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A novel sustainable and ecofriendly UV-Spectrophotometric method for the determination of Naringenin in bulk and vaginal niosomal nanoformulation

Research Article

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Abstract

Naringenin, a flavonoid found in several fruits like oranges, grapefruit, and bergamot, exhibits a wide range of biological effects. These include antidiabetic, antidepressant, antiatherogenic, antitumor, immunomodulatory, anti-inflammatory, hypolipidemic, antioxidant, peroxisome proliferator-activated receptors (PPARs) activation, hepatoprotective properties, memory enhancement and DNA protective properties. The aim of this current study is to develop a simple and precise UV spectrophotometric technique for measuring Naringenin Active Pharmaceutical Ingredient (API) in bulk and vaginal niosomal nanoformulation using PBS (Phosphate buffer solution) pH 4.5. The absorption peak of Naringenin was identified at 287 nm, and it adhered to Beer's law within the concentration range of 2 to 14 µg/ml. The calibration curve demonstrates a linear relationship between absorbance and concentration within the range of 2 to 14 µg/ml. The determined limit of detection and limit of quantification were found to be 0.587672µg/mL and 1.780824µg/mL, respectively. The validity of the method was assessed for repeatability, accuracy and precision. The results obtained indicated minimal intraday and interday variation. The excipients in the nanoformulation did not interfere the analysis. The developed analytical UV spectrophotometric method is simple, rapid and consistent, making it suitable for estimating the drug in both bulk and nanoformulation.

Keywords: PCOS, Naringenin, Niosomes, Forced degradation studies, Analytical method validation, Phosphate buffer pH 4.5.

Introduction

Naringenin, a type of flavonoid compound, is found in abundance in various fruits like oranges, grapefruit, and bergamot.(1)The IUPAC name of Naringenin is 2, 3-dihydro-5, 7- dihydroxy-2-(4-hydroxyphenyl) - 4H-1-benzopyran-4-one. (2)It has a molecular weight of 272.256 g/mol (C15 H12 O5). The chemical structure of Naringenin is presented in figure 1.Naringenin possesses three hydrogen donors and five acceptors. It is a solid compound with a melting point ranging from 248 to 251 degrees Celsius. This compound demonstrates weakly basic characteristics, as evidenced by its two pKa values of 7.05 and 8.84. It readily dissolves in organic solvents such asdimethyl formamide, ethanol and dimethyl sulfoxide, its solubility in aqueous buffers is limited. (3) Clinical investigations into various dosing schedules of this medication have faced challenges due to its highly limited solubility in water. Consequently, it's not surprising that the absolute bioavailability of Naringenin was found to be merely 4% in rabbits following oral ingestion. (4)

Naringenin is the product of the enzymatic hydrolysis of naringin. (5) Naringenin is classified as a flavone, a subtype of flavonoid, is recognised for its potential bioactive impact on human have proposed a negative correlation between flavonoid consumption, including naringenin, and oxidative stress effects. (6) The hydroxyl groups within the naringenin structure have the capability to provide hydrogen to reactive oxygen species (ROS) and neutralize free radicals. (7)

Naringenin exhibits a range of biological properties such as antidiabetic, antidepressant, antiatherogenic, antitumor, immunomodulatory, anti-inflammatory, hypolipidemic, antioxidant, peroxisome proliferator-activated receptors (PPARs) activation, hepatoprotective properties, memory enhancement and DNA protective properties. (6,8)

Polycystic ovary syndrome (PCOS) stands as the prevailing endocrine disorder among women, impacting approximately 8% to 13% of women of reproductive age. (9) Both genetic and environmental factors contribute to the development of polycystic

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ovary syndrome (PCOS). Unfavourable lifestyle choices, dietary habits, or exposure to infectious agents can heighten the likelihood of PCOS. Insulin resistance, accompanied by elevated insulin levels, disrupts ovarian function, leading to increased level of androgen and following anovulation. Furthermore, PCOS often involves disturbances in the levels of follicle-stimulating hormone (FSH), gonadotropin-releasing hormone, prolactin and luteinising hormone (LH). (10) Hyperandrogenism and high body mass index are identified as risk factors associated with the onset of non-alcoholic fatty liver disease (NAFLD) in individuals diagnosed with polycystic ovary syndrome (PCOS). (11)

Research findings indicated that naringenin effectively prevented the weight gain typically linked with PCOS and led to decreased serum glucose levels in rats with PCOS. Naringenin treatment in a rat model of polycystic ovary syndrome (PCOS) exhibited antioxidant properties and influenced the activity of steroidogenic enzymes. (12)

Oral administration remains widely preferred due to its high patient acceptance. However, it is noteworthy that over 90% of newly developed active pharmaceutical ingredients face challenges with poor water solubility. Consequently, upon oral intake, only a fraction of the drug enters systemic circulation owing to its limited water solubility and slow dissolution rate. To address these issues, various nanotechnologies have been explored. (13)

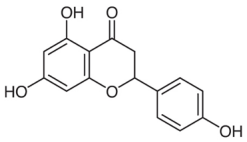
Niosomes are vesicles composed of non-ionic surfactants, typically featuring a bilayer structure. They often include components such as cholesterol and charge-inducing agents, which are usually biocompatible and non-toxic. Niosomes exhibit greater stability compared to liposomes and possess the capability to encapsulate both hydrophilic and lipophilic molecules with biological activity. (14)Therefore due to low oral bioavailability, naringenin was incorporated into a niosomal nanoformulation for encapsulation. (15)So administration through vaginal route for conditions like PCOS could improve the bioavailability.

In women of reproductive age, the typical pH range for the vagina is between 4.0 to 4.5, though it might be slightly elevated beyond 4.5 in premenarchal and postmenopausal women. (16) In this research study, an analytical method was developed in pH 4.5 phosphate buffer and methanol, as literature review reveals that no research articles were published using methanol and pH 4.5 phosphate buffer.

Based on the literature review, various techniques such asUV spectroscopy(17,18),high performance liquid chromatography (HPLC)(17,19), have been utilised to detect Naringenin in bulk as well as pharmaceutical formulation.Here, methanol was utilised as a dilution solvent, which is less cost-effective and more toxic than an aqueous solvent.

Therefore, the primary aim of this current study is to employ green solvent for the determination of Naringenin API and niosomal nanoformulation.

Figure 1: Chemical structure of Naringenin



Materials and Methods

Instrumentation

The analysis of Naringenin was conducted using a Shimadzu UV-1900 UV-Spectrophotometer equipped with UV probe software. To ensure precise measurement of the samples, a calibrated weighing balance was utilised throughout the experiment. The particle size and zeta potential were analysed using a Nano ZS ZS90 device (Malvern Instruments, UK). The measurements were conducted at 25°C with the aid of folded capillary cells (DTS 1060). The instrumentation was utilised at KLE College of Pharmacy, Belagavi.

Chemicals and solvents

Naringenin was acquired from the Sisco Research Laboratories, Mumbai, while methanol and a phosphate buffer with a pH of 4.5 were obtained from KLE College of Pharmacy in Belagavi. The chemicals utilised in the study were exclusively of analytical grade.

Method development

Preparation of the standard stock solution

The solvent system chosen for the experiment was pH 4.5 phosphate buffer solution. A precise amount of 10mg of Naringenin was weighed and placed into a clean, dried 10ml volumetric flask. Naringenin exhibited limited solubility in this phosphate buffer solution of pH 4.5, so Naringenin was dissolved in methanol, and the volume was adjusted to 10ml with methanol. This solution, with a concentration of 1000µg/ml, was established as the standard stock solution. Subsequently secondary and tertiary stock solutions were prepared with phosphate buffer solution of pH 4.5.

Determination of λ_{max}:

A UV-Spectrophotometer was employed to analyse a working standard solution of Naringenin with a concentration of 10µg/ml. The wavelength scanning range was set from 400nm to 200nm, and the solution exhibited its maximum absorption at 287nm.

Plotting of calibration curve

Serial dilutions containing concentrations of 2, 4, 6, 8, 10, 12, 14 mg/ml were prepared using secondary stock solution. The solutions were analysed and the absorbance were measured at 287nm. The calibration curve was plotted as concentration on x-axis and absorbance on y-axis, and linear regression equation was determined.

Method Validation

The UV-spectroscopy method for quantifying the concentration of Naringenin was developed and then subjected to validation according to the ICH Q2 (R1) guidelines. This validation process involved evaluating multiple parameters, including linearity, range, limit of quantification (LOQ), limit of detection (LOD), precision, accuracy, ruggedness and robustness. The validation utilised pre-established calibration standards, as outlined in the following description. (20)

Linearity and range

The linearity of an analytical method indicates its ability to precisely measure data that correlates directly with the concentration of the analyte. To evaluate the linearity of the method, serial dilutions ranging from 2 to 14 µg/ml were prepared using the secondary stock solution. These dilutions were subsequently analysed at 287nm, with each measurement performed in triplicate. (21)

LOD and LOQ

The limit of detection (LOD) signifies the lowest concentration of the analyte in a sample that can be identified. On the other hand, the limit of quantification (LOQ) represents the minimum amount of analyte in a sample that can be accurately measured and quantified. To calculate these values, the following formula was utilised: LOD= 3.3 X σ / S and LOQ = 10 X σ / S, where σ represents standard deviation of response and S stands for the slope of calibration curve. (22)

Precision

The precision of an analytical method refers to its level of repeatability under typical operating conditions. Precision was evaluated by assessing the method's consistency through intraday and interday analyses of samples. Dilutions containing Naringenin with concentrations 2, 8, 14µg/mL were prepared for this evaluation. The absorbance of each solution at 287nm was determined. Subsequently, the %RSD (relative standard deviation), Mean, and SD (standard deviation) were calculated to gauge precision. (23)

Intraday Precision: The intraday precision was measured by analysing the samples three times within a single day using UV Spectroscopy at different time intervals. Mean, SD, and %RSD values were calculated based on these analyses.

Inter day Precision: It was assessed by preparing and analysing aliquot solutions over three consecutive days. Mean, SD, and %RSD values were then calculated based on these analyses. (17)

Accuracy

Accuracy of the proposed UV-Visible method was confirmed through recovery trials, which involved adding known amounts of the analyte to the samples. Three distinct Naringenin solutions were prepared in triplicate, each at concentrations of 50%, 100%, and 150% relative to the standard Naringenin concentration of 8µg/ml. The percentage recovery was subsequently determined based on these experiments.(24)

Ruggedness

When evaluating ruggedness, dilutions containing Naringenin were created, and the absorbance of each solution at 287nm was measured by different analysts and with different instruments. Subsequently, the %RSD, Mean, and SD were calculated. (17)

Robustness

A key aspect of robustness is devising methods that accommodate anticipated variations in separation parameters. To evaluate method robustness, parameters such as detector wavelength variation are deliberately altered within defined ranges, and the quantitative impact of these variables is assessed. (25)The detector wavelength was varied ±2nm, absorbance is measured and mean, SD and %RSD was calculated.

Forced degradation studies

To evaluate the stability of the UV-Spectroscopic method developed, samples underwent degradation tests by exposure to acid, base, oxidation, and photolytic conditions. It is generally desirable to control the degradation conditions to prevent it from excessive degradation. Therefore 2 hrs is preferred for the stress degradation studies. In the present study, stress degradation assays were performed for 2 hrs as per ICH recommended stress conditions.(29)

Acid degradation study

Naringenin solution was prepared by using 1ml of secondary stock solution and 1ml of 0.1 N HCl in a 10mL volumetric flask. The solution was then subjected to stress conditions at 80°C for 2 hours. Then made up the volume with PBS pH 4.5 and spectra was analysed. (26,27,29)

Base degradation study

Naringenin solution was prepared by using 1ml of secondary stock solution and 1ml of 0.1 N NaOH in a 10mL volumetric flask. The solution underwent stress conditions for 2 hours at 80°C. Then volume was made up with PBS pH 4.5 and the spectra was scanned. (28,29)

Oxidation degradation study

Naringenin solution was prepared by using 1ml of secondary stock solution and 1ml of 30% hydrogen peroxide. Subsequently, the solution was subjected to stress conditions at 80°C for 2 hours. Then made up the volume with PBS pH 4.5 and spectra was recorded. (30,29)

Photo degradation study

In the photodegradation study, solution of Naringenin at a concentration of 10µg/mL was prepared with PBS pH 4.5 in a transparent volumetric flask to a final volume of 10mL. The flask was then sealed,

exposed to sunlight for 2 hours and spectra was scanned. (29)

Preparation of Naringenin loaded niosomes

Naringenin loaded niosomes were formulated using the thin film-hydration technique coupled with sonication. The niosomes were formulated with Span 80 and cholesterol at a specific molar ratio. Preliminary trials were performed to select the molar ratio for niosomal formulation. Molar ratios from low to high for span 80 : cholesterol (1.35:1,1.50:1,1.79:1)for batches B1, B2 and B3 respectively were selected for analysis. The niosomal compositions, along with Naringenin (20mg), were taken in round bottom flask and dissolved in chloroform. Subsequently, the solvent was evaporated at room temperature (27 ± 2 °C) (31,32) using a rotary evaporator to create a thin film. At this temperature, the formation of a uniform thin film is facilitated, which is crucial for the consistent formulation of niosomes. The thin film ensures that the solvent evaporates evenly, leading to a more homogeneous product. Any remaining solvent was then dried overnight in a vacuum desiccator. Following this, phosphate buffer solution pH 4.5 was introduced to the thin film at a temperature of 50 ± 2 °C, and the mixture was stirred for 30 minutes. It improves the drugs solubility and dispersion in the aqueous phase and promotes the formation of multilamellar niosomes with high entrapment efficiency. The obtained dispersion underwent sonication using a microtip probe sonicator for a duration of 10 minutes.(31,32,33)The prepared niosomes were stored in ambered coloured bottle in refrigerator.

Particle Size and Zeta Potential Analysis

The average particle size and zeta potential of the Naringenin niosomes were evaluated using the Malvern Zetasizer. Specifically, 1ml of the Naringenin niosomes was withdrawn and diluted with 10ml of Milli-Q water for analysis.

Entrapment Efficiency

To determine the encapsulation efficiency (EE), the niosomal dispersion containing Naringenin (1 ml) was disrupted by adding 0.1 ml of 0.1% Triton X-100 in 2 ml PBS (pH 4.5) and agitated for 5 minutes. The resulting solution underwent centrifugation at 3000 rpm for 5 minutes using a High-Speed Refrigerated Centrifuge (Floor Model, 7000 Kubota, Japan). Then 1ml of supernatant was pipetted, appropriately diluted, and analysed using UV/Visible spectroscopy at 287 nm. The percentage EE was then calculated. (34)

$$\%EE = \frac{\text{Total entrapped drug} - \text{Amount of drug in supernatant}}{\text{Total entrapped drug}} \times 100$$

Assay of Naringenin loaded niosomal nanoformulation

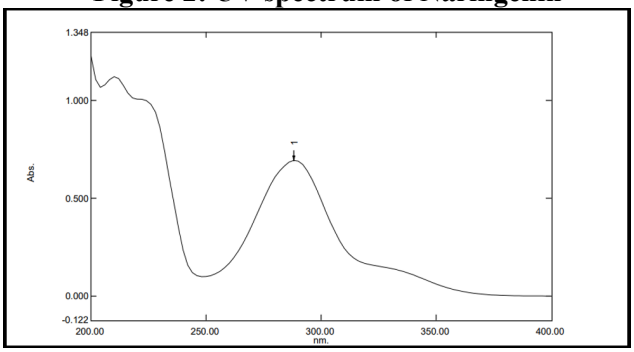
The newly developed analytical technique was employed to determine the concentration of Naringenin in niosomal formulation. The dilution was prepared with concentration of 10µg/ml and absorbance was measured. Then the amount of Naringenin was determined by utilising the calibration curve. (24)

Results and Discussion

Determination of maximum wavelength

The wavelength of maximum absorption after scanning was found to be 287nm. The UV visible spectroscopy spectrum of Naringenin is depicted in Figure 2.

Figure 2: UV spectrum of Naringenin



Linearity and range

The linearity and range for Naringenin were established by analysing triplicates of concentrations ranging from 2 to 14 µg/ml. These concentrations were measured at 287 nm, yielding a correlation coefficient of 0.9989. Linearity data is signified in Table 1 and the standard calibration curve of Naringenin is shown in figure 3. The overlay spectrum graph illustrating the results from UV-visible spectroscopy analysis is presented in Figure 4.

The limit of detection (LOD) and limit of quantification (LOQ) of Naringenin were determined following the approach outlined in the International Conference on Harmonization (ICH) guidelines, which involves utilising the standard deviation of the response and slope. The obtained values for LOD and LOQ were 0.587672µg/mL, 1.780824µg/mL, respectively. These results highlight the high sensitivity of the UV method proposed.

Table 1: Linearity and range data of Naringenin

Sr. No.	Concentration (µg/	Absorbance
1	0	0
2	2	0.149
3	4	0.271
4	6	0.376
5	8	0.494
6	10	0.636
7	12	0.747
8	14	0.874
R^2= 0.9989		
y = 0.0614x + 0.0133		

Precision

Precision was assessed through intraday and interday precision tests, demonstrating excellent repeatability, intraday and interday precision of the proposed method. The mean % RSD values for both interday and intraday precision studies were calculated represented in the table 2 and 3.

Figure 3: Standard calibration curve of Naringenin

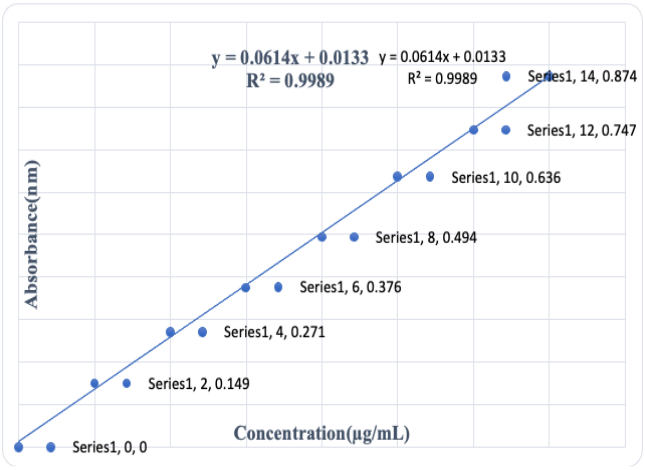


Figure 4: The overlay spectrum graph of Naringenin

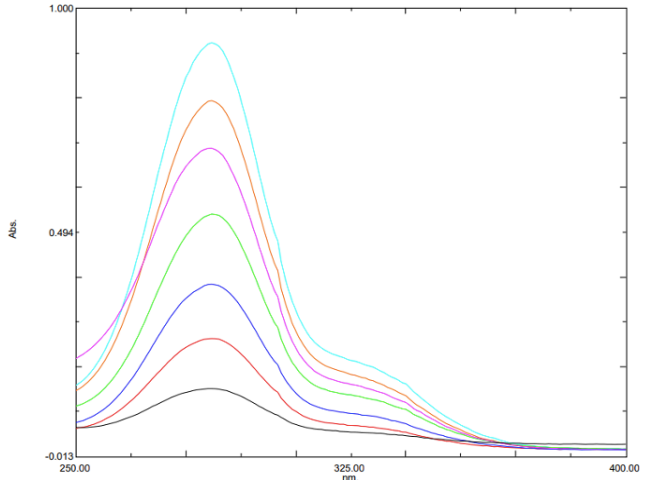


Table 2: Intraday precision data of Naringenin

Replicates	Concentration (µg/ mL)	Absorbance Morning	Absorbance Noon	Absorbance Evening
1	**2**	0.148	0.149	0.150
2	**2**	0.151	0.147	0.151
3	**2**	0.149	0.150	0.148
Mean± S.D.		0.14933±0.001528	0.148667±0.001528	0.149667±0.001528
%RSD		1.022896	1.027483	1.020618
1	**8**	0.492	0.491	0.492
2	**8**	0.496	0.490	0.494
3	**8**	0.495	0.493	0.495
Mean± S.D.		0.49433±0.002082	0.49133±0.001528	0.493667±0.001528
%RSD		0.421106	0.310894	0.309424
1	**14**	0.876	0.873	0.875
2	**14**	0.871	0.876	0.871
3	**14**	0.873	0.874	0.873
Mean±S.D.		0.87333±0.002517	0.874333±0.001528	0.873±0.002
%RSD		0.288162	0.174707	0.229095

Table 3: Interday precision data of Naringenin

Replicates	Concentration (µg/mL)	Absorbance Day 1	Absorbance Day 2	Absorbance Day 3
1	**2**	0.149	0.134	0.131
2	**2**	0.150	0.140	0.130
3	**2**	0.149	0.140	0.129
Mean± S.D.		0.14933±0.001528	0.14933±0.000577	0.138±0.003464
%RSD		1.022896	0.386618	2.510219
1	**8**	0.492	0.482	0.476
2	**8**	0.491	0.482	0.476
3	**8**	0.495	0.485	0.475
Mean± S.D.		0.49433±0.002082	0.492667±0.002082	0.483±0.001732
%RSD		0.421106	0.42253	0.358603
1	**14**	0.871	0.862	0.856
2	**14**	0.874	0.861	0.855
3	**14**	0.872	0.865	0.854
Mean±S.D.		0.87333±0.002517	0.872333±0.001528	0.862667±0.002082
%RSD		0.288162	0.175108	0.241306

Accuracy

The accuracy of the sample was evaluated through recovery experiments, where the average recovery of the sample fell within acceptable limits. This indicates that the proposed method is reliable for estimating Naringenin. The data are shown in table 4.

Table 4: Accuracy data of Naringenin

Conc. added µg/ml	Level	Vol of std(ml)	Vol of formulation(ml)	Abs	Mean	Conc. found	% recovery
12	50	4	8	0.748	0.754333	12.06895	100.5746
	50	4	8	0.762			
	50	4	8	0.753			
16	100	8	8	0.98	1.005667	16.16232	101.0145
	100	8	8	1.014			
	100	8	8	1.023			
20	150	12	8	1.26	1.236667	19.92454	99.62269
	150	12	8	1.22			
	150	12	8	1.23			

Ruggedness

The ruggedness of the method was assessed by changing the analyst and instrument, and the percentage relative standard deviation (% RSD) was computed. The findings are presented in Table 5.

Table 5: Ruggedness data for Naringenin

Replicates	Concentration (µg/mL)	Absorbance (change of Analyst)	Absorbance (change of instrument)
1	**2**	0.148	0.151
2	**2**	0.150	0.153
3	**2**	0.152	0.150
Mean±S.D.		0.150±0.002	0.15133±0.001528
%RSD		1.3333	1.009378
1	**8**	0.490	0.493
2	**8**	0.495	0.489
3	**8**	0.491	0.491
Mean±S.D.		0.492±0.002646	0.491±0.002
%RSD		0.537754	0.407332
1	**14**	0.871	0.874
2	**14**	0.869	0.868
3	**14**	0.872	0.870
Mean±S.D.		0.870667±0.001528	0.870667±0.003055
%RSD		0.175443	0.350886

Robustness

For the estimation of method robustness, variation in detector wavelength was done. Naringenin was estimated at 285nm and 289nm and results are given in the table 6.

Table 6: Robustness data for Naringenin

Replicates	Concentration (µg/ml)	Absorbance at 285nm	Absorbance at 289nm
1	**2**	0.146	0.151
2	**2**	0.142	0.154
3	**2**	0.145	0.153
Mean±S.D.		0.150±0.002	0.1443±0.002082
%RSD		1.3333	1.442263
1	**8**	0.486	0.504
2	**8**	0.489	0.507
3	**8**	0.491	0.509
Mean±S.D.		0.492±0.002646	0.4886±0.002517
%RSD		0.537754	0.514996
1	**14**	0.854	0.886
2	**14**	0.852	0.884
3	**14**	0.854	0.886
Mean±S.D.		0.870667±0.001528	0.8533±0.001155
%RSD		0.175443	0.135316

Forced degradation studies

Naringenin showed varying levels of degradation when exposed to different stress conditions.

When Naringenin was exposed to acidic conditions (0.1N HCl), it underwent degradation to an extent of 13.99% % due to protonation of its hydroxyl groups. However, in a basic environment (0.1N NaOH), it degrades to

larger extend i.e. 19.02%, as the alkaline conditions promote deprotonation. Naringenin exposed to oxidation degradation showed extreme degradation as compared to acid and base degradation i.e. 24.37%. This is due to oxidation disrupting the molecular structure by cleaving double bonds and oxidising hydroxyl groups.Photolytic degradation resulted in minimal changes in the spectra, with a degradation rate of 12.26% as Naringenin is relatively photostable and less affected by light exposure. (29) These findings emphasise Naringenin is more susceptible to degradation in basic and oxidative environments, while it demonstrates greater stability under acidic and photolytic conditions.The spectrums after degradation studies are represented in figures 5a, 5b, 5c, 5d and degradation data is represented in table 7.

Table 7: Degradation studies of Naringenin

Sr.No.	Degradation Parameters	Absorbance	% Degradation
1	After photolytic	0.558	12.26
2	After acid	0.547	13.99
3	After base	0.515	19.02
4	After oxidation	0.481	24.37

Figure 5a: Spectrum after photolytic degradation

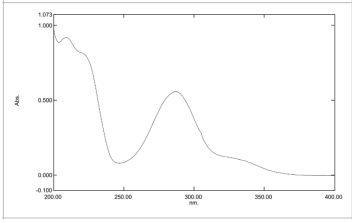


Figure 5b: Spectrum after acid degradation

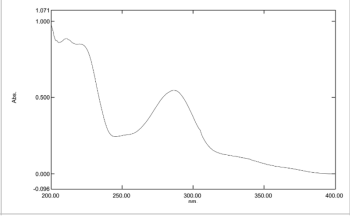


Figure 5c: Spectrum after base degradation

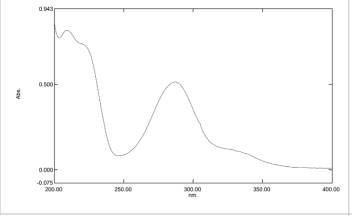
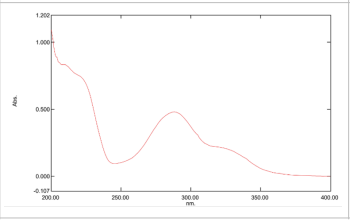


Figure 5d: Spectrum after oxidation degradation



Particle Size and Zeta Potential Analysis

The optimal niosomal batch was chosen based on the particle size and zeta potential range required for vaginal delivery.Particle Size of the optimised batch(B2) was found to be 180.9nm indicating the nano-size of the particles and zeta potential was found to be -33.04mV, indicating that particles are strongly anionic in nature and the formulation is stable enough and without any aggregate formation. The particle size and zeta potential distribution is given in figure 6a and 6b.

Figure 6a: The particle size distribution

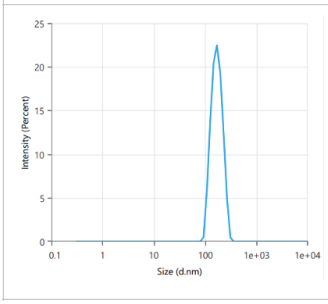
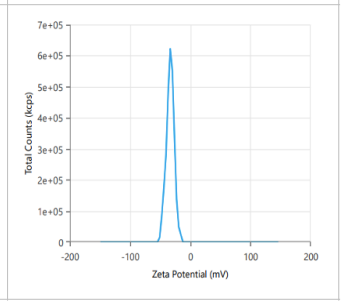


Figure 6b: Zeta potential distribution



Entrapment Efficiency

The measurement of entrapment efficiency is essential to know the formulation's ability to retain the drug, preventing early release or degradation. The %EE of the prepared Naringenin loaded niosomes was found to be 81.98%. A higher entrapment efficiency signifies better formulation performance, ensuring more effective retention of the drug within the delivery system for controlled and targeted release.

Assay of Naringenin loaded niosomal nanoformulation

The UV method developed was effectively utilised for determining the Naringenin in niosomal nanoformulation. The average assay percentage of Naringenin in the nanoformulation was determined to be 99.62% and represented in table 8.

Table 8: Assay of Naringenin loaded niosomal nanoformulation

Formulation	Amount of drug	Amount of drug estimated	Assay
Naringenin niosomes	10mg	9.962mg	99.62%

Conclusion

A straightforward, precise, and accurate UV-Visible spectrophotometric method was developed and validated for the estimation of Naringenin. The developed method demonstrated specificity, as no interference was detected from any excipients present in the niosomes during the analysis of Naringenin. Validation of the proposed method was conducted following the guidelines outlined by the International Council for Harmonisation (ICH), and both accuracy and precision were found to be within acceptable limits, with consistent recovery rates observed. Additionally, forced degradation studies revealed that Naringenin underwent degradation under acidic, basic, and photolytic conditions. Notably, Naringenin showed significant degradation under oxidative stress conditions. Overall, the UV method demonstrated excellent applicability for determining Naringenin content in bulk and niosomes, including other dosage

formulations, and all results meeting the predefined acceptance criteria.

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References

1. Yang Y, Trevethan M, Wang S, Zhao L. Beneficial effects of citrus flavanones naringin and naringenin and their food sources on lipid metabolism: An update on bioavailability, pharmacokinetics, and mechanisms. The Journal of nutritional biochemistry. June, 2022;104;108967.
2. Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya-Martínez M.T, Gutiérrez-Salinas J, Bautista M, Morales-González Á, González-Rubio M.G, Aguilar-Faisal J.L, Morales-González J.A. Review of natural products with hepatoprotective effects. World journal of gastroenterology. October, 2014;20(40);14787.
3. Joshi R, Kulkarni Y.A, Wairkar S. Pharmacokinetic, pharmacodynamic and formulations aspects of Naringenin: An update. Life sciences. December, 2018;215; 43-56.
4. Hsiu S.L, Huang T.Y, Hou Y.C, Chin D.H, ChaoP.D. Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits. Life sciences. February, 2002;70(13);1481-1489.
5. Ribeiro I.A, Ribeiro M.H. Naringin and naringenin determination and control in grapefruit juice by a validated HPLC method. Food Control. April, 2008; 19(4);432-438.
6. Kiran S.D, Rohini P, Bhagyasree P. Flavonoid: A review on Naringenin. Journal of Pharmacognosy and Phytochemistry. 2017; 6(5); 2778-83.
7. Shen S.C, Ko C.H, Tseng S.W, Tsai S.H, Chen Y.C. Structurally related antitumor effects of flavanones in-vitro and in-vivo: involvement of caspase 3 activation, p21 gene expression, and reactive oxygen species production. Toxicology and Applied pharmacology. June, 2004;197(2);84-95.
8. Kaur A, Dang S. Development of a nanoemulsionloaded with naringenin. Nanotechnology Perceptions. March, 2022;18(1); 53-63.
9. Hoeger K.M, Dokras A, Piltonen T. Update on PCOS: consequences, challenges, and guiding treatment. The Journal of Clinical Endocrinology & Metabolism. March, 2021;106(3); e1071-83.
10. Ajmal N, Khan S. Z, Shaikh R. Polycystic ovary syndrome (PCOS) and genetic predisposition: A review article. European journal of obstetrics &gynecology and reproductive biology: X. July, 2019;3; 100060.
11. Spremović R.S, Pupovac M, Andjić M, Bila J, Srećković S, Gudović A, Dragaš B, Radunović N. Prevalence, risk factors, and pathophysiology of nonalcoholic fatty liver disease (NAFLD) in women with Polycystic Ovary Syndrome (PCOS). Biomedicines. January, 2022;10(1);131.
12. Hong Y, Yin Y, Tan Y, Hong K, Zhou H. The flavanone, naringenin, modifies antioxidant and steroidogenic enzyme activity in a rat model of letrozole-induced polycystic ovary syndrome. Medical science monitor: international medical journal of experimental and clinical research. January, 2019; 25; 395-401.
13. Zhang G, Guan H, Li J, Li M, Sui X, Tian B, Dong H, Liu B, He Z, Li N, Zhao M. Roles of effective stabilizers in improving oral bioavailability of naringenin nanocrystals: Maintenance of supersaturation generated upon dissolution by inhibition of drug dimerization. Asian Journal of Pharmaceutical Sciences. August, 2022;17(5); 741-50.
14. Liga S, Paul C, Moacă E.A, Péter F. Niosomes: Composition, Formulation Techniques, and Recent Progress as Delivery Systems in Cancer Therapy. Pharmaceutics. February, 2024;16(2); 223.
15. Zhang P, Lin R, Yang G, Zhang J, Zhou L, Liu T. Solubility of naringenin in ethanol and water mixtures. Journal of Chemical & Engineering Data. September, 2013;58(9); 2402-4.
16. Lin Y.P, Chen W.C, Cheng C.M, Shen C.J. Vaginal pH value for clinical diagnosis and treatment of common vaginitis. Diagnostics. October, 2021;11(11);1996.
17. Jha D.K, Shah D.S, Talele S.R, Amin P.D. Correlation of two validated methods for the quantification of naringenin in its solid dispersion: HPLC and UV spectrophotometric methods. SN Applied Sciences. April, 2020;2; 1-1.
18. Bhandari R, Kuhad A, Paliwal JK, Kuhad A. Development of a new, sensitive, and robust analytical and bio-analytical RP-HPLC method for in-vitro and in-vivo quantification of naringenin in polymeric nanocarriers. Journal of Analytical Science and Technology. December 2019;10(1); 1-4.
19. Metkari V, Shah R, Salunkhe N. Development and validation of UV spectrophotometric method for estimation of naringenin in phytosomal formulation: interlaboratory comparison, capability, and statistical analysis. Analytical Sciences. November, 2023;39(11); 1917-28.
20. Peerzade M.Y, Memon S, Bhise K, Aamer A.I. Development and validation of UV-Visible spectrophotometric method for estimation of ritonavir in bulk and formulation. Pharma Innovation J. 2019;8(4):30-34.
21. Kudatarkar N, Jalalpure S, Singadi R, Gurao R, Dinnimath B. Development And Validation Of UV-Visible Spectrophotometric Method For Estimation Of Chrysin In Bulk Form. Journal of Pharmaceutical Negative Results. December, 2022;13(8);4758-63.
22. Taleuzzaman M. Limit of blank (LOB), limit of detection (LOD), and limit of quantification (LOQ). Org. Med. Chem. Int. J. August, 2018;7(5);555722.

23. Shetti P, Jalalpure S.S. Optimization of a validated UV-spectrophotometric methodology for assessment of apigenin in bulk powder. IJPER. January, 2022;56(1);281-6.
24. Kumar H.B.C, Kurangi B, Soudagar M, Chimgave S, Patil S. Development and Validation of UV-Spectrophotometric Method for Estimation of Vinpocetine in Marketed Formulation and Nanoformulation. International Journal of Ayurvedic Medicine, 2023; 14 (3);717-723
25. Ravisankar P, Sulthana M.S, Babu PS. Development and validation of stability-indicating UV spectrophotometric method for determination of Apremilast in bulk and pharmaceutical dosage form. Ind J Res Pharm Biotechnol. January, 2017;5(1);47-53.
26. Kurangi B, Jalalpure S, Jagwani S. A validated stability-indicating HPLC method for simultaneous estimation of resveratrol and piperine in cubosome and human plasma. Journal of Chromatography B. August, 2019;1122;39-48.
27. Jadhav K.V, Dhamecha D.L, Asnani G.P, Patil P.R, Patil M.B. Stability-indicating stress degradation studies of lafutidine using UV spectrophotometric method. Pharm Methods. 2013;4(1);21-5.
28. Agrawal P.Y, Agrawal M.Y, Jadhav S.B, Shinde R.J. Development and validation of novel UV spectrophotometric method for the determination of evogliptintartarate in pharmaceutical dosage form. Indian J Pharm Educ Res. 2020;54(4);1174-9.
29. Kurangi B, Jalalpure S. A validated stability-indicating RP-HPLC method for piperine estimation in black pepper, marketed formulation and nanoparticles. Indian J Pharm Educ Res. 2020;54(3s):s677-86.
30. Chimagave S.S, Jalalpure S.S, Patil A.K, Kurangi B.K. Development and validation of stability indicating UV-spectrophotometric method for the estimation of hesperidin in bulk drugs, plant extract, Ayurveda formulation and nanoformulation. Indian J Pharm Educ Res. July, 2022;56(3);865-72.
31. Chaikul P, Khat-Udomkiri N, Iangthanarat K, Manosroi J, Manosroi A. Characteristics and in vitro anti-skin aging activity of gallic acid loaded in cationic CTAB niosome. European Journal of Pharmaceutical Sciences. April, 2019;131;39-49.
32. Manosroi A, Chaikul P, Abe M, Manosroi W, Manosroi J. Melanogenesis of methyl myristate loaded niosomes in B16F10 melanoma cells. Journal of biomedical nanotechnology. April, 2013;9(4);626-38.
33. Bhat M.I, Ganesh N.S, Majeed T, Chandy V. Niosomes a controlled and novel drug delivery system: A brief review. World journal of Pharmaceutical sciences. 2019;3(8);481-97.
34. Mavaddati M.A, Moztarzadeh F, Baghbani F. Effect of formulation and processing variables on dexamethasone entrapment and release of niosomes. Journal of Cluster Science. November, 2015;26;2065-78.

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