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INSTRUMENTAL METHOD DEVELOPMENT AND STABILITY STUDY OF CATECHIN IN GREEN TEA

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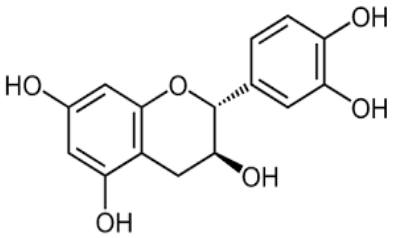
ABSTRACT:

Green tea was originated in China, tracing back to 2737 B.C. For centuries, all tea was green tea. Green tea is simply the leaves of the camelia sinensis plant placed to steep in hot water. The green tea is obtained from the tea plant Camelia sinensis (L.) which belongs to the family Theaceae. Catechin is the active ingredient of green tea. Catechin exist widely in plants, which can be used as medicine and treated for cardiovascular disease, cancer and other features. This experiment established a method development for the determination of catechin, so as to provide references for its development and utilization. The method was developed by HPLC. The HPLC conditions were as follows: A C18 column as separation column (4.6 x 250 mm) di mobile phase of methanol-acetic acid (V:V:= 80:20), flow rate of 0.7 mL/min, detection wavelength of 278 nm and column temperature of 30 ºC, HPLC standard curve: y=62.03 x + 2.536, R2 = 0.991, the linear relationship between catechin concentration and peak area was good in the range of 5-25µg/l, the recovery rates of the catechin was 88.45 % with RSD of 0.54 %. The determination of Catechin was done by HPLC. Force Degradation study was carried out by using HPLC.

Key Words: Catechin, HPLC

Introduction

Green Tea is one of the most popular beverages consumed worldwide. Tea, from the plant Camellia sinensis, is consumed in different parts of the world as green, black, or Oolong tea. The main components of tea are polyphenolic compounds, the main quality parameters for teas, commonly known as catechins, which represent a group of compounds belonging to the flavonoid family. Catechins, may be contained in (5 to 27%) of the dried tea leaf, which are divided into four primary compounds epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), epicatechin (EC). EGCG constituting (10 to 50%) of catechins and being the most potent due to its degree of gallation and hydroxylation.



IUPAC name: (2R, 3S) -2-(3,4-dihydroxyphenyl) -3,4-dihydro-2H-chromene-3, 5,7.

The Molecular Formula of Catechin is C15H14O6, whereas, Molecular weight is 290.26 g/mol. The Appearance is solid and Colour is Peach to light brownish. It is soluble in Water, Ethanol, Methanol. Catechins are natural polyphenolic phytochemicals and provides Antioxidant effect. Other health benefits of catechin provides Anticarcinogenic property, Anti-obesity property, Antidiabetic property. The health benefits of polyphenols found in green tea (GT), the unfermented leaves of the tea plant, Camellia sinensis, have been extensively investigated in the last fifteen years. The components in green tea undergo metabolic processing such as glucuronidation, methylation and sulfation in the body, which produces some active metabolites. The catechins and their metabolites may be identified in blood plasma, urine, and several tissues. However, the bioavailability of catechins is very poor due to various reasons such as poor stability to heat, light, alkaline pH. Recently, the health benefits of green tea and its constituents such as prevention of cancer and cardiovascular diseases and the antioxidative, anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antiviral, neuroprotective and cholesterol-lowering are under investigation. Catechins are sensitive to several environmental factors such as heat and light, and readily undergo degradation in oxidation with a consequent loss in activity. A number of studies have shown the instability of catechins compounds.

Experimental Work

Chromatographic conditions: A RP C18 column (Agilent) (4.6 x 250 mm)), mobile phase of methanol - acetic acid (V: V = 80:20), flow rate of 0.7 mL/ min, detection wavelength of 278 nm and 30 ºC as column temperature, sample amount of 20 µL, retention time of 3.350 min, theoretical plate number is 43510.

Preparation of standard stock solution and selection of detecting wavelength standard stock solution.

An accurately weighed quantity of about 10 mg of Catechin was taken in 10.0 ml volumetric flask, dissolved in HPLC grade methanol and volume was made up to mark with same solvent (conc. 1mg/ml).

An aliquot portion of standard stock solution was diluted appropriately with same solvent to obtain conc. of 10µg/ml. This solution was scanned in 1cm cell using double beam UV visible Spectrophotometer over the range of 400-200nm and the UV absorbance spectrum was recorded. From the spectrum the detecting wavelength selected for estimation of drug was 278.0 nm as shown in **Figure 1**.

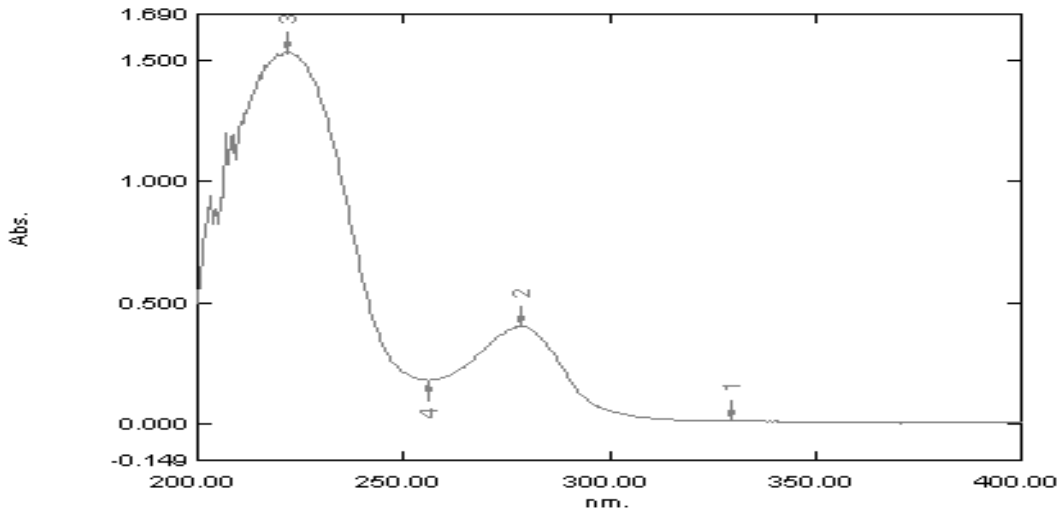


Figure 1: Spectra of Catechin with ethanol (10 µg/mL)

Materials and method

Mobile phase was prepared by mixing 20ml (0.1) in Acetic acid in 80 ml methanol. The mobile phase was sonicated for 15 min on ultrasonic bath and then it was filtered through 0.45 µm nylon membrane filter. The aliquot portion of standard stock solution was diluted appropriately with HPLC grade methanol to obtain conc. of 100 µg/ml. Appropriate aliquot of Catechin standard solution was transferred in series of 10 ml volumetric flask and volume was made with methanol to obtain various conc. of 5-25 µg/ml. These solutions were injected using a 40 µl fixed loop system separately in triplicate and chromatographed under conditions described above and peaks were recorded. The graph was plotted as conc. of drug Vs peak area and depicted in figure where Correlation coefficient = 0.999, Slope = 62.03, Intercept = 2.536, Retention time = 3.350, Theoretical plates = 43510, Tailing Factor = 0.66.

This proposed method was applied to marketed formulation.

To determine the content of catechin In Tablet formulation; capsule content (Label claim: 500mg) were weighed accurately. 100 mg sample in 100 ml Methanol which is equal to 1000µg/ml. this is the stock 2. The solution was filtered through Whatman filter paper. The solution was further diluted with mobile phase to obtain concentration 20µg/mL. The sample solutions were injected into column for 2 times and the content of capsule was calculated by comparing a sample peak with that of standard.

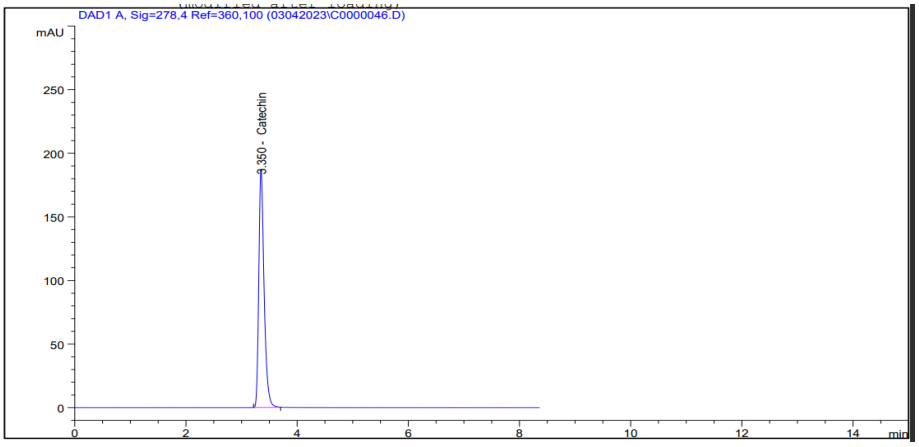


Figure 2: Typical chromatogram of Catechin (RT 3.3 respectively)

Table 1: Analysis of capsule formulation

Sr. No.		Concentration µg/ml	Area	Amount Found	Label claim	% Label Claim
	Standard	**20**	**1238.99**		**20**	
1		**20**	**1200.01**	**19.37**	**20**	**96.85**
2		**20**	**1212.09**	**19.56**	**20**	**99.08**
3	**Sample**	**20**	**1324.01**	**21.37**	**20**	**91.54**
4		**20**	**1280.08**	**20.66**	**20**	**103.3**
5		**20**	**1216.06**	**19.62**	**20**	**98.00**
		Mean	**1413.21**	**22.74**		**2.27**
		SD	**17.706**	**0.285**		**0.029**
		%RSD	**1.253**	**1.255**		**1.255**

System Suitability test

System Suitability parameter is a pharmacopeial requirement and is used to verify whether the reproducibility of the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

Table 2: Regression analysis of calibration curve for Catechin and system suitability parameters.

Sr.no	Parameters	Catechin
1.	**Detection Wavelength**	**278 nm**
2.	**Linearity Range (µg/ml)**	**5-25**
3.	**Slope**	**62.03**
4.	**Intercept**	**2.536**
5.	**Correlation Coefficient**	**0.999**
6.	**Retention Time**	**3.350**
7.	**Theoretical plates**	**43510**
8.	**Tailing Factor**	**0.66**

Validation of Developed method.

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. It was ascertained based on recovery studies performed bv standard addition method. Standard drug ***solution of*** concentration 4, 5, 6 µg/mL were spiked and % recovery was calculated from the ***absorbances*** at 278 nm.

For accuracy solution, Prepare 5 µg/ml from tab stock solution II

5X 80 % = 4 µg/ml

5 X 100 % = 5 µg/ml

5 X 120 % = 6µg/ml

80 % = 0.1 ML EXT SOLUTION and ADD 4 µgm/ml STds (0.04 ml) And make up volume 10 ml with Diluent

100 % =0.1 ML EXT SOLUTION and ADD 5 µgm/ml STds (0.05 ml) And make up volume 10 ml with Diluent

120 % = 0.1 ML EXT SOLUTION and ADD 6 µgm/ml STds (0.06ml) And make up volume 10 ml with Diluent.

The results of recovery studies and statistical data are recorded in **table 3**

Table 3: Results of Recovery Studies for Catechin.

80%, y=62.03 x + 2.536

Sr no.	µgm/ml	Amount added	Area	Amount found	Amount recovered	% Recovery
1	5	4	532.9874	8.55153	3.55153	88.79
2	5	4	531.3198	8.524646	3.524646	88.12
			Mean	8.54	3.54	88.45
			SD	0.019	0.019	0.48
			%RSD	0.223	0.537	0.54

100%

Sr no.	µgm/ml	Amt added	Area	Amount found	Amount recovered	% Recovery
1	5	5	588.2798	9.442911	4.442911	88.86
2	5	5	586.3541	9.411867	4.411867	88.24
			Mean	9.43	4.43	88.55
			SD	0.022	0.022	0.44
			%RSD	0.233	0.496	0.50

120%

Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recvd	% Recv
1	5	6	640.3988	10.28313	5.283134	88.05
2	5	6	645.0095	10.35746	5.357464	89.29
			Mean	10.32	5.32	88.67
			SD	0.053	0.053	0.88
			%RSD	0.509	0.988	0.99

Precision

Precision of an analytical method is expressed as S.D. or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

Intra –day Result

Intra – day precision were performed by the applicate of the drug three different dilutions on the same day and the values of relative standard deviations were calculated. The studies were also repeated on different days.

Inter – day precision were performed by the applicate of the drug three different dilutions on different day and the values of relative standard deviations were calculated. The studies were also repeated on different days.

Table 4: Results for precision are shown below.

Drug	Amount Taken [µg/ml]	Intra- day [n=3]		Inter- day [n=3]	
		Amount Found [µg/ml]	% RSD	Amount found [µg/ml]	% RSD
Catechin	10	9.83	0.17	9.81	0.10
	15	15.40	0.49	15.43	0.06
	20	19.99	0.11	19.99	0.04

Repeatability

Repeatability expresses the precision under the same operating conditions over a small interval of time. Repeatability is also termed intra-assay precision.

Table 5: Result and statistical data for Repeatability

Conc.	Area I	Area II	Mean	Amount found	SD	%SD
20	1242.416	1242.724	1242.57	19.99	0.22	0.02

Robustness

The robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in resolution, capacity factor, peak height and peak width (tailing factor). Robustness was studied for Catechin, results obtained was displayed in following tables. As resulted the flow rate of the mobile phase was changed from 0.6 ml/min to 0.8 ml/min. Similarly, the effect of deliberate changes in organic modifier (methanol) composition was evaluated. In this study, the percentage composition of methanol was altered by ± 1nm.

Limit of Detection

The Limit of detection (LOD) of an individual analytical procedure is the lowest amount of an analyte that can be detected in given sample, which can be detected but not necessarily quantitated under the stated experimental conditions as an exact value.

Equation $y=62.03 x + 2.536$

Slope 107.4

Regression 0.999

Standard deviation 0.67

$LOD = 3.3 \times SD / Slope$

$LOD = 0.035$

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte that can be detected quantitatively from sample, with suitable acceptable precision and accuracy under the stated experimental conditions.

$LOQ = 10 \times SD / Slope$

$LOQ = 0.108$

Stability Study

Table 6: Force degradation conditions.

Sr.no.	Conditions	Optimum stress conditions	Time
1.	Acid Degradation	0.1 N HCL	1hr
2.	Base Degradation	0.1 N NAOH	1hr
3.	H2O2 Degradation	3 %	1hr
4.	Neutral	-	1hr

Acid Degradation

Take 0.5 ml (30 mcg) Sample from Stock (API). Add 05 ml 0.1N HCL and Make up volume with Diluent after 60 min, and take HPLC reading.

Base Degradation

Take 0.5 ml (30 mcg) Sample from Stock (API), add 05 ml 0.1N HCL and Make up volume with Diluent after 60 min and take HPLC reading.

H_2O_2 Degradation

Take 0.5 (30 mcg) ml Sample from Stock (API)add 05 ml 0.1N HCL and Make up volume with Diluent after 60 min and take HPLC reading.

Neutral

Take 0.53ml (30 mcg) Sample From Stock (API) add 05 ml Water and Make up volume with Diluent after 60 and take HPLC reading.

Result and Discussion

Investigation of linear relationship: As shown below the result indicated catechin had good linear relationship with peak area in the range of 5-25 µg, the regression equation is y = 62.032x + 2.536, R^2 = 0.9991

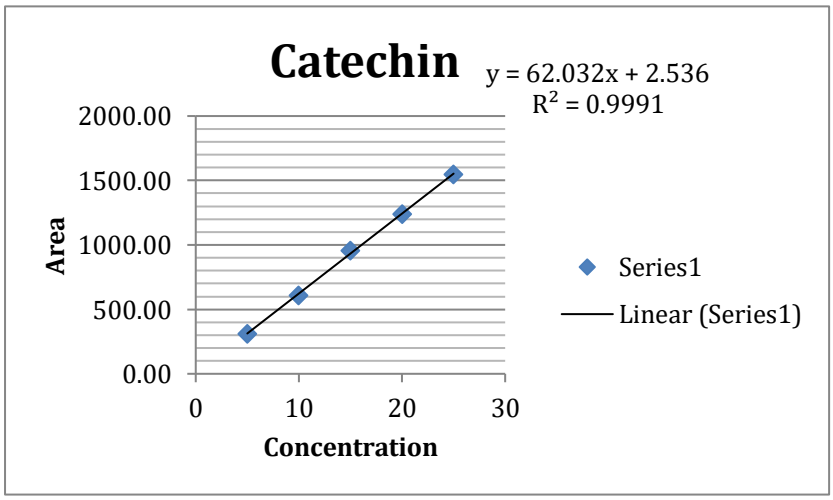


Figure 3: Standard curve of Catechin by HPLC

Result of precision test: The RSD of reference catechin peak areas was within limit (n = 3), which suggested the instrument was of good precision.
Result of Repeatability: The % SD was found to be 0.02 which was within limit.
Result of Robustness:

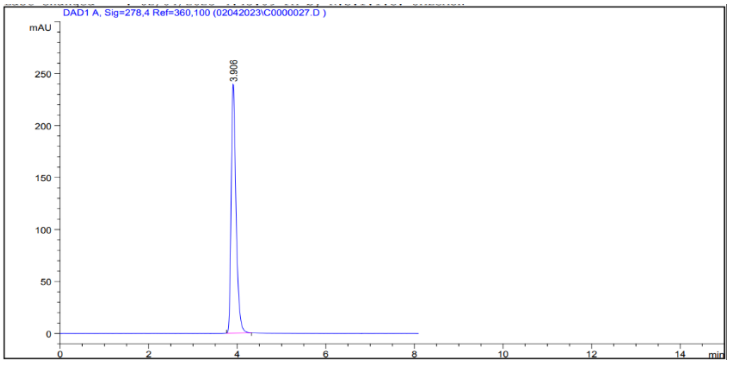


Figure 4: Chromatogram of Catechin at flow rate 0.6 ml/min

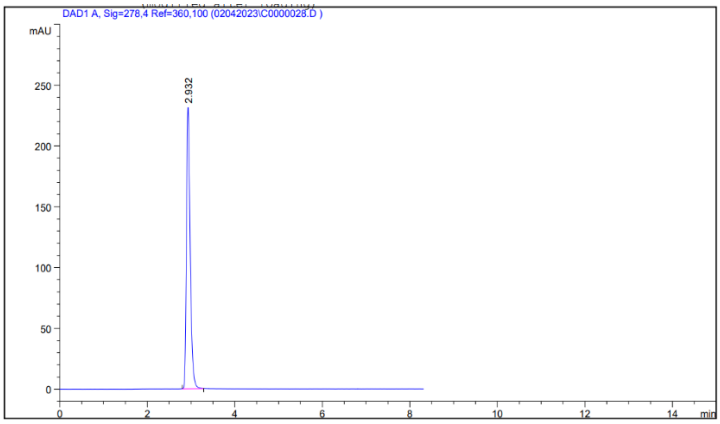


Figure 5: Chromatogram of Catechin at flow rate 0.8 ml/min

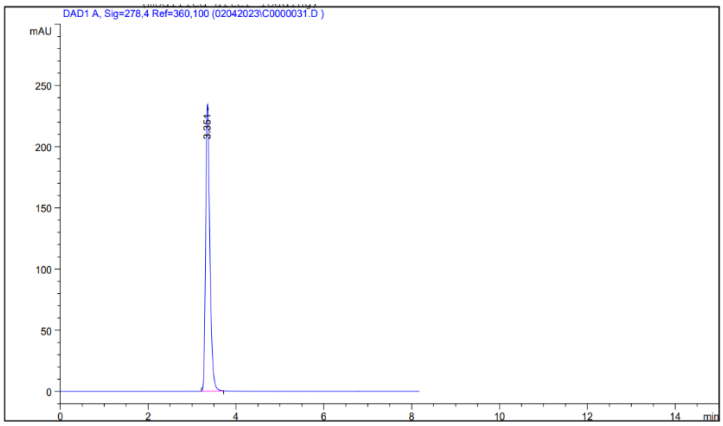


Figure 6: Chromatogram of Catechin with +1% mobile phase

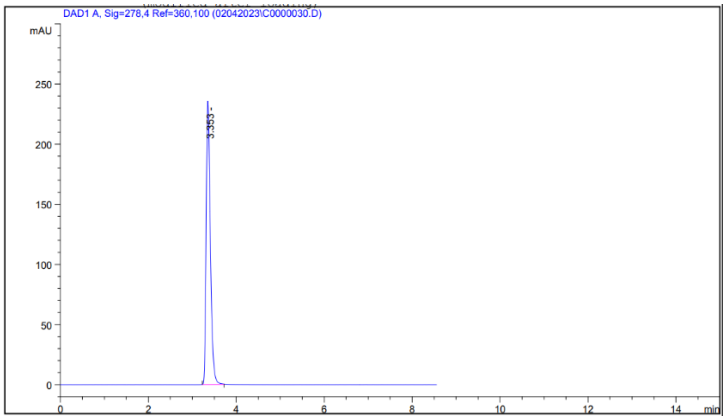


Figure 7: Chromatogram of Catechin with -1% mobile phase

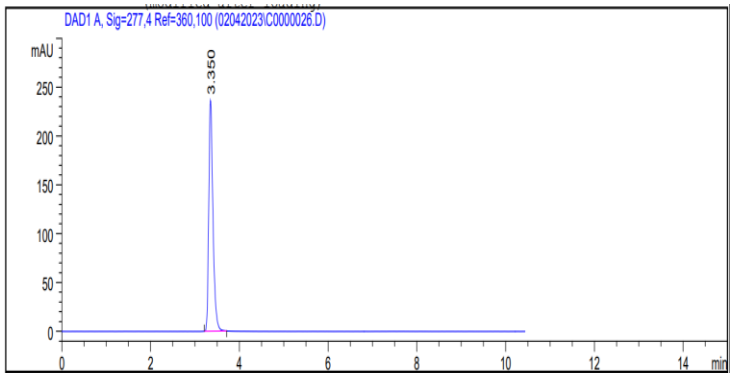


Figure 8: Chromatogram of Catechin at wavelength (277nm)

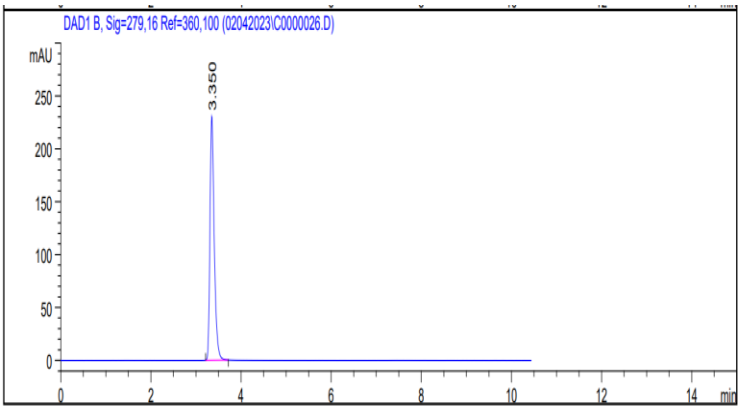


Figure 9: Chromatogram of Catechin at wavelength (279nm)

From all above studies, after making deliberate changes in flow rate (±0.1mL.min $^{-1}$), organic modifier concentration, Catechin (± 1%) and wavelength (± 1nm) have not made any significant changes in theoretical plate counts. Standard deviation for all robustness studies were found to be within the limits.

Result of LOD and LOQ:

The limit of Detection (LOD) and Limit of Quantitation (LOQ) for Catechin were separately determined and reported, based on the calibration curve was found to be 0.035 µg/ml and 0.108 µg/ml respectively.

Result of stability study:

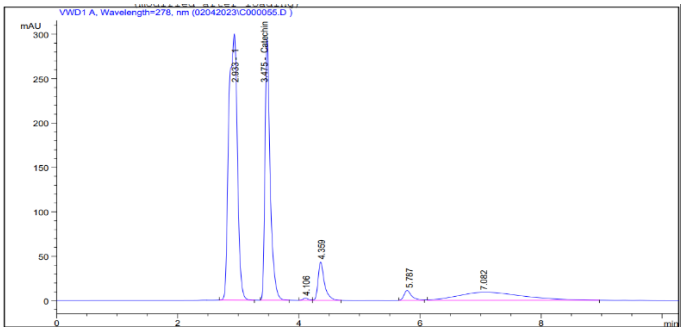


Figure 10: Chromatogram of Catechin after 1hr (Acid Degradation)

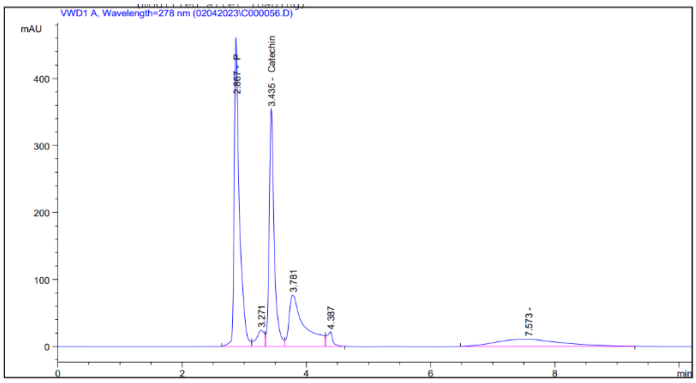


Figure 11: Chromatogram of Catechin after 1hr (Base Degradation)

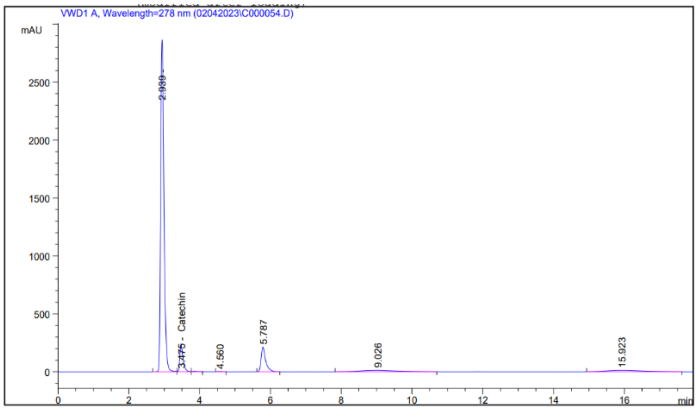


Figure 12: Chromatogram of Catechin after 1hr (H_2O_2 Degradation)

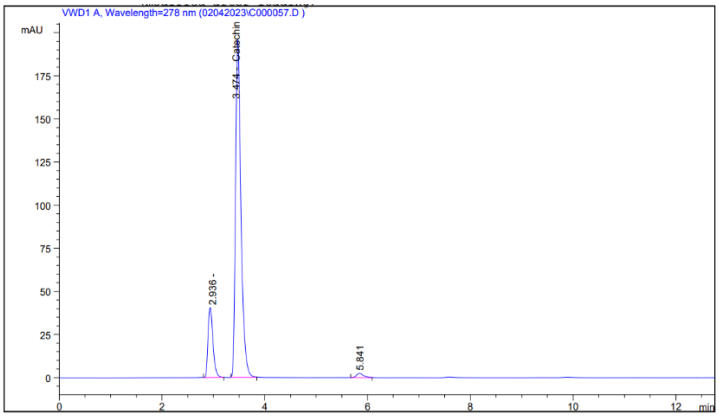


Figure 13 : Chromatogram of catechin after 1hr (Neutral)

Table 7: Degradation Calculation

Sr. No.	Degradation	Area of Standard	% Recovery	Actual % Degradation
1.	**Acid Degradation**	**1546.9**	**81.31**	**18.69**
2.	**Base Degradation**	**1546.9**	**90.46**	**9.54**
3.	**H2O2 Degradation**	**1546.9**	**90.95**	**9.05**
4.	**Neutral**	**1546.9**	**96.41**	**3.59**

Conclusion

In the present work, and High-performance liquid chromatographic methods have been developed and validated for the estimation of catechin in green tea. Simple HPLC method have been developed and validated chromatographic conditions for Catechin. Method was developed using C_{18} (4.6 x 250 mm) column. Flow rate adjusted was 0.7 ml/min. The mobile phase used was methanol: acetic acid (80:20 v/v). Detecting wavelength was 278 nm. After application of the developed methods to the marketed preparations, % R.S.D. was found to be less than 2 indicating high degree of precision. Methodology investigation indicated HPLC standard curve: y=62.03 x + 2.536, R2 = 0.991, the linear relationship between catechin concentration and peak area was good in the range of 5-25 µg.

The RP HPLC methods validated for parameters like accuracy, precision, linearity for estimating the Catechin. Stability test are carried out so that the recommended storage conditions and shelf life can be included on the label to ensure that the drug is safe, effective throughout the shelf life. The information, thus, obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing and packaging, where knowledge of chemical behaviour can be used to improve the quality of drug product.

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