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(RESEARCH ARTICLE)

Method development and Validation of Catechin hydrate by using UV-Visible Spectroscopy

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Abstract

Catechin hydrate, a polyphenolic molecule, is widely present in tea leaves, fruits, and vegetables. The present study aims to develop an innovative, uncomplicated, precise, and reliable UV-visible spectroscopic technique to estimate Catechin Hydrate. The λ_{max} of catechin hydrate in methanol: water (90:10) was 278 nm. The drug exhibits linearity within the range of 2–12 µg/ml, with a R^2 value of 0.9991. The accuracy of selected method was assessed using a recovery study at three distinct levels: 80%, 100%, and 120%. The % recovery was determined to be between 99.83% and 101.08%. The minimal % RSD values indicate the method's accuracy and repeatability. The precision study was examined using intraday and interday changes. A % RSD value of less than 2 indicates the procedure's precision. The robustness of the suggested approach was examined by varying its wavelength. The LOD and LOQ were found to be 0.314 and 1.148 µg/ml, respectively. The results clearly show that the UV-visible spectroscopic method may effectively be used to estimate the concentration of Catechin hydrate.

Keywords: UV-Visible Spectroscopy; Method Development; Validation; Catechin Hydrate

1. Introduction

Analytical approaches are essential for drug design, development, standardization, and quality control. Pharmacokinetics and drug metabolism research consider them equally important. Both of these factors are essential for evaluating the bioavailability and durability of the therapeutic response [1]. Analytical instrumentation is crucial in the manufacturing and assessment of new products, as well as in safeguarding consumers and the environment [2]. Analytical techniques are employed universally across several domains to produce data. These facts are the foundation for decision-making, so their legitimacy is highly significant. Therefore, it is imperative to obtain consistent data quality through various analytical approaches [3]. A method validation of the analytical method is to verify the appropriateness of the proposed analytical method for its intended purpose. The suggested approach conducted validation according to International Council on Harmonization (ICH) for various characteristics, including linearity, accuracy, robustness, precision, LOQ and LOD. They provide a foundation for implementing measures to enhance the environmental sustainability of chemical materials and processes [4,5]. Chemists from several disciplines establish these procedures, including organic synthesis, chemical engineering, and analytical chemistry. The method validation process provides greater confidence that the analytical procedure suits its intended purpose. This process involves documenting evidence to support the appropriateness of the procedure [6,7].

The IUPAC name of Catechin (Figure 1) is 2-(3,4-dihydrophenyl)-3,4-dihydro-(2H)-benzopyran-3,5,7-triol Catechin a prevalent flavan-3-ol compound that occurs naturally in several sources. Catechin hydrate is a significant natural flavonoid that plants produce as a secondary metabolite. It has attracted considerable interest because of its potential

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therapeutic activity, including anti-inflammatory and antioxidative properties [8]. Additionally, it plays a crucial role in prevention and treatment of diseases caused by oxidative damage. It also demonstrates potential biological impacts such as cardioprotective, neurological, and anticancer properties. In addition to these benefits, it has been claimed to improve the decrease of lipids on the walls of arteries, offer protection against induced DNA damage, cling to cellular walls, disrupt microbiological growth, and effectively scavenge alkyl peroxyl radicals [9].

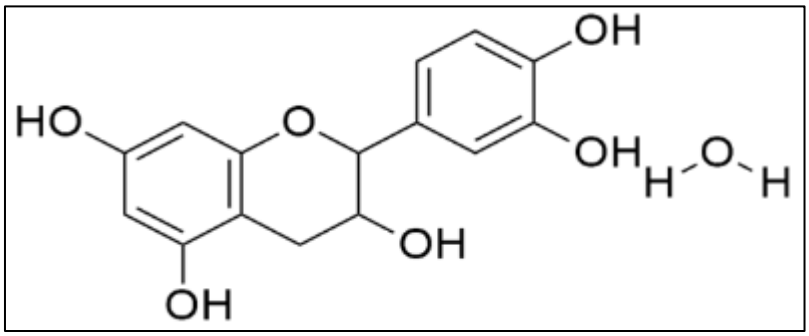

Figure 1 Structure of catechin

As per the literature from various database such as Google Scholar, PubMed or Science Direct, various methods are available for the estimation of catechin hydrate obtained from various natural sources. It includes HPLC [10-12], UV/Vis Spectroscopy and FT-IR spectroscopy [13], UPLC [14], UHPLC [15], UHPLC–MS/MS [16], HPLC with UV and MS-electrospray detection [17] and HPLC/fluorescence [18]. Therefore, to develop a simple, sensitive, precise, accurate, robust, and economical technique to quantify using UV-visible spectroscopy, as per ICH guidelines, is needed.

2. Materials and methods

The study utilized an UV-Visible Spectroscopy system (UV/VIS LasanyLI-2702), Ultra Sonicator (Lifecare equipment's with 3210 model), Analytical Balance (Wear with PGB 301 model).

2.1. Chemicals and Reagents

The research employed analytical-grade materials which are essential to ensure the accuracy and reliability of the Spectroscopic analysis.

2.2. Selection of Solvent for Method Development

Different solvents, including Methanol, ethanol, water, and phosphate buffer, were selected according to the drug's solubility. Based on the absorbance given by different solvents, Methanol and Water in the ratio of 90:10 were selected for further estimation of catechin hydrate by UV-visible spectroscopy.

2.3. Preparation of Standard Solution

Accurately weighed 25mg of catechin hydrate and dissolved in 15 ml methanol and water (90:10) in a 25 ml volumetric flask. Volume was made up to 25ml with the same solvent and sonicated for 10 minutes to dissolve catechin hydrate. Further dilutions were made as per the requirement for different purposes of method validation.

2.4. Determination of λ max

A prepared standard solution of catechin hydrate was scanned from 200-400nm by a UV-visible spectrophotometer, and maximum absorbance was recorded.

2.5. Development of standard curve

Calibration curves were prepared by preparing the concentration range of 2-12 µg/ml in a 10 ml volumetric flask from a previously prepared stock solution. The volume was increased up to 10 ml with the help of methanol: water (90:10). The absorbance of the resultant solution was taken at 278nm and a graph was created by taking absorbance on the Y axis and concentration on the X-axis.

3. Results and discussion

3.1. Determination of λ max

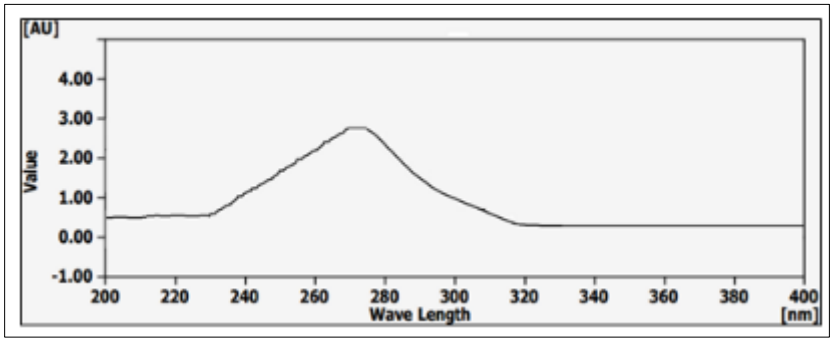

Figure 2 Absorbance maxima of Catechin hydrate

The maximum absorbance of catechin hydrate was 278nm in methanol: water (90:10) which is considered the wavelength of a standard drug. The spectrum of catechin is depicted in Figure 2.

3.2. Development of standard curve

Table 1 mention an absorbance of 2, 4, 6, 8, 10, 12 ug/ml of catechin hydrate. The concentration versus absorbance graph is plotted (Figure 3). From the calibration curve, the regression equation was found to be y = 0.096x+0.0433, with an R^2 value of 0.9991. This calibration curve is considered as linearity and used for further validation parameter.

Table 1 Different Concentration and absorbance of Catechin hydrate

Concentration (ppm)	Absorbance
2	0.246
4	0.412
6	0.613
8	0.822
10	1.009
12	1.190

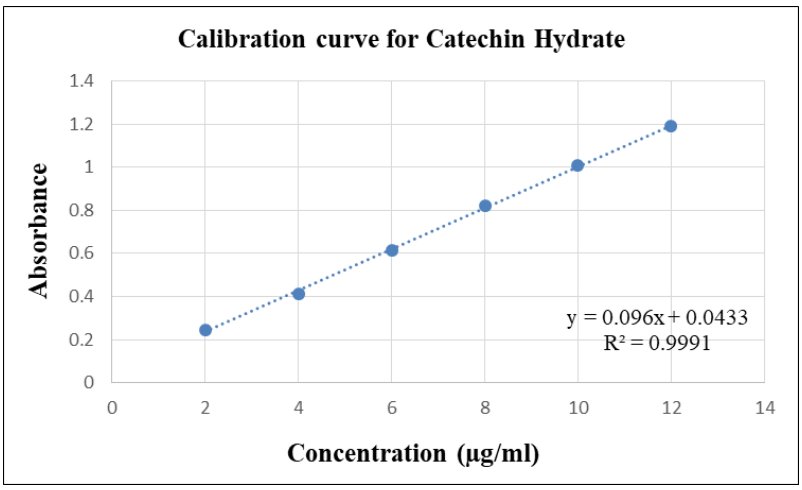

Figure 3 Standard Curve for Catechin hydrate

3.3. Method Validation of Catechin hydrate

3.3.1. Linearity

A previously obtained calibration curve is considered a linearity result for catechin hydrate. The correlation coefficient suggests that the range of 2-12ug/ml of catechin hydrate was linear.

3.3.2. Accuracy

The proposed UV-Vis method's accuracy was tested using the standard addition method, and % recovery was calculated. The results are recorded in Table 2. It shows a better mean %recovery for catechin hydrate with a % RSD less than 2%.

Table 2 Evaluation data of Accuracy study of Catechin hydrate

Catechin hydrate					
Level (%)	**Origin level (µg/ml)**	**Amount added (µg/ml)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	2	1.6	101.63	101.08	1.674
100	2	2	99.19		
120	2	2.4	102.44		
80	6	4.8	100.65	99.83	0.907
100	6	6	100.00		
120	6	7.2	98.86		
80	10	8	100.30	100.79	0.547
100	10	10	100.69		
120	10	12	101.39		

3.3.3. Precision

A precision study of the selected method was carried out using intra-day and inter-day precision studies. Three concentration levels viz.2, 6 and 10 ug/ml were taken in the morning, afternoon, and evening as intra-day and day 1 to day 3 with the same concentration as inter-day precision study. The results of the study are shown in Table 3. The results suggest that the method was precise, with % assay within the range and %RSD less than 2% for all selected levels.

Table 3 Evaluation data precision study of Catechin hydrate

Intra-day	**Morning**			**Afternoon**			**Evening**		
Concentration (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
2	0.248	98.12	1.813	0.251	99.26	1.164	0.253	99.48	0.967
6	0.614	99.40	0.658	0.618	98.49	0.786	0.622	99.16	0.738
10	1.014	99.46	0.549	1.021	99.47	0.728	1.019	98.23	0.587
Inter-day	**Day 1**			**Day 2**			**Day 3**		
2	0.217	99.53	1.219	0.219	99.23	1.401	0.214	100.00	1.401
6	0.650	100.30	0.307	0.650	100.30	0.387	0.649	99.58	0.387
10	1.049	100.28	0.285	1.049	100.03	0.543	1.047	99.55	0.543

3.3.4. Robustness

The robustness study was evaluated by adjusting the wavelength to 277nm and 279nm. The results were less than 2%, which suggests that the selected method was robust for the analysis of catechin hydrate. The results are mentioned in Table 4.

Table 4 Evaluation data for Robustness of Catechin hydrate

Catechin hydrate			
Concentration (µg/ml)	**Wavelength**	**Absorbance**	**% RSD**
6	277	0.622	0.371
6	278	0.619	0.323
6	279	0.619	0.323

3.3.5. Determination of LOD and LOQ

The study's LOD and LOQ suggest that catechin hydrate can be analyzed and estimated at the sub-microgram level, indicating the method's sensitivity (Table 5).

Table 5 Evaluation data for LOD & LOQ of Catechin hydrate

Catechin hydrate	
LOD	0.314 µg/ml
LOQ	1.148 µg/ml

4. Conclusion

The results clearly show that the UV-visible spectroscopic method may effectively estimate the concentration of Catechin hydrate. The approach was verified to comply with the ICH criteria. The current research demonstrates that the method exhibits excellent reproducibility. Additionally, this approach is accurate, reproducible, sensitive, precise and cost-effective for analyzing Catechin hydrate. Therefore, this method can be readily and conveniently utilized for regular quality control examination of Catechin hydrate.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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