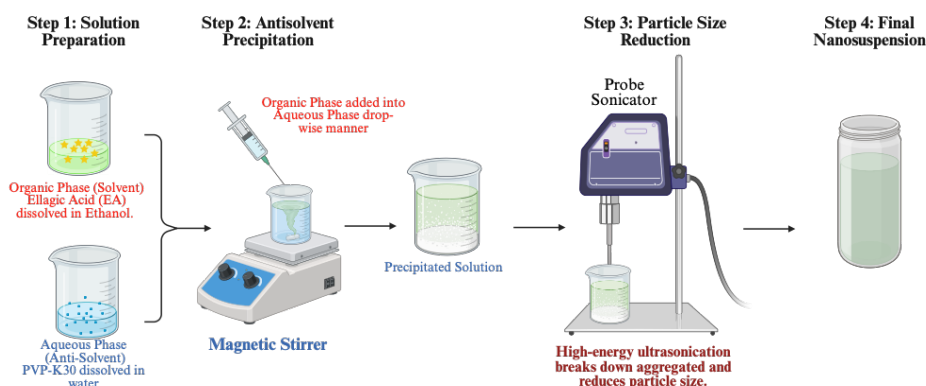


Figure 1: Preparation of the PVP K30 Coated Ellagic Acid Nanosuspension

Design of experiment (DoE)

For the optimization of formulation and evaluation particle size, FC-CCD was implemented. The experimental design included 9 runs using Design Expert® version 13 software. PVP K30 (A) and Xanthan Gum (B) served as the independent variables, while particle size (Y1) and viscosity (Y2) were the dependent variables. The influence of these critical factors on the key responses was systematically analyzed using Analysis of Variance (ANOVA) and multilinear regression[10].

Characterization of Ellagic Acid Nanosuspension

The Ellagic Acid nanosuspension (EANS) was characterized for mean particle size, polydispersity index (PDI), and zeta potential. Measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK), following the manufacturer's guidelines. Before analysis, the optimized nanosuspension was suitably diluted with deionized water to prevent multiple scattering. Mean particle size and PDI were assessed to determine size distribution and homogeneity, while zeta

potential was measured to evaluate surface charge and physical stability[18].

Determination of Entrapment Efficiency (EE%)

EE was assessed to determine the amount of Ellagic Acid encapsulated in the nanosuspension. Free (unentrapped) drug was separated by centrifugation, and its concentration in the supernatant was measured using a validated UV spectrophotometric method. Specifically, 5 mL of the optimized nanosuspension was centrifuged at 15,000 rpm for 15 minutes at 4°C. The resulting clear supernatant, containing unentrapped drug, was carefully collected, diluted with DMSO:Ethanol (50:50, v/v), and its absorbance measured at 277 nm[18]. The amount of free Ellagic Acid was determined from the calibration curve, and entrapment efficiency was calculated using the following equation: Entrapment efficiency

$$(EE\%) = \frac{\text{Total Drug} - \text{Free drug}}{\text{Total Drug}} \times 100$$

Determination of Drug Loading (DL%)

Drug loading was determined for the optimized Ellagic Acid nanosuspension after the entrapment efficiency

analysis. The amount of entrapped Ellagic Acid was calculated by subtracting the quantity of free drug in the supernatant from the total amount initially used in the formulation. The concentration of free drug was measured using a validated UV-Visible spectrophotometric method at 277 nm after suitable dilution with DMSO:Ethanol (50:50, v/v)[18]. Drug loading was then calculated using the following equation:

$$\text{Drug Loading (\%)} = \frac{\text{Entrapped drug}}{\text{Entrapped drug} + \text{Wt of Polymer}} \times 100$$

Method Validation

The UV-based detection method for ellagic acid was validated by assessing linearity, accuracy, robustness, precision, limit of detection (LOD), limit of quantification (LOQ), and specificity, as detailed below[12,13,14].

Linearity

Six values ranging from 2 to 32 µg/mL were analyzed in order to create a standard calibration curve for ellagic acid. A UV-Visible Spectrophotometer (UV-1800, Shimadzu, Japan) was used to test each standard's absorbance at 277 nm. Plotting the calibration curve as absorbance vs ellagic acid concentration[12,14].

Accuracy

The accuracy of the UV method was confirmed through recovery studies using the standard addition technique. In triplicate, three separate ellagic acid solutions were prepared at 80%, 100%, and 120% of the standard concentration (10 µg/mL).

The accuracy was calculated by using the following equation 1:

$$\%RC = \left(\frac{SPC - S}{SP} \right) \times 100 \dots\dots\dots 1$$

Where, SPS= Amount obtained in the spiked sample, %RC= Percent recovery, SP= Amount added to the sample and S= Amount found in the sample[13,14].

Precision

The precision of the developed UV spectrophotometric method was assessed following ICH Q2(R2) guidelines. Precision was evaluated as repeatability (intra-day) and intermediate precision (inter-day). A 20 µg/mL Ellagic acid standard solution was analyzed six times, and absorbance values were recorded, with precision expressed as percentage relative standard deviation (%RSD). For intra-day precision, a 10 µg/mL solution was analyzed six times on the same day. For inter-day precision, the same concentration was analyzed over three consecutive days under identical conditions. The %RSD was calculated using equation 2:

$$\%RSD = \frac{SD}{\text{Mean}} \times 100 \dots\dots\dots 2$$

Where, SD = Standard deviation, Mean = Mean absorbance

Robustness

Robustness refers to an analytical method's ability to remain unaffected by small, deliberate changes in

parameters, indicating its reliability during routine use. The robustness of the developed UV spectrophotometric method was assessed according to ICH Q2(R2) guidelines by introducing slight variations in wavelength and solvent composition during the analysis of the optimized Ellagic Acid nanosuspension. A sample equivalent to 10 µg/mL of Ellagic Acid was transferred to a volumetric flask, extracted with DMSO:Ethanol (50:50 v/v) by sonication for 10 minutes, and diluted to volume with the same solvent. The solution was filtered through a 0.45 µm membrane filter before analysis. Robustness was evaluated by making the following deliberate changes: Detection wavelength: 275 nm, 277 nm, and 279 nm, Solvent composition: DMSO:Ethanol (48:52, 50:50, and 52:48 v/v).

Absorbance data were recorded after each condition was tested three times. Calculations were made for the mean, standard deviation (SD), and percentage relative standard deviation (%RSD).[12,14].

Limit of Detection (LOD)

LOD is the lowest analyte concentration that can be detected, though not necessarily quantified, under specified conditions. The LOD for the developed UV spectrophotometric method was established according to ICH Q2(R2) guidelines using the standard deviation of the response (σ) and the slope (S) of the calibration curve[12,13,14].

$$\text{Formula: } LOD = 3.3 \times \sigma / S$$

Where: σ = Standard deviation of the response, S = Slope of the calibration curve

Limit of Quantification (LOQ)

The LOQ is the lowest analyte concentration that can be quantitatively determined with acceptable precision and accuracy. LOQ was calculated based on ICH Q2(R2) guidelines using the standard deviation of the response (σ) and the slope (S) of the calibration curve[12,13,14].

$$LOQ = 10 \times \sigma / S$$

Where: σ = Standard deviation of the response, S = Slope of the calibration curve

Specificity

Specificity is the ability of an analytical method to selectively measure the analyte while excipients are present.

impurities, or degradation products. The specificity of the developed UV spectrophotometric method was tested to confirm that formulation excipients did not interfere with Ellagic Acid detection at 277 nm. A blank solution (DMSO:Ethanol, 50:50 v/v) and a placebo solution (containing all excipients except Ellagic Acid) were prepared and scanned from 200–400 nm. Similarly, standard (10 µg/mL Ellagic Acid) and sample solutions (optimized nanosuspension, equivalent to 10 µg/mL Ellagic Acid) were scanned. The absorption spectra of all solutions were compared to identify any interference at 277 nm[19].

Results

Ellagic Acid nanosuspension was prepared by the nanoprecipitation method employing PVP-K30 as the

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stabilizing polymer and Xanthan Gum as the viscosity-enhancing agent. Preliminary formulation trials were conducted to identify the suitable concentration range of the formulation variables for obtaining a stable nanosuspension with desirable physicochemical characteristics. Based on the preliminary investigations, a FC-CCD was employed to systematically optimize the formulation variables. The experimental design consisted of nine experimental runs generated using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA).

The concentrations of PVP-K30 (Factor A) and Xanthan Gum (Factor B) were selected as the independent variables, while particle size (Y_1) and viscosity (Y_2) were chosen as the dependent responses. The formulation variables were varied according to the experimental design, and nanosuspensions were prepared under

identical processing conditions. The prepared formulations were subsequently evaluated for particle size and viscosity to determine the influence of the independent variables on the critical quality attributes of the formulation.

Multiple linear regression analysis and Analysis of Variance (ANOVA) were used to quantitatively examine how the formulation factors affected the measured responses. Response surface plots were created to illustrate the impact of formulation factors, and mathematical models were created to explain the relationship between the independent variables and the responses. In order to achieve the lowest possible particle size and viscosity while preserving formulation stability, the optimal formulation was chosen using the desirability function

Table 01: FTIR spectrum of Ellagic Acid, Physical Mixture, and Formulation Compatibility Studies of Drug and excipient

Characteristic peak position (cm-1)	Functional group	Peak position observed in Drug (cm-1)	Peak position observed in Physical mixture (cm-1)	Peak position observed in Formulation (cm-1)
1600-1900	C=O (stretch)	1820.47	1739.48	1692.23
1600-1700	C=C (stretch)	1553.38	1656.55	1560.13
3000-2850	C-H (stretch)	2938.98	2926.45	3090.37
1470-1430	CH ₂ (scissoring vibrations)	1454.06	1459.85	1508.06

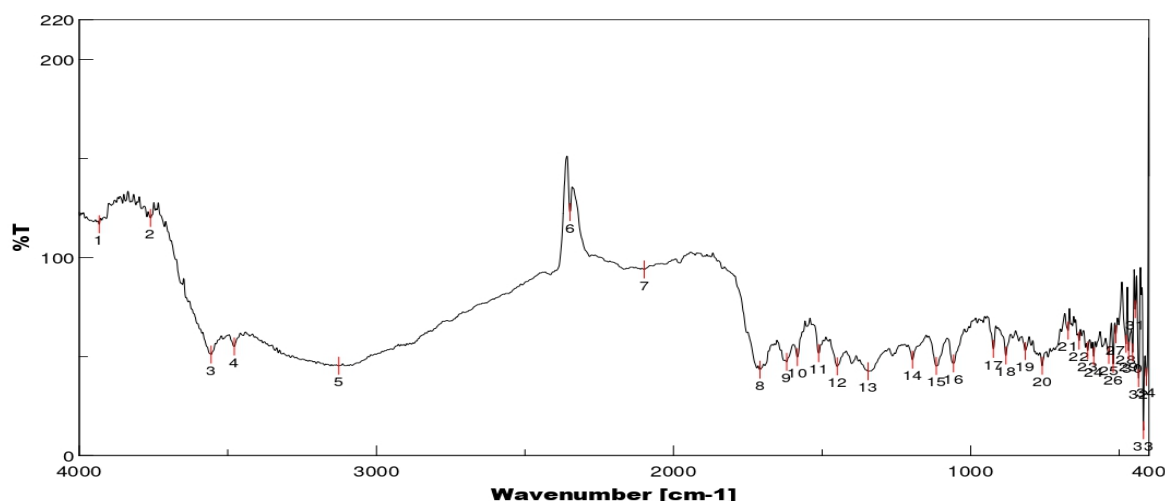


Figure 2: FTIR spectrum of Ellagic Acid, Physical Mixture, and Formulation

The FTIR spectra of pure Ellagic Acid, the physical mixture, and the optimized PVP-K30-coated Ellagic Acid

nanosuspension were compared to investigate possible drug–excipient interactions. Pure Ellagic Acid exhibited characteristic absorption bands corresponding to the

carbonyl (C=O) group's stretching vibration, aliphatic C–H stretching, aromatic C=C stretching, and CH₂ scissoring vibrations[21,22]. These characteristic bands were also observed in the physical mixture and optimized formulation, although slight shifts in peak positions and variations in peak intensity were noted. Such minor spectral changes are generally attributed to intermolecular hydrogen bonding and physical interactions between the hydroxyl groups of Ellagic Acid and the carbonyl groups of PVP-K30 during the nanoprecipitation process[23]. Importantly, no additional peaks appeared and none of the characteristic peaks of Ellagic Acid disappeared in the optimized formulation, indicating the absence of chemical degradation or covalent interaction between the drug and polymer. The preservation of the principal functional group vibrations confirms that Ellagic Acid remained chemically stable throughout the formulation process. Similar observations have been reported in polymer-based nanosuspensions and solid dispersions, where PVP acts as a steric stabilizer through hydrogen bonding without altering the chemical structure of the incorporated drug[24]. The interaction between PVP-K30 and poorly water-soluble drugs has also been shown to reduce particle

aggregation and improve physical stability by preventing crystal growth during precipitation[21].

The slight peak shifts observed in the formulation further suggest successful adsorption of PVP-K30 onto the surface of Ellagic Acid nanoparticles, thereby providing steric stabilization and improving dispersion stability. Collectively, the FTIR results demonstrate excellent compatibility between Ellagic Acid and PVP-K30 and support the suitability of PVP-K30 as a stabilizing polymer for the preparation of Ellagic Acid nanosuspension.

Differential Scanning Calorimetry (DSC)

The DSC thermogram of pure Ellagic Acid demonstrated a sharp endothermic peak at 142.5°C, corresponding to its characteristic melting point and confirming its crystalline nature. The optimized Ellagic Acid nanosuspension showed a broad endothermic peak at 122.0°C with reduced intensity. The shift in melting temperature and decreased peak intensity indicate a reduction in drug crystallinity, which may be attributed to nanosization and molecular dispersion of the drug within the PVP-K30 matrix. No additional thermal events were observed, suggesting the absence of significant drug–polymer interactions and confirming the compatibility of Ellagic Acid with PVP-K30. The partial conversion of the drug to a less crystalline state is expected to contribute to improved solubility and dissolution behavior.

DSC Analysis

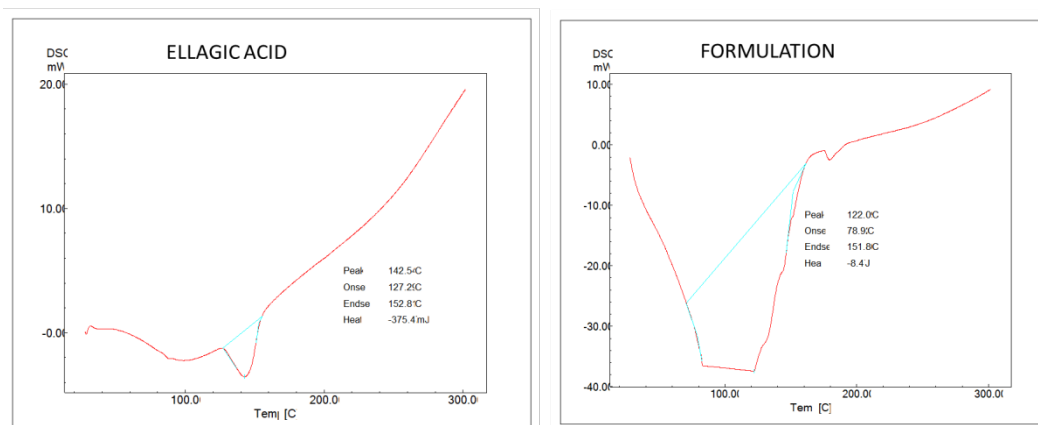


Figure 3: DSC Analysis of Ellagic Acid and Formulation

The DSC thermogram of pure Ellagic Acid displayed a sharp endothermic peak at 142.5°C, confirming its crystalline nature. In contrast, the optimized nanosuspension exhibited a broader endothermic peak at 122.0°C with reduced intensity. The reduction in melting temperature and peak intensity indicates partial loss of crystallinity following nanoprecipitation, which is commonly observed due to particle size reduction and molecular dispersion of the drug within the polymeric matrix. The absence of any additional thermal events further indicates that no chemical interaction occurred between Ellagic Acid and PVP-K30 during formulation.

The reduced crystallinity is expected to improve the apparent solubility and dissolution rate of Ellagic Acid, thereby enhancing its oral bioavailability. Comparable DSC findings have been reported for polymer-stabilized nanosuspensions, where a reduction in crystallinity contributed to improved dissolution characteristics and formulation stability[23].

Design of Experiment (DoE)

A Quality by Design (QbD) approach was employed to optimize the formulation variables affecting the characteristics of the Ellagic Acid nanosuspension. A

Development And Statistical Optimization Using Face-Centered Central Composite Design With Validated Uv Spectrophotometric Method For Quantification Of An Ellagic Acid Nanosuspension

Face- FC-CCD was generated using Design-Expert® software. The experimental design consisted of 9 experimental runs, allowing systematic evaluation of the effects of the selected formulation variables on the CQAs. In the present study, the concentration of PVP-K30 (A) and Xanthan Gum (B) were selected as the independent variables, while particle size (Y₁) and Viscosity (Y₂) were considered as the dependent responses. The influence of the independent variables on the responses was evaluated

using Analysis of Variance (ANOVA) and multiple linear regression analysis. The optimized formulation was selected based on the desirability function to achieve minimum particle size and Viscosity.

The factors and the corresponding levels have been represented in Table 02. The different method parameters along with responses in different runs as per FC-CCD are captured in Table 03.

Table 02: Independent variables and the levels as per FC-CCD

Independent variables	Low level (%)	High level (%)
A: PVP K30	2	4
B: Xanthan Gum	1	3
Dependent Variables	Constraints*	
Y1: Particle Size (nm)	Minimize	
Y2: Viscosity (cps)	Minimize	

Table 03:

RUN	PVP K30	Xanthan	Particle Size (nm) (Y1)	Viscosity (cP) (Y2)
1	4	1	498	14.8
2	2	1	552	18.6
3	4	3	619	32.4
4	2	3	629	36.8
5	3	1	525	16.9
6	3	3	615	34.6
7	2	2	598	24.5
8	4	2	605	22.1
9	3	2	608	26.3

Experimental runs as per FC-CCD in preparation of nanosuspension of Ellagic acid along with responses

Impact of independent variables on dependent variables

Impact on particle size

The mathematical model generated for particle size is represented in equation 3. The particle size of the prepared formulation ranges between 498-629 nm.

$$Y1 = +558.22 - 9.50 * A + 48.00 * B \dots\dots\dots 3$$

A and B represent the concentration of suspending agent and stabilizer (% w/w), respectively

Figure 1 illustrates how xant (A) and PVP (B) affect particle size. The graph and equation demonstrate that while PVP K30 was directly proportional to PS, Xanthan Gum was inversely proportional to PS.

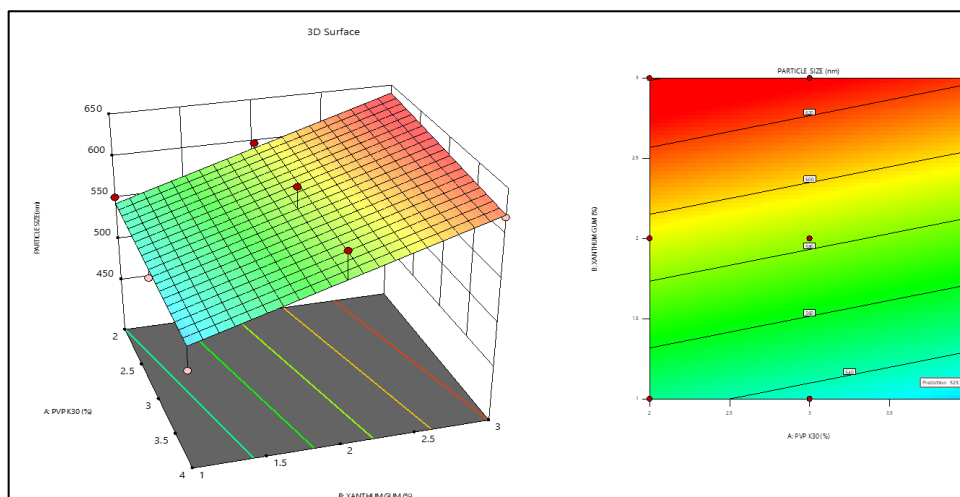


Figure 4: 2D contour plot and 3D surface plot showing the effect of Xantham Gum and PVP on particle size

Impact on Viscosity

The mathematical model generated for Viscosity is represented in equation 4. The viscosity of the prepared formulation ranges between 14.8-36.8cp.

$$Y_2 = +25.22 - 1.77A + 8.92B \dots\dots\dots 4$$

A and B represent the concentration of suspending agent and stabilizer (% w/w), respectively

Figure 2 illustrates how xant (A) and PVP (B) affect viscosity. The graph and equation demonstrate that while Xanthan gum was directly proportional to Viscosity, PVP K30 was inversely proportional to Viscosity.

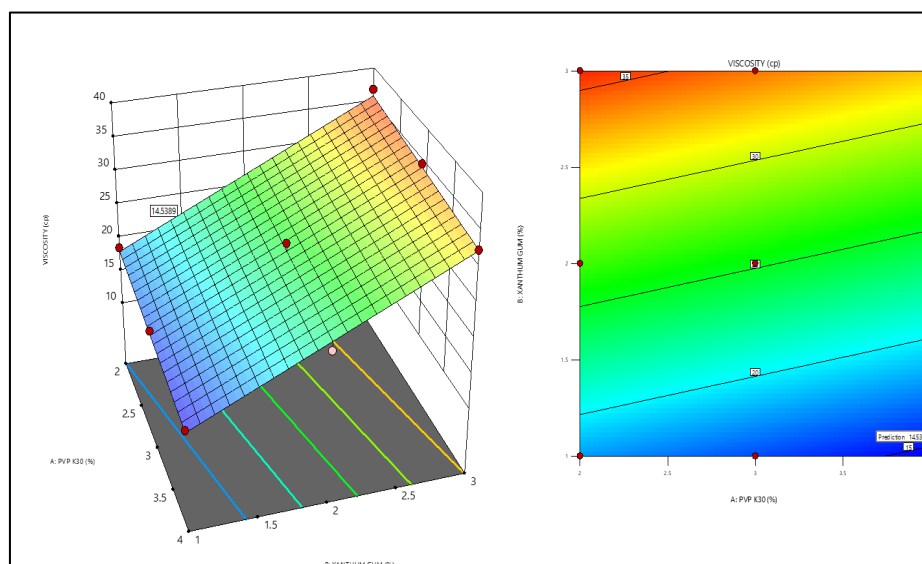


Figure 5: 2D contour plot and 3D surface plot showing the effect of Xanthan Gum and PVP on Viscosity

Optimization of nanoformulation

A numerical optimization using the desirability approach was applied to identify the optimal formulation settings. Feasibility studies and grid searches were conducted to refine process parameters, resulting in a nanoformulation with the most desirable critical quality attributes (CQAs). The optimized formulation was evaluated, and its experimental response values were compared to those predicted by the mathematical models. As shown in Table 4, the prediction errors for particle size and viscosity were 4.68 and 4.08, respectively. These low prediction error values confirm the validity and predictive power of the mathematical models generated by MLRA and ANOVA.

Table 04: Comparative Analysis of Model-Predicted Versus Experimental Values for Optimized Parameter Characteristics and Quality Attribute

RUN	Optimal settings X1:X2	RESPONSES	Experimental value	Predicted value	% Prediction error
13	X1: 3.881 X2: 1	Y1	552.7	526.8	4.68
		Y2	15.3	14.7	4.08

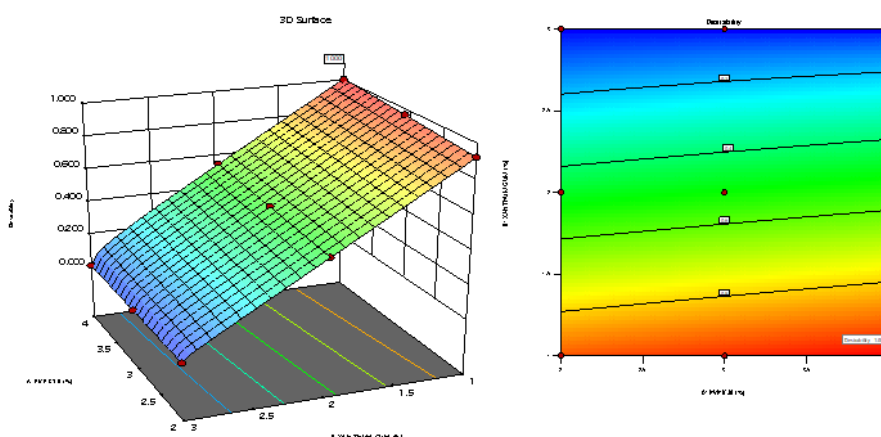


Figure 6: the desirability plot representing the optimization of method variables using FC-CCD within the desirable range.

Entrapment Efficiency (EE%)

The optimized Ellagic Acid nanosuspension exhibited a high entrapment efficiency of $90.85 \pm 0.56\%$, indicating efficient incorporation of the drug within the polymeric matrix. The high entrapment efficiency can be attributed to the optimum concentration of PVP-K30, which effectively stabilized the nanoparticles and minimized drug diffusion into the external aqueous phase during the nanoprecipitation process. In addition, the presence of Xanthan Gum increased the viscosity of the dispersion medium, thereby reducing drug leakage and enhancing drug retention within the nanosuspension. The high entrapment efficiency obtained demonstrates the suitability of the optimized formulation for efficient drug encapsulation and suggests its potential to improve drug stability and provide sustained drug release.

Drug Loading (DL%)

The optimized Ellagic Acid nanosuspension demonstrated a drug loading of $31.24 \pm 0.39\%$, confirming efficient incorporation of the drug within the nanosuspension matrix. The optimized concentrations of PVP-K30 and Xanthan Gum provided an appropriate balance between nanoparticle stabilization and drug incorporation, resulting in satisfactory drug loading. The formulation minimized drug loss during nanoprecipitation while maintaining particle stability, thereby improving the overall loading efficiency. Adequate drug loading is an important quality attribute because it allows higher drug content within the carrier system, reduces the proportion of excipients, and contributes to improved dosage efficiency. The obtained results indicate that the optimized nanosuspension is suitable for sustained drug delivery and may enhance the therapeutic potential of Ellagic Acid.

Scanning Electron Microscopy (SEM)

SEM was used to observe the surface morphology of the particles. The micrographs showed that the particles were uniform in size, roughly spherical, and approximately 500 nm. The size distribution was consistent with other

nanosuspension systems reported in the literature. The observed aggregates may explain the relatively high polydispersity indices.

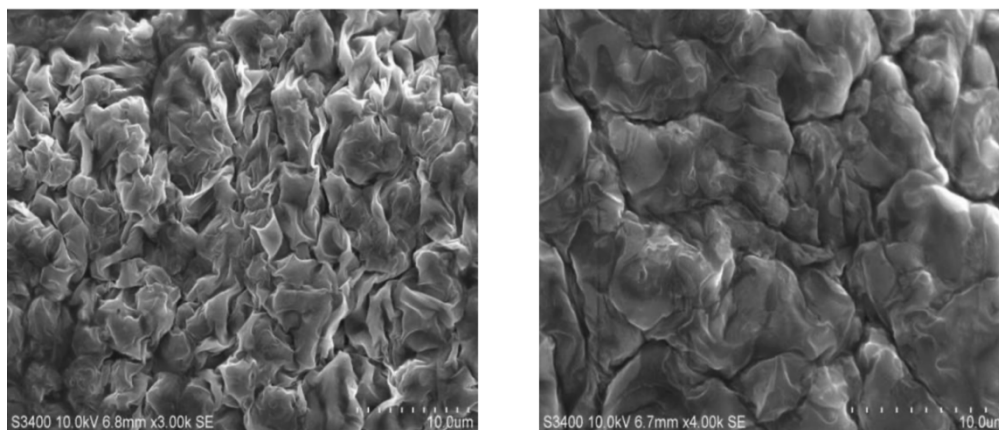


Figure 7: SEM Images of Ellagic Acid Nanosuspension Method Development

Determination of Maximum Absorption of Ellagic acid

Using a UV spectrophotometer, the medication solution (20 μ g/ml) was scanned between 400 and 200 nm to determine the maximal absorption of ellagic acid. Figure 8 illustrates the acquired spectra, which revealed that the absorption maxima for ellagic acid was 277 nm.

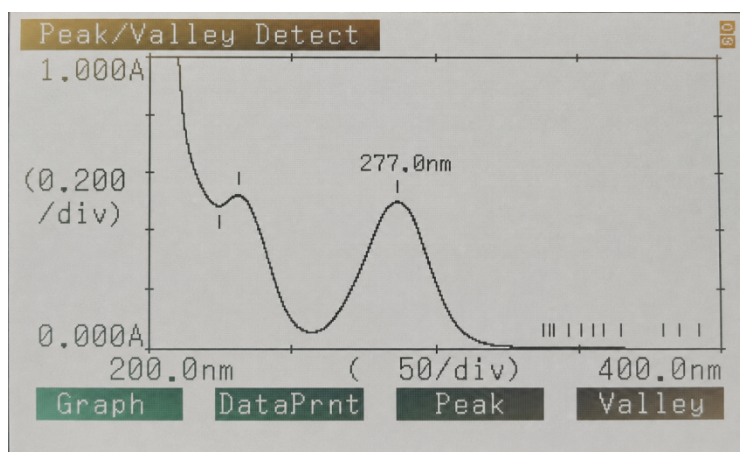


Figure 8: λ_{max} of Ellagic Acid

Method Validation

Determination of standard calibration curve Ellagic acid

The method exhibited good linearity over the concentration range studied. The linear regression equation obtained for the calibration curve was $y = 0.0248x + 0.0714$ with a correlation coefficient ($R^2 = 0.9931$), indicating a strong linear relationship between concentration and absorbance. The linearity data, calibration curve, and regression analysis are presented in Table and Figure respectively.

Table 05: Linearity of Ellagic Acid Nanosuspension

Sl.No.	Concentration (μ g mL ⁻¹)	Absorbance
1.	2	0.046 $\pm$ 0.002
2.	4	0.095 $\pm$ 0.4
3.	6	0.131 $\pm$ 0.007
4.	8	0.188 $\pm$ 0.003
5.	16	0.349 $\pm$ 0.006

6.	32	0.734±0.006
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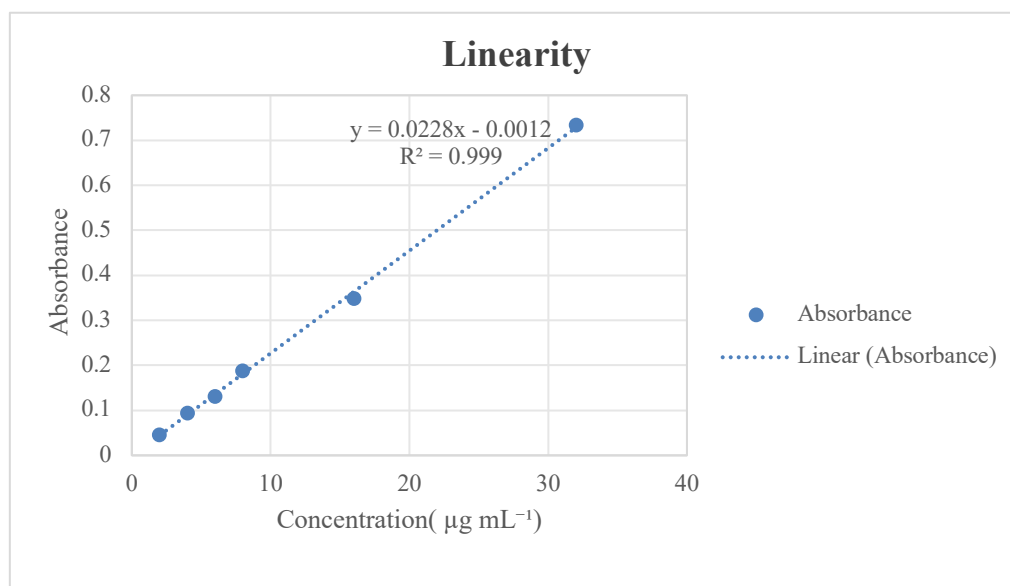


Figure 9:Linearity of Ellagic Acid Nanosuspension

Accuracy

The accuracy of the proposed analytical method was assessed by standard addition at three concentration levels (80%, 100%, and 120%). The percentage recovery ranged from 98–102%, with %RSD values below 2.0%, indicating excellent recovery and reproducibility. The results demonstrate that the developed method is accurate, reliable, and unaffected by the formulation matrix, thereby confirming its applicability for routine analysis of Ellagic Acid in nanosuspension formulations.

Table 6. Statistical analysis of Accuracy of the proposed method.

Level	Replicate	Amount Added (SP) (µg/mL)	Amount Found (S) (µg/mL)	Amount Obtained in Spiked Sample (SPS) (µg/mL)	% Recovery
80%	1	8.0	9.87	17.72	98.13
80%	2	8.0	10.10	18.23	101.63
80%	3	8.0	9.95	17.98	100.38
100%	1	10.0	10.15	20.34	101.90
100%	2	10.0	9.88	19.70	98.20
100%	3	10.0	10.05	20.14	100.90
120%	1	12.0	9.82	21.67	98.75
120%	2	12.0	10.20	22.43	101.92
120%	3	12.0	9.96	21.97	100.08
%RSD < 2%					

Precision

Intra-day and inter-day precision studies showed satisfactory repeatability and intermediate precision for the developed analytical method. The %RSD values for Ellagic Acid at 20 µg/mL were below the ICH acceptance limit of 2.0%, confirming the method's consistency and reproducibility. The low variability demonstrates the reliability and precision of the proposed UV spectrophotometric method for quantifying Ellagic Acid in nanosuspension formulations.

Table 7: Statistical analysis of Intraday Assay of the proposed method

Replicate	Absorbance		
	Morning	Afternoon	Evening
1	0.486	0.483	0.490
2	0.493	0.495	0.482
3	0.481	0.479	0.496
4	0.489	0.488	0.484

5	0.478	0.492	0.487
6	0.491	0.485	0.479
Mean	0.4863	0.4870	0.4863
SD	0.0059	0.0061	0.0063
%RSD	1.21	1.25	1.30

Table 8: Statistical analysis of Interday Assay of the proposed method

Replicate	Absorbance		
	Day 1	Day 2	Day 3
1	0.485	0.481	0.489
2	0.491	0.497	0.480
3	0.478	0.486	0.495
4	0.489	0.492	0.486
5	0.482	0.479	0.492
6	0.494	0.488	0.483
Mean	0.4865	0.4872	0.4875
SD	0.0061	0.0070	0.0058
%RSD	1.25	1.44	1.19

Robustness

Robustness studies demonstrated that deliberate variations in the analytical wavelength (275 and 279 nm) and solvent composition (DMSO:Ethanol, 48:52 and 52:48 v/v) did not produce any significant changes in the absorbance values of Ellagic Acid. The %RSD values remained within the ICH acceptance criterion (<2.0%) for all experimental conditions, confirming that the developed UV spectrophotometric method is robust, reproducible, and unaffected by minor operational variations. These results support the applicability of the method for routine pharmaceutical analysis and quality control of Ellagic Acid nanosuspension formulations.

Table 9: Statistical analysis of Robustness by Variation in Wavelength

Wavelength (nm)	Absorbance 1	Absorbance 2	Absorbance 3	Mean $\pm$ SD	%RSD
275	0.548	0.551	0.546	0.548 $\pm$ 0.0025	0.46
277	0.554	0.557	0.555	0.555 $\pm$ 0.0015	0.27
279	0.550	0.552	0.554	0.552 $\pm$ 0.0020	0.36

Table 10: Statistical analysis of Robustness by Variation in Solvent Composition

DMSO:Ethanol (v/v)	Absorbance 1	Absorbance 2	Absorbance 3	Mean $\pm$ SD	%RSD
48:52	0.552	0.555	0.553	0.553 $\pm$ 0.0015	0.27
50:50	0.554	0.557	0.555	0.555 $\pm$ 0.0015	0.27
52:48	0.551	0.553	0.556	0.553 $\pm$ 0.0025	0.45

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

By establishing the LOD and LOQ, the analytical sensitivity of the new approach was determined. The suggested UV spectrophotometric method's great sensitivity was demonstrated by the computed LOD and LOQ values, which were 0.189 $\mu\text{g/mL}$ and 0.573 $\mu\text{g/mL}$, respectively. The approach is appropriate for regular pharmaceutical analysis and quality control applications because of its low detection and quantification limits, which show that it can precisely detect and quantify Ellagic Acid at trace concentration levels.

Table 11: Results of LOD and LOQ

Drug	LOD	LOQ
Ellagic Acid	0.189 $\mu\text{g/mL}$	0.573 $\mu\text{g/mL}$

Specificity

Specificity studies revealed that the blank and placebo solutions produced negligible absorbance at the selected analytical wavelength (277 nm), while the standard and optimized formulation exhibited a well-defined absorption peak corresponding to Ellagic Acid. No additional peaks or spectral overlap attributable to the formulation excipients were observed, confirming the absence of analytical interference. These results demonstrate the excellent specificity of the developed UV spectrophotometric method and establish its suitability for the selective quantification of Ellagic Acid in nanosuspension formulations.

Table 12: Statistical analysis Specificity of Proposed Method

Sample	λ_{max} (nm)	Absorbance at 277 nm
Blank (DMSO:Ethanol, 50:50 v/v)	—	0.000
Placebo (PVP-K30 solution)	—	0.003
Standard Ellagic Acid (10 $\mu\text{g/mL}$)	277	0.452
Sample (Nanosuspension equivalent to 10 $\mu\text{g/mL}$)	277	0.449

Discussion

The UV spectrophotometric method developed for the quantitative estimation of Ellagic Acid was successfully validated according to the International Council for Harmonisation (ICH Q2(R2)) guidelines and demonstrated satisfactory analytical performance for routine pharmaceutical analysis[20]. The absorption spectrum of Ellagic Acid exhibited a well-defined maximum absorbance (λ_{max}) at 277 nm in a DMSO:Ethanol (50:50, v/v) solvent system, indicating that this wavelength provided optimum sensitivity and minimal spectral interference. The use of the mixed solvent system significantly improved the solubility of Ellagic Acid, a poorly water-soluble polyphenolic compound, resulting in stable and reproducible absorbance measurements[20]. With a regression equation of $y = 0.0248x + 0.0714$ and a correlation coefficient ($R^2 = 0.9931$), the devised approach followed Beer-Lambert's law over the concentration range of 2–32 $\mu\text{g/mL}$, confirming a strong linear relationship between concentration and absorbance. Although the correlation coefficient was slightly lower than the ideal value of 1.000, it remained well within the acceptable range for quantitative analytical methods and demonstrated the suitability of the selected concentration range for routine drug estimation[20,21].

Accuracy studies performed using the standard addition method at 80%, 100%, and 120% concentration levels produced percentage recoveries between 98% and 102%, with %RSD values below 2%, confirming that the developed method accurately quantified Ellagic Acid without significant interference from formulation excipients[24]. Similarly, the intra-day and inter-day precision studies yielded %RSD values below the acceptable limit of 2%, indicating excellent repeatability and intermediate precision. The robustness of the method was further confirmed by introducing deliberate variations in analytical wavelength (275 and 279 nm) and solvent composition (DMSO:Ethanol, 48:52 and 52:48, v/v). These minor changes produced negligible variations in

absorbance, with all %RSD values remaining below 2%, demonstrating that the method is reliable under normal laboratory conditions[22]. The calculated LOD (0.189 $\mu\text{g/mL}$) and LOQ (0.573 $\mu\text{g/mL}$) values indicated good analytical sensitivity, enabling the detection and accurate quantification of Ellagic Acid even at low concentration levels. Furthermore, specificity studies showed negligible absorbance for blank and placebo solutions, whereas the standard and nanosuspension samples exhibited distinct absorption at 277 nm, confirming that PVP-K30 and other formulation components did not interfere with the determination of Ellagic Acid[20].

Conclusion

The present study successfully developed and optimized a PVP-K30-coated Ellagic Acid nanosuspension using the antisolvent precipitation technique and a Face-Centered Central Composite Design (FC-CCD) approach. The optimized formulation exhibited a particle size of 552.7 nm, viscosity of 15.3 cP, entrapment efficiency of $90.85 \pm 0.56\%$, and drug loading of $31.24 \pm 0.39\%$. FTIR and DSC analyses confirmed drug–excipient compatibility and reduced crystallinity of Ellagic Acid, while SEM demonstrated the formation of nearly spherical particles with uniform morphology. In addition, a simple, rapid, economical, and reliable UV spectrophotometric method was successfully developed using DMSO:Ethanol (50:50, v/v), with Ellagic Acid exhibiting a maximum absorbance (λ_{max}) at 277 nm. The method showed good linearity over the concentration range of 2–32 $\mu\text{g/mL}$ ($R^2 = 0.9931$) and was successfully validated according to ICH Q2(R2) guidelines, demonstrating acceptable accuracy, precision, robustness, specificity, sensitivity, and reproducibility. Overall, the optimized nanosuspension and validated analytical method provide a reliable platform for routine quality control and support the further pharmaceutical development of Ellagic Acid formulations.

Conflict Of Interest:

The authors have no conflicts of interest regarding this investigation.

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