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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR EPICATECHIN ESTIMATION WITH SOLUBILITY ANALYSIS

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ABSTRACT

Objectives: This study aimed to develop and validate an ultraviolet (UV) spectrophotometric method for the accurate and reproducible quantification of epicatechin in bulk form. In addition, the solubility profile of epicatechin in various pharmaceutical solvents and oils was systematically investigated to support formulation development.

Methods: Method development was carried out using pure epicatechin with methanol as the solvent. The method was validated according to international council for harmonization guidelines by evaluating linearity, precision (intra-day and inter-day), accuracy, limit of detection, limit of quantification, and robustness. Solubility studies were performed by equilibrating excess epicatechin with selected solvents (methanol, isopropyl alcohol, chloroform, ethyl acetate, water, and 1-butanol) and oils (castor oil, coconut oil, eucalyptus oil, olive oil, and clove oil), followed by quantitative analysis using UV spectrophotometry. Statistical analysis was applied where applicable, with significance considered at $p<0.05$.

Results: The developed method exhibited excellent linearity over the studied concentration range ($R^2=0.9982$), with high precision (percent relative standard deviation [%RSD]<3.1%), acceptable accuracy (recovery 98.5–106.3%), and robust performance (%RSD<2.0%). All validation parameters were statistically acceptable ($p<0.05$). Solubility studies revealed that methanol demonstrated significantly higher solubility for epicatechin (12.33±0.05 mg/mL) compared with other solvents ($p<0.05$), followed by isopropyl alcohol and chloroform. Among the oils evaluated, castor oil (4.75±0.02 mg/mL) and coconut oil (3.89±0.01 mg/mL) showed significantly greater solubilizing capacity than other oils ($p<0.05$).

Conclusion: The validated UV spectrophotometric method is reliable, economical, and suitable for routine quantification of epicatechin in pharmaceutical and research applications. Furthermore, the statistically significant solubility findings ($p<0.05$) provide valuable guidance for the rational formulation design of epicatechin-based dosage forms.

Keywords: Epicatechin, Ultraviolet spectrophotometry, Method validation, Solubility, Pharmaceutical analysis, *Acacia catechu*, flavonoids.

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INTRODUCTION

Chronic lifestyle-related diseases, such as diabetes mellitus, cardiovascular disorders, and obesity, are increasing worldwide, partly due to dietary changes, including the consumption of high-fat foods [1,2]. These conditions often require long-term pharmaceutical management, which may lead to adverse effects such as fatigue, hepatotoxicity, and gastrointestinal disturbances [3]. Consequently, there is growing interest in natural bioactive compounds derived from plants, which may offer therapeutic benefits with fewer side effects [4,5]. Flavonoids, a major class of polyphenolic compounds widely distributed in the plant kingdom, have been extensively studied for their antioxidant, anti-inflammatory, cardioprotective, and antidiabetic properties [6,7]. Epicatechin, a member of the flavanol subclass, is abundant in various medicinal plants, notably in *Acacia catechu*, a tree traditionally used in Ayurvedic and ethnomedicinal systems for its health benefits [8,9]. Epicatechin exhibits significant antioxidant activity by scavenging free radicals and modulating signaling pathways associated with oxidative stress and inflammation [10]. It has shown potential in improving endothelial function, lowering blood glucose levels, and attenuating the production of inflammatory cytokines [11,12]. Chemically, epicatechin is identified by the IUPAC name (2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol, with the molecular formula $C_{15}H_{14}O_6$ and a molecular weight of 290.27 g/mol [13]. It contains multiple hydroxyl groups that contribute to its strong antioxidant capacity but also influence its solubility and bioavailability [14,15]. In the human body, epicatechin undergoes metabolic transformations such as methylation, glucuronidation, and sulfation, which impact its pharmacokinetic profile [16,17]. Understanding the solubility characteristics of epicatechin is essential for developing effective formulations that optimize its bioavailability [18]. Poor aqueous solubility is a common limitation of many bioactive flavonoids, necessitating solubility evaluation across a range of solvents to inform formulation design [19]. Oils and non-polar solvents are particularly relevant for lipid-based drug delivery systems, which can enhance the absorption of poorly soluble compounds [20]. Quantitative analysis of epicatechin has traditionally relied on chromatographic techniques such as high-performance liquid chromatography (LC) and LC-mass spectrometry, which, although highly sensitive and specific, require expensive instrumentation and complex sample preparation [21,22]. In contrast, ultraviolet (UV) spectrophotometry, based on the Beer-Lambert law, offers a rapid, cost-effective, and non-destructive alternative for routine analysis [23,24]. However, validated UV spectrophotometric methods specifically targeting epicatechin quantification in bulk materials remain limited in the literature [25]. This study aims to develop and validate a simple UV spectrophotometric method for the estimation of epicatechin concentration in bulk form, followed by a comprehensive solubility evaluation in various polar and non-polar solvents and oils. Method validation is conducted in accordance with international council for harmonization (ICH) Q2 (R1) guidelines to ensure accuracy, reliability, and reproducibility suitable for pharmaceutical and research applications [26]. An overview of UV spectrophotometric determination of epicatechin is illustrated in Fig. 1.

METHODS

Epicatechin, the primary bioactive flavonoid of *A. catechu*, was procured from HiMedia, India. All reagents and solvents used in the experiment were of analytical grade. Fresh methanol was used for the preparation of all validation solutions. The active pharmaceutical ingredient was weighed using a Shimadzu digital balance (AX 200, India) to ensure precise measurement. UV absorbance measurements and method validation for epicatechin were conducted using a double-beam spectrophotometer (Labindia Analytical 3200 UV/VIS spectrophotometer, India). A standard stock solution was prepared by dissolving 10 mg of epicatechin in 10 mL of methanol in a volumetric flask, yielding a concentration of 1 mg/mL. A working stock solution of 100 µg/mL was obtained by transferring 1 mL of the standard stock solution into another flask and diluting it with methanol. This working stock was further diluted to prepare a series of concentrations for the calibration curve. UV-visible spectra were recorded for the working stock solution, using methanol as the blank. Epicatechin exhibited a maximum absorbance peak at 278 nm, which was used as the analytical wavelength throughout the study, shown in Fig. 2 [25].

Serial dilutions were prepared from the working stock solution within the concentration range of 10–50 µg/mL, and absorbance was measured at 278 nm using methanol as the blank. The calibration curve was constructed by plotting the mean absorbance values against the corresponding concentrations, with each concentration measured in triplicate. Data analysis, including regression analysis, was performed using Microsoft Excel software. Standard deviation (SD) was calculated to assess variability and the dispersion of data relative to the mean. Method validation was conducted in accordance with ICH Q2 (R1) guidelines [26].

The following parameters were evaluated:

1. Linearity: The method's capacity to produce absorbance directly proportional to epicatechin concentration within the tested range. Regression parameters were derived from the calibration plot.
2. Precision: Assessed through intraday and interday reproducibility studies, expressed as percent relative SD (%RSD), calculated by:%RSD = (SD/mean) × 100
3. Accuracy: Determined at three concentration levels 80%, 100%, and 120% of the nominal concentration by calculating percentage recovery and %RSD.
4. Ruggedness: Ruggedness of the developed UV-spectrophotometric method for epicatechin was evaluated to ensure reliability under normal usage. The analysis was conducted using 20 µg/mL epicatechin solutions by three different analysts under identical laboratory conditions. The consistency in absorbance values and low %RSD confirmed that the method is robust, reproducible, and unaffected by minor variations in operating conditions.
5. Robustness: Evaluated by measuring absorbance at wavelengths of 277 nm, 278 nm, and 279 nm for selected concentrations to observe the effect of slight instrumental variations. The corresponding %RSD values were computed.
6. Limit of detection (LOD) and limit of quantification (LOQ): Calculated based on the SD of the response and the slope of the calibration curve using the formulas:
   - LOD=3.3×(SD/s)
   - LOQ=10×(SD/s).

Where *s* is the slope of the calibration curve.

Solubility studies of epicatechin

Solubility studies of epicatechin were carried out using the developed and validated UV-spectrophotometric method. An excess amount of epicatechin was added to 1 mL of each solvent (methanol, isopropyl alcohol, butan-1-ol, ethyl acetate, chloroform, and water) and oils (castor oil, coconut oil, eucalyptus oil, olive oil, and clove oil) to obtain saturated solutions. The samples were vortexed for 5 min and then equilibrated for 24 h at 25±0.5°C in a temperature-controlled orbital shaker to ensure equilibrium solubility. After equilibration, the samples were filtered through 0.45 µm nylon membrane filters, previously verified to show no adsorption of epicatechin. The filtrates were suitably diluted with methanol, and the concentration of epicatechin was determined by measuring absorbance at 278 nm using the validated UV-spectrophotometric method. Each solubility determination was performed in triplicate (n=3).

Statistical analysis

All experimental data are expressed as mean±SD. Method validation parameters such as precision, accuracy, ruggedness, and robustness were evaluated using %RSD, in accordance with ICH Q2 (R1) guidelines. For solubility studies, one-way analysis of variance was applied to

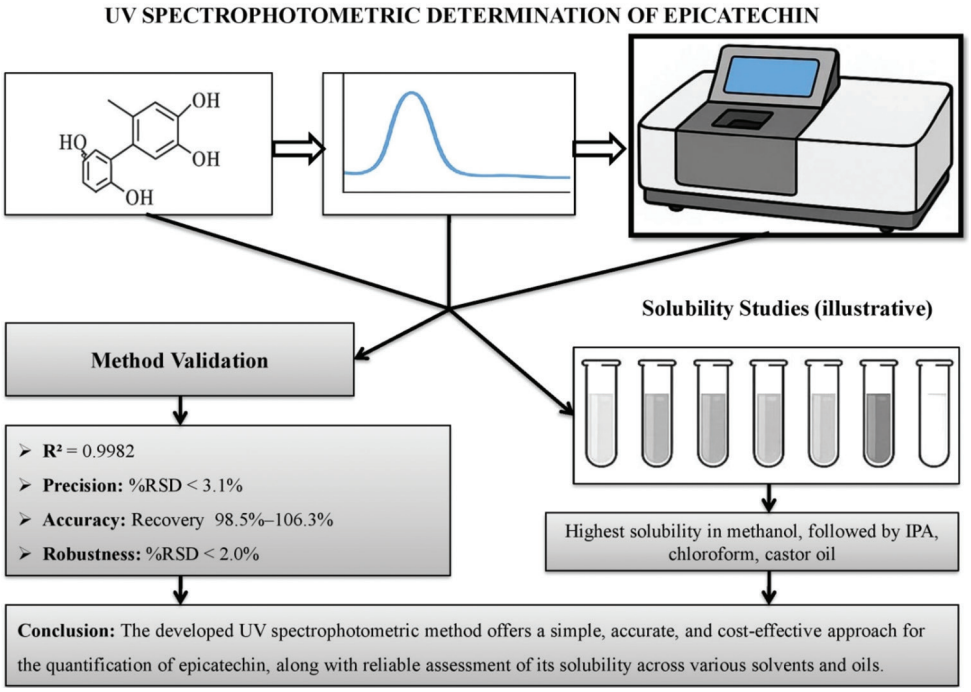

Fig. 1: Overview of the ultraviolet spectrophotometric determination of epicatechin

assess differences among solvents and oils, followed by Tukey's multiple comparison *post hoc* test to identify statistically significant pairwise differences between individual solvents and oils. Statistical analysis was performed using GraphPad Prism software, and differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The validation of the analytical procedure is fundamental to demonstrate its reliability and applicability for estimating epicatechin as a bioactive constituent of *A. catechu*. Several critical parameters were examined to validate the developed UV-spectrophotometric method for epicatechin quantification.

Linearity: The linearity of the developed UV-spectrophotometric method for epicatechin was evaluated using standard solutions prepared in methanol at concentrations of 5, 10, 15, 20, 25, and 30 µg/mL. Each concentration was analyzed in triplicate (n=3), and absorbance was measured at 278 nm. The mean absorbance values, SD, and %RSD are summarized in Table 1, and the calibration curve is shown in Fig. 3.

The calibration curve followed the regression equation y=×0.0011+0.034 with an excellent correlation coefficient (R^2=0.9989), confirming a strong linear relationship between concentration and absorbance. The low %RSD values further indicate consistency of measurements at each concentration level. These results confirm the linearity of the method over the tested range, in accordance with ICH Q2 (R1).

Precision: The precision of the developed UV-visible spectrophotometric method for estimating epicatechin was assessed by evaluating both intraday and interday precision at three concentration levels: 10, 20, and 30 µg/mL. Each concentration was analyzed in triplicate (n=3). The results, shown in Tables 2 and 3, include mean absorbance, SD, %RSD, and statistical significance using p-values.

Intraday precision

The intraday precision showed %RSD values of 2.50%, 1.58%, and 1.32% for 10, 25, and 50 µg/mL, respectively, indicating excellent repeatability of the method within a single day.

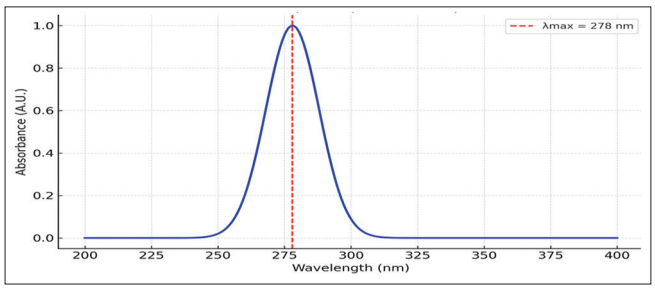

Fig. 2: Ultraviolet (UV)-visible absorption spectrum of epicatechin recorded at 278 nm using the Labindia Analytical 3200 UV/VIS Spectrophotometer (India)

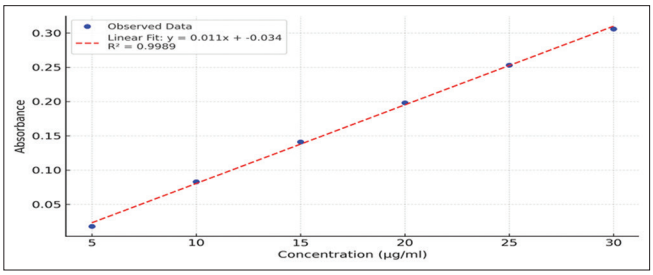

Fig. 3: Epicatechin linearity curve at 278 nm

Inter-day precision

The inter-day precision assessed across three different days produced %RSD values of 2.53%, 1.59%, and 1.63%, which again confirmed the intermediate precision and method stability over time.

The low %RSD values (<3%) in both intraday and interday studies indicate excellent precision**,** and the statistically significant p-values confirm consistent absorbance variations corresponding to concentration. Thus, the method is precise, repeatable, and stable over time in accordance with ICH guidelines.

Accuracy

Accuracy was verified through recovery studies at 80%, 100%, and 120% concentration levels using a 20 µg/mL epicatechin standard solution. Mean recoveries were as follows:

- 99.75% at 80% concentration level
- 101.54% at 100% concentration level
- 106.88% at 120% concentration level.

The recovery study of epicatechin demonstrated excellent accuracy, with mean recoveries of 99.75%, 101.54%, and 106.88% at 80%, 100%, and 120% levels, respectively. The %RSD values were all below 2%, indicating minimal variability. The accuracy recovery results demonstrate that the method provides highly reliable and reproducible estimation of epicatechin as shown in Table 4. The low % RSD values (<2%) confirm the validity, accuracy, and sensitivity of the method across all tested concentrations. These results confirm the method's reliability and suitability for accurate quantitative analysis of epicatechin.

Ruggedness

Ruggedness of the proposed method is determined for 20 µg/mL concentration of epicatechin by analysis of aliquots from a homogenous slot by three analysts using the same operational and environmental conditions.

The ruggedness study for epicatechin at 20 µg/mL revealed consistent and reproducible results across all three analysts. The %RSD values were 1.20%, 1.55%, and 0.52% for Analyst 1, Analyst 2, and Analyst 3, respectively. The ruggedness study of the UV method for epicatechin

Table 1: Statistical linearity data for UV-spectrophotometric estimation of epicatechin (n=3)

Concentration (µg/mL)	Mean absorbance±SD	%RSD
5	0.018±0.0006	3.33
10	0.083±0.0015	1.81
15	0.141±0.0017	1.21
20	0.198±0.0020	1.01
25	0.253±0.0019	0.75
30	0.306±0.0021	0.69

SD: Standard deviation, UV: Ultraviolet, %RSD: Percent relative SD

Table 2: Intraday precision analysis of epicatechin (n=3)

Concentration (µg/mL)	Absorbance values	Mean±SD	%RSD
10	0.078, 0.080, 0.081	0.080±0.002	2.50
20	0.190, 0.187, 0.193	0.190±0.003	1.58
30	0.305, 0.296, 0.309	0.303±0.004	1.32

%RSD: Percent relative SD, SD: Standard deviation

Table 3: Interday precision analysis of epicatechin (n=3)

Concentration (µg/mL)	Absorbance values	Mean±SD	%RSD
10	0.077, 0.081, 0.080	0.079±0.002	2.53
20	0.189, 0.186, 0.192	0.189±0.003	1.59
30	0.308, 0.299, 0.312	0.306±0.005	1.63

SD: Standard deviation, %RSD: Percent relative SD

Table 4: Accuracy assessment of epicatechin by UV-visible spectrophotometry (n=3)

Pre-analyzed sample (µg/mL)	Drug added (µg/mL) (%)	Drug recovered (µg/mL)	Percentage recovery	Mean±SD (%)	%RSD
20	16 (80)	35.95	99.86	99.75±0.015	0.015
20	16	35.96	99.88		
20	16	35.92	99.52		
20	20 (100)	40.32	100.80	101.54±0.643	0.63
20	20	40.82	102.05		
20	20	40.73	101.77		
20	24 (120)	44.60	104.57	106.88±1.864	1.74
20	24	45.80	108.18		
20	24	46.10	107.90		

UV: Ultraviolet, %RSD: Percent relative SD, SD: Standard deviation

Table 5: Ruggedness evaluation of epicatechin at 20 µg/mL using UV-visible spectrophotometry (n=6)

Analyst	Absorbance values	Mean±SD	%RSD
Analyst 1	0.191, 0.194, 0.195, 0.190, 0.192, 0.196	0.193±0.002	1.20
Analyst 2	0.193, 0.190, 0.197, 0.188, 0.194, 0.196	0.193±0.003	1.55
Analyst 3	0.192, 0.195, 0.193, 0.194, 0.194, 0.194	0.194±0.001	0.52

UV: Ultraviolet, %RSD: Percent relative SD, SD: Standard deviation

Table 6: Robustness evaluation of epicatechin at 20 µg/mL under deliberate method variations (n=3)

Condition	Parameter	Absorbance	Mean	SD	%RSD
Change in wavelength	282 nm	0.192	0.193	0.002	1.04
	283 nm	0.193			
	284 nm	0.195			
Change in temperature	10°C	0.192	0.192	0.001	0.52
	25°C	0.191			
	40°C	0.193			
Change in solvent brand	Brand A	0.193	0.193	0.002	1.03
	Brand B	0.192			
	Brand C	0.194			

%RSD: Percent relative SD, SD: Standard deviation

showed consistent absorbance with %RSD below 2% across three analysis as shown in Table 5. Thus, the method is rugged, reliable, and suitable for reproducible analysis under routine laboratory conditions. The low variability demonstrates that the developed UV-spectrophotometric method is rugged and unaffected by minor changes in operator handling, thereby ensuring reliability in routine analysis.

Robustness

The robustness of an analytical technique is an indicator of its ability to remain unchanged by limited, yet deliberate changes in process parameters and demonstrates its reliability during daily use. Robustness of the UV spectrophotometer analytical method was determined by analyzing the 20 µg/mL Epicatechin solutions at different wavelength, temperature, and solvent brand.

Robustness was evaluated by introducing small deliberate changes in wavelength (± nm), temperature, and solvent and solvent brand as shown in Table 6. The % RSD values remained below 2%, indicating method stability.

LOD and LOQ

Using the calibration curve slope and SD, LOD and LOQ values were calculated as:

Table 7: Solubility of epicatechin in various solvents and oils (n=3)

Medium	Type	Solubility (mg/mL)±SD	Appearance
Methanol	Solvent	12.33±0.05	Clear solution
Isopropyl alcohol	Solvent	7.85±0.02	Clear solution
Chloroform	Solvent	5.47±0.01	Slightly turbid
Ethyl acetate	Solvent	2.12±0.01	Turbid suspension
Water	Solvent	<0.1	Turbid suspension
Butan-1-ol	Solvent	<0.1	Turbid suspension
Castor oil	Oil	4.75±0.02	Slightly cloudy
Coconut oil	Oil	3.89±0.01	Slightly cloudy
Eucalyptus oil	Oil	1.67±0.01	Oily suspension
Olive oil	Oil	0.92±0.01	Oily suspension
Clove oil	Oil	2.34±0.02	Slightly turbid

- LOD = 1.742 µg/mL
- LOQ = 5.278 µg/mL.

These low values highlight the sensitivity of the method, suitable for detecting and quantifying epicatechin in low concentrations.

Solubility studies of epicatechin

The developed UV-spectrophotometric method was employed to evaluate the solubility profile of epicatechin in various solvents and oils. An excess quantity of epicatechin was introduced into 1 mL of each solvent, namely, methanol, isopropyl alcohol, ethyl acetate, chloroform, and water, and oils such as castor oil, coconut oil, eucalyptus oil, olive oil, and clove oil. The mixtures were vortexed, equilibrated, and filtered before UV analysis. The solubility data (Table 7) revealed the highest solubility in methanol (12.33±0.05 mg/mL), followed by moderate solubility in isopropyl alcohol (7.85±0.02 mg/mL) and chloroform (5.47±0.01 mg/mL). Poor solubility was observed in water (<0.1 mg/mL) and butan-1-ol (<0.1 mg/mL). Among the oils, castor oil (4.75±0.02 mg/mL) and coconut oil (3.89±0.01 mg/mL) demonstrated better solubilizing potential compared to olive oil and eucalyptus oil. Microscopic observations revealed clear solutions in methanol and isopropyl alcohol, whereas turbid dispersions were seen in ethyl acetate and water, indicating incomplete solubilization.

Although solvents such as methanol and isopropyl alcohol show higher solubility values for epicatechin compared to oils such as olive oil or eucalyptus oil. Therefore, while solvents may appear more effective in dissolving epicatechin, this difference may be due to random variation in the samples rather than a true underlying difference in solubility performance.

CONCLUSION

This study successfully established and validated a UV-visible spectrophotometric method for the accurate quantification of epicatechin in bulk form. The method met all validation criteria as per ICH Q2 (R1) guidelines, confirming its reliability in terms of linearity, precision, accuracy, robustness, and sensitivity. In addition to method validation, solubility profiling across various solvents and oils revealed methanol, isopropyl alcohol, and chloroform as the most effective solvents, while castor oil and coconut oil showed the highest solubilizing capacity among oils. These findings not only support the method's suitability for routine quality control but also provide essential data to guide formulation development aimed at improving the solubility and bioavailability of epicatechin. Future work should extend this method's application to dosage forms and biological matrices to further broaden its utility in pharmaceutical and pharmacokinetic studies.

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AUTHOR CONTRIBUTIONS

The sole author was responsible for conceptualization, methodology, data analysis, investigation, writing of the original draft, and reviewing and editing of the manuscript.

CONFLICTS OF INTEREST

The author confirms no conflicts of interest.

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