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DEVELOPMENT AND VALIDATION OF A SIMPLE AND NOVEL UV SPECTROPHOTOMETRIC APPROACH FOR THE QUANTITATIVE ESTIMATION OF ELLAGIC ACID IN BULK AS PER ICH GUIDELINES

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Keywords:

Ellagic acid, Anti-inflammatory, UV Spectroscopy, Ethanol, Validation, Method development

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ABSTRACT: Ellagic acid, a naturally occurring polyphenolic compound is found in various fruits and vegetables and has attracted considerable attention due to its broad spectrum of biological and pharmacological properties. Several key aspects of ellagic acid, including its antioxidant activity, anti-cancer properties, anti-inflammatory effects, protective role in cardiovascular health, as well as its antibacterial and antiviral activities, were highlighted in prior research. The proposed UV method, found to be simple and novel. The developed method aligns with the concept of "green" solvents, aiming to minimize the environmental damage typically caused by solvent use in chemical production. Ethanol was chosen as the solvent Instead of all other alternatives. The absorbance of ellagic acid was measured in the UV range, and a λmax of 254 nm was identified. Recovery studies and statistical validation further supported the findings of the study. The drug follows linearity in the concentration range of 2–10µg/ml. The low values of % RSD are indicative of the accuracy and reproducibility of the method. Precision was assessed through repeatability tests. Furthermore, adherence to the rigorous guidelines outlined in ICHQ2(R2) was ensured throughout the comprehensive validation process, consolidating the method's credibility and suitability for analytical applications.

INTRODUCTION: Traditional medicine-based herbal remedies are frequently utilized to treat a variety of ailments. Ellagic acid (EA), a polyphenol metabolite used in herbal medicine, is present in many medicinal plants.

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Ellagic acid (EA) is a polyphenol extractive (tannin) that is extracted from various fruits, nuts, and plants, including raspberries, strawberries, grapes, pomegranates, black currants, longan seeds, and green tea.

It is also known as 2,3,7,8-tetrahydroxy [1]-benzopyranol [5,4,3-cde] benzopyran-5,10-dione. Ellagitannin represent the largest group of hydrolysable tannins, with over 1000 known compounds. EA's antioxidant and anti-inflammatory qualities play a key role in protecting against natural and chemical toxicities.

Ellagic acid, with a molecular mass of 302.197 g/mol, is a highly thermostable molecule with low solubility in water and moderate solubility in alcohol. The human body can metabolize ethanol; thus, it doesn't need to be eliminated during further processing due to safety concerns. Because of its comparatively low toxicity and the fact that bioethanol, which includes all ethanol intended for food use, is produced from crops that offset carbon emissions, ethanol is referred to as a "green" solvent. Being one of the greenest organic solvents, ethanol is especially preferred for use in green spectroscopy. Ethanol is less hazardous and has a lower vapor pressure than Acetonitrile and Methanol. This results in less evaporation and, ultimately, less inhaled amounts. In addition to its antioxidative qualities and anti-inflammatory capabilities, EA has been shown to be anti-inflammatory *in-vitro* and *in-vivo* studies.

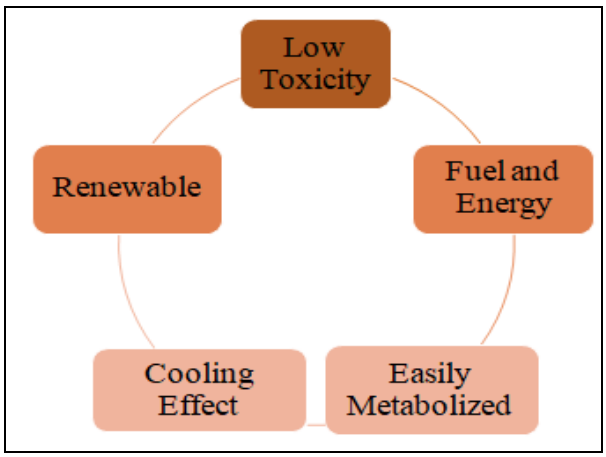

FIG. 1: BENEFITS OF ETHANOL AS GREEN SOLVENT

MATERIALS AND METHODS:

Equipment and Chemicals: Jasco v-730 spectrophotometer was used for the analysis. Labman Sonicator used for performing sonication. Ellagic acid was procured from Yucca enterprises. Ethanol was used as a solvent. For weighing and measuring of chemicals, calibrated analytical balance and glassware were used. Double distilled water was used where needed.

Preparation of Solution:

Stock A: Ellagic Acid Solution (500 ppm): Accurately weighed 5mg of Ellagic acid was transferred it into a 10ml cleaned and dried volumetric flask, and dissolved in sufficient quantity of ethanol using ultrasonicator for 10 minutes. The final volume was adjusted upto the mark with ethanol to obtain a standard stock solution of 500ppm (Stock A).

Stock B Solution (100 ppm): Two milliliters of the solution were pipetted from stock A and transferred to a 10-milliliter volumetric flask. Dilution using Ethanol was done to obtain 100 ppm solution (Stock B).

Working Standard Solutions (2-10µg/ml): A 2,4,6,8 & 10 µg/ml was prepared from Stock B. A graph was plotted between absorbance (Y-axis) and concentration (µg/ml) on the X-axis. Based on this plot, linearity was determined using the formula y = mx + c. From the calibration curve, calculate the coefficient of determination (r^2), slope (m), and intercept (c).

Selection of Wavelength of Maximum Absorption: The 10µg/ml working standard solution was scanned in the 200–400 nm range. Using ethanol as a blank, the wavelength of ellagic acid was found to be 254 nm.

Calibration Curve: A series of working standard solutions as 2, 4, 6, 8, and 10 ppm were prepared using ethanol as solvent.A 1 cm quartz cuvette was used to measure absorbance at 254 nm following ethanol baseline correction. Plotting a calibration curve with concentration on the X-axis and absorbance on the Y-axis was done.

Validation Parameters: ICH Q2 (R2): The developed method was validated for following parameters: Linearity, accuracy, precision, detection limit, quantitation limit, and robustness.

Linearity: Linearity is determined by filling cuvettes with that are prepared in the range of 2–10 µg/ml of concentration, spanning at least five distinct concentrations. Ellagic Acid's regression line equation and correlation coefficient were calculated by plotting the absorbance vs. concentration calibration curve.

Accuracy: In order to determine the accuracy of the method, Ellagic Acid recoveries were calculated at three different levels: 80,100 and 120 percent of the test concentration (6 µg/ml). A mean percentage recovery was calculated and recovery values were calculated.

Detection Limit: As the name suggests, the detection limit refers to the lowest concentration of analyte that can be identified but not always quantified as an exact value.

The formula for the detection limit (LOD) = 3.3xσ/S. where σ is the relative standard deviation of the response. S stands for the calibration curve's slope (for the analyte).

Quantitation Limit: Quantitative limits are the lowest concentrations of analytes in a sample that can be quantitatively identified with appropriate precision and accuracy.

In order to calculate the Quantitation Limit (LOQ), we use the formula LOQ = 10xσ/S. where, σ is the relative standard deviation of the response. S stands for the calibration curve's slope (for the analyte).

Precision:

Repeatability: The standard solution was placed in cuvettes six times and the absorbance values of all six concentrations were measured using UV spectrophotometer. The %RSD for the area of six replicate concentrations was determined to be within the prescribed limits.

Robustness: Robustness incorporates deliberate modifications in procedure parameters and indicates the approach's reliability in typical use.

The wavelength was changed between plus and minus, and the solutions were prepared in triplicate and examined to determine the percentage of RSD.

RESULT AND DISCUSSION:

Analytical Method for Determining Drug Concentration (UV/VIS Method):

Selection of Detection Wavelength: Fig. 1 shows that Ellagic Acid has a wavelength of 254 nm.

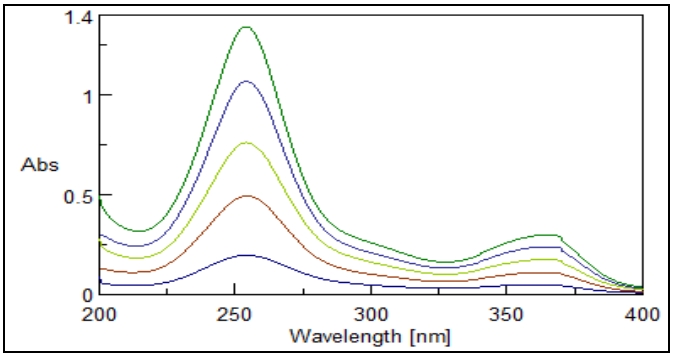

FIG. 1: OVERLAY SPECTRA OF ELLAGIC ACID

Construction of Calibration Curve: A series of working standard solutions of 2, 4, 6, 8, and 10 µg/ml was scanned at 254 nm. A graph was made showing the concentrations and their related absorbance. These concentrations showed a linear response across the investigated range of 2-10µg/ml. The calibration curve for absorbance (optical density) and concentration (ppm) was y =0.1918x+0.014, with a correlation coefficient of r^2 = 0.9938 (see **Fig. 2**).

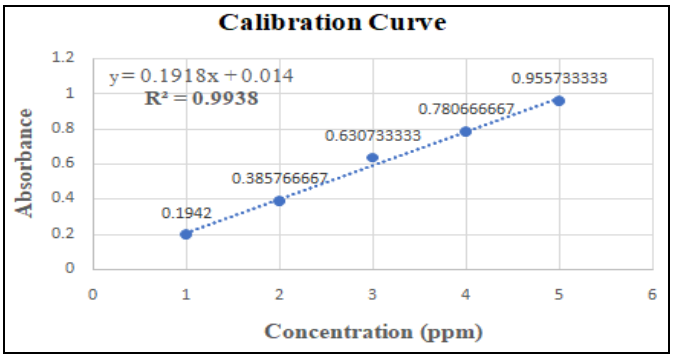

FIG. 2: CALIBRATION CURVE OF ELLAGIC ACID

Method Validation:

Linearity: Ellagic Acid's linearity was determined to be linear between 2 and 10µg/ml. As indicated in **Table 1**, in this study y=0.1918x+0.014 was determined as the regression equation, with r^2= 0.9938 as the correlation coefficient.

TABLE 1: LINEARITY RESULT DATA

Sr. no.	Conc. (µg/ml)	Absorbance	Std Deviation	% RSD
1.	2ppm	0.1942	0.00191572	0.9865
2.	4ppm	0.38577	0.0059702	1.548
3.	6ppm	0.63073	0.00638931	1.013
4.	8ppm	0.78067	0.00452364	0.5795
5.	10ppm	0.95573	0.00115902	0.1213

Accuracy: The %Recovery was found to be in the range of 96.87-102.25% **Table 2** for Ellagic Acid which indicates a good accuracy of the method.

TABLE 2: SHOWS % RECOVERY OF EA

Level	Amount Taken (µg/ml)	Amount Spiked	Amount Found	%Recovery
I	6	4.8	10.46	96.87%
II	6	6.0	11.84	98.65%
III	6	7.2	13.50	102.25%

LOD & LOQ: The results were computed using the slope (S=0.1918) and the standard deviation of the response (σ) from six replicates of the lowest concentration (2 µg/ml).

The calibration curve is used to compute the LOD using the formula:

$$LOD = 3.3x\sigma/S,$$

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

TABLE 3: LOD & LOQ RESULT

Parameter	Ellagic Acid
LOD	0.06869
LOQ	0.2080

Precision

Repeatability: Instead of utilizing six different concentrations, repeatability was evaluated using six replicate readings of the same concentration (6µg/ml). Good precision is confirmed by the low %RSD (0.366%) **Table 4.**

TABLE 4: REPEATABILITY RESULT

Concentration	Absorbance
6	0.6554
6	0.6586
6	0.6587
6	0.6582
6	0.6575
6	0.6526
Mean	0.65683
S.D.	0.0024
%RSD	0.36598

Robustness: Even with little wavelength variations, the technique remained reliable. Since wavelength variation data is the typical robustness parameter for UV methods, the previously stated inaccuracy in the "by Labman" condition has been fixed.

TABLE 5: ROBUSTNESS, ΛMAX VARIABLE CHANGED BY ±2NM

Conc.	252 nm			256 nm			by Labman		
	Set I	Set II	Set III	Set I	Set II	Set III	Set I	Set II	Set III
6	0.6895	0.6944	0.6998	0.7278	0.7369	0.7476	0.8250	0.7470	0.8371
Mean		0.694567			0.73743			0.8030	
Std. Deviation		0.005152			0.0091			0.79567	
%RSD		0.741761			1.33611			0.81189	

CONCLUSION: The UV-Visible spectroscopic method was successfully developed for the quantification of Ellagic Acid method using Ethanol as a Green solvent. Ethanol as a solvent reduces the use of hazardous solvents and is consistent with green chemistry concepts. It was discovered that the linearity range of Ellagic Acid was 2-10µg/ml.

The λmax at 254 nm provided a reliable analytical wavelength, and recovery studies further supported the accuracy of the procedure. The correlation coefficient was 0.9938, and the percentage RSD was 0.15, both of which are within acceptable bounds. Compliance with ICH Q2(R2) guidelines throughout the validation process ensures the robustness and reliability of the method for routine analytical applications. Future work should include specificity studies and application to real samples (e.g., pomegranate extract) to fully establish utility. Given its simplicity, cost-effectiveness, and minimal environmental impact, the proposed method is highly suitable for the analysis of ellagic acid in various pharmaceutical and research settings, contributing to greener and more sustainable analytical practices.

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Borge, SCES's Indira College of Pharmacy, Tathawade, Pune. All authors read and approve the final manuscript.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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