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Method development and validation of Catechin in aqueous medium by using ultraviolet-visible spectrophotometer

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Abstract

Background: Catechin, a flavonoid compound with significant antioxidant activity, requires a reliable analytical method for its quantitative determination in pharmaceutical and research applications. **Objective**: In this study, a simple and rapid UV-Visible Spectrophotometry method was developed and validated for the determination of catechin dissolved in aquadest for quality control and laboratory applications. **Methods:** A UV-Visible spectrophotometry method was developed for the determination catechin dissolved in aquadest. The maximum wavelenght (λmax) of catechin was determined by scanning in the range of 200-400 nm. calibration curves were prepared at concentrations ranging from 20-60 ppm. method validation was carried out according to AOAC (Association of Official Analytical Chemists) guidelines, including asessment of linearity, precision, accuracy, limit of detection (LoD) and limit of quantification (LoQ). **Results:** Catechin showed maximum absorbantion at 275 nm. The Calibration curve exhibited excellent linearity with a correlation coefficient (R2) of 0,99. precision studies showed %RSD values 7,3%, while accuracy testing yielded recovery values between 95,72±2,42 %- 102,05±1,74%. The LoD and LoQ were calculated to be 4,16 ppm and 12,60 ppm, respectively. **Conclusion:** the developed UV-Vis Spectrophotometry method is simple, precise, and accurate. it is suitable for routine determination of catechin in aqueous samples and can be applied in research as well as quality control of pharmaceutical formulation.

Keywords: catechin; validation; spectrophotometer; aqueous

Cite This Article

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INTRODUCTION

Catechin is a natural flavonoid compound widely found in various plants, including green tea, cocoa, and fruits. It possesses potent antioxidant properties that contribute to its pharmacological activities such as antioxidant, anti-inflammatory, cardioprotective, and anticancer effects [1–3]. Due to these significant biological roles, catechin has gained increasing attention in the pharmaceutical and nutraceutical fields, necessitating reliable analytical methods for its quantitative determination in different formulations and research samples[4].

Spectrophotometric analysis remains one of the most commonly used methods for determining active compounds because of its simplicity, cost-effectiveness, and rapid analytical capability. Among these, UV-Visible spectrophotometry is particularly advantageous for compounds like catechin, which exhibit distinct absorbance in the ultraviolet range. However, method validation is essential to ensure that analytical results are accurate, precise, and reproducible according to international standards such as those established by the association of official analytical chemists (AOAC) [4].

This study aimed to develop and validate a simple, rapid, and reliable UV-Visible spectrophotometric method for the determination of catechin dissolved in aquadest. The method validation included evaluation of linearity, precision, accuracy, limit of detection (LoD), and limit of quantification (LoQ), with the goal of establishing a standardized approach suitable for quality control and laboratory applications in pharmaceutical analysis.

METHODS

Materials

(+)-catechin hydrate (purity ≥98%, Pcode 102687710) and ethanol analitical grade (CAS-NO-64-1745)were obtained from Sigma Aldrich; Distilled water (aquadest) was used as solvents throughout the study was purchased from Medika Laborta.

Determination of Maximum Wavelenght

a standard catechin solution with a concentration of 1000 ppm was first prepared. 50 mg of catechin was dissolved in distilled water (aquadest) and 5 mL of ethanol in a 50 mL volumetric flask, diluted to the mark, and shaken until homogeneous. The solution was then sonicated for 10 minutes. The 1000 ppm catechin standard solution was subsequently diluted to 500 ppm by pipetting 5 mL of the standard solution into a 50 mL volumetric flask and diluting to the mark with distilled water. The solution was scanned within the wavelength range of 200–400 nm using a UV-Visible spectrophotometer to determine the maximum absorbance wavelength [5,6].

Linearity

Linearity testing was performed by constructing a calibration curve from a 500 ppm catechin standard solution. 25 mg catechin was dissolved in distilled water and 5 mL of ethanol in a 50 mL volumetric flask, diluted to the mark, and shaken until homogeneous. The solution was then sonicated for 10 minutes. The catechin standard solution was serially diluted to concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, each in triplicate. The absorbance of each solution was measured at the selected wavelength, and the resulting data were used to construct a calibration curve and obtain the linear regression equation [5–7].

Precision

Precision by calculating the relative standard deviation (%RSD) or coefficient of variation. The test was performed at three concentration levels (20 ppm, 40 ppm, and 60 ppm) with three replicates for each concentration using the repeatability method. Precision was evaluated both intra-day (replicate measurements within the same day) and inter-day (measurements over three consecutive days). The standard deviation (SD) and relative standard deviation were then calculated with equation below [6].

%RSD= SDMean x 100% (1)

Accuracy

Accuracy testing was performed at three concentration levels (20 ppm, 40 ppm, and 60 ppm), each with three replicates. The results were expressed as the mean percentage recovery with equation below [4].

%Recovery= Measured Concentration Actual Concentration x 100% (2)

Limit of Detection (LoD) and Limit of Quantification (LoQ)

LOD and LOQ were determined using a 500 ppm catechin standard solution, which was diluted to concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, each prepared in triplicate (36). The absorbance of each solution was measured at the selected wavelength, and a calibration curve was plotted to obtain the linear regression equation (37). The results were then processed using formula to calculate LOD and LOQ values based on the following formulas [4].

LoD= 3.3 x SD Slopex 100% (3)
LoQ= 10 x SD Slopex 100% (4)

Notes
SD : Standard deviation (Sy/x)
LOD : Limit of Detection
LOQ : Limit of Quantification
Slope : Value of *b* in the linear regression equation ($y = bx + a$)

RESULTS

The maximum wavelength (λmax) of catechin was determined using a UV-Visible spectrophotometer in the range of 200–400 nm. The spectrum showed that catechin exhibited maximum absorbance at a wavelength of 275 nm, as presented in Figure 1.

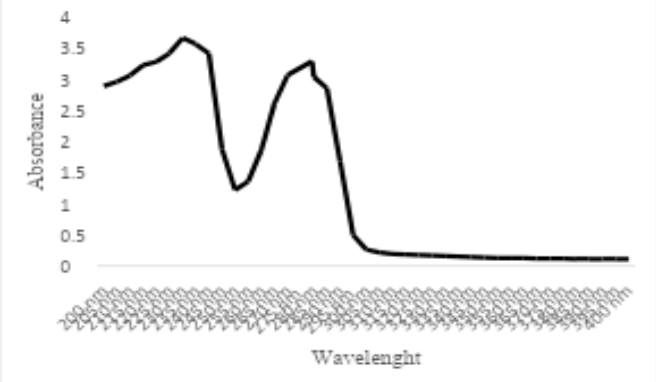

Figure 1. maximum wavelenght of catechin

A calibration curve was prepared for catechin standard solutions at concentrations ranging from 20 to 60 ppm. The resulting regression equation was y = 0.0132x + 0.0206, with a correlation coefficient (R^2) of 0.9963, as shown in Figure 2.

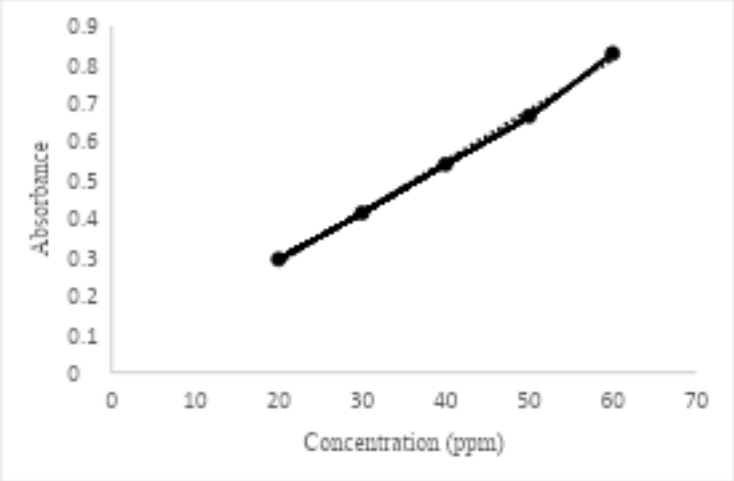

Figure 2. calibration curve of catechin standard solution

Precision testing was performed using three concentrations (20, 40, and 60 ppm). The intra-day %RSD values were 1.701%, 2.526%, and 1.233%, while the inter-day %RSD values were 3.585%, 2.808%, and 2.075%, respectively (Table 1).

Table 1. Precisions

Concentration (ppm)	*Intraday precision* (n=3)		*Interday precision* (n=9)	
	Measured concentration (mean±SD)	RSD (%)	Measured concentration (mean±SD)	RSD (%)
20	20,409±0,347	1,701	21,345±0,765	3,585
40	38,288±0,967	2,526	38,231±1,073	2,808
60	60,308±0,744	1,233	58,971±1,224	2,075

Accuracy was carried out at three concentration levels (20, 40, and 60 ppm), each analyzed in triplicate. The percentage of recovery values obtained were 102.045%, 95.720%, and 100.513%, respectively (Table 2).

Table 2. Percentage of Recovery

Concentration (ppm)	Measured concentration (mean±SD)	Recovery (%) (mean±SD)
20 ppm	20,409±0,347	102,045±1,735824
40 ppm	38,288±0,967	95,720±2,41802
60 ppm	60,308±0,744	100,513±1,239262

The Limit of Detection (LOD) and Limit of Quantification (LOQ) values obtained for catechin were 4.157 ppm and 12.597 ppm, respectively.

DISCUSSION

The results of this study demonstrate that the developed UV-Visible spectrophotometric method is suitable for the quantitative determination of catechin in aqueous solution. The spectrum of catechin exhibited a maximum absorbance at a wavelength of 275 nm [4,8], indicating that the electronic transition of the compound occurred most efficiently at this point. Measurement at the maximum wavelength ensures optimal analytical sensitivity, as small concentration changes result in significant variations in absorbance, which is consistent with previous findings for flavonoid compounds.

The linearity assessment revealed a strong linear correlation between catechin concentration and absorbance, with a regression equation of $y = 0.0132x + 0.0206$ and a correlation coefficient (R^2) of 0.9963. This value meets the AOAC acceptance criteria ($R^2 > 0.99$), confirming that the analytical response is directly proportional to concentration within the tested range. A good linear relationship demonstrates that the developed method is capable of producing reliable quantitative measurements for catechin [6]. Precision testing further confirmed the repeatability and reproducibility of the method, as indicated by low %RSD values for both intra-day (1.233–2.526%) and inter-day (2.075–3.585%) analyses. These values are well below the AOAC acceptance limit of 7.3% for analyte concentrations ≥10 ppm, suggesting that the method is consistent and stable across repeated measurements [6,7].

Accuracy evaluation showed that the method provided satisfactory recovery values ranging from 95.72% to 102.05%, all within the acceptable range of 80–110% as established by AOAC guidelines. Recovery values close to 100% indicate that the method yields accurate results that closely reflect the true catechin concentration in the sample, confirming its reliability for quantitative analysis. The sensitivity of the method was demonstrated by the low values of the Limit of Detection (LOD) and Limit of Quantification (LOQ), which were determined to be 4.157 ppm and 12.597 ppm, respectively. These results indicate that the instrument and the developed method possess sufficient sensitivity to detect and quantify catechin even at low concentrations. Overall, the validation parameters—including wavelength determination, linearity, precision, accuracy, and sensitivity—fulfilled the AOAC criteria, confirming that the developed UV-Visible spectrophotometric method is simple, precise, accurate, and reliable for the routine analysis of catechin in pharmaceutical and research applications [6,8].

CONCLUSIONS

The developed UV-Visible spectrophotometric method for catechin analysis is simple, accurate, and sensitive. It demonstrated excellent linearity, good precision, and acceptable recovery values. This validated method is suitable for routine quantitative determination of catechin in pharmaceutical and research applications..

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

This study used artificial intelligence (AI) tools and methodologies in the following capacities Manuscript writing support: AI-based language models, ChatGPT was employed to: Language refinement (improving the grammar, sentence structure, and readability of the manuscript). Technical writing assistance (providing suggestions for structuring complex technical descriptions more effectively) and draw conclusions. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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