Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
CODEN: JPCBBG
Impact Factor (GIF): 0.701
Impact Factor (SJIF): 3.905
September- November 2017; 5(3):238-245
Published on: October 29, 2017

Research Article

Development and Validation of UV Spectrophotometric Method for Estimation of Catechin in *Acacia catechu* Methanolic Extract against Marker Compound

Pooja Bhardwaj, Manpreet Kaur, Amit Sharma, Navharman Singh, Manoj Kumar Katual, Rajesh Kumar*

Rayat-Bahra Institute of Pharmacy, Hoshiarpur, Punjab, India

***Corresponding Author:** Rajesh Kumar, Rayat-Bahra Institute of Pharmacy, Hoshiarpur, Punjab, India

Received: 31 July 2017 **Revised:** 08 September 2017 **Accepted:** 15 September 2017

ABSTRACT

A simple, rapid, precise and accurate UV-spectrophotometric method was developed for quantitative analysis of Catechin in methanolic extract. The initial stock solution of catechin was prepared in phosphate buffer pH 6.8 solvent and subsequent dilutions were made thereafter. The standard solution of catechin marker showed maximum absorption at wavelength 278.4 nm. The drug was found to obey Lambert-Beer's law in a concentration range 5-40 µg/mL with coefficient of correlation (R^2) of 0.9991. The method was validated as per the ICH guidelines. Since the developed method involves relatively economical solvents and no complex extraction techniques, it can be employed in routine analysis of catechin in bulk as well as its subsequent formulations.

Keyword: Catechin; UV spectrophotometry; phosphate buffer; validation, ICH.

INTRODUCTION

Since long, the pharmaceutical industries are investing enormous efforts and resources in identifying potentially beneficial phytoconstituents that can be used to treat various physiological and pathological conditions. This is the reason why several plants are being exhausted based on their traditional uses [1].

The plant selected for the present study is *Acacia catechu* (family Leguminosae), which is the second largest genus in this family, that comprises of more than 1200 species in almost all habitats of the world [2]. Insufficient information is available about the chemistry of most of these species inspite of their large and widespread presence in the warm sub-arid and arid parts of the world. Acacia can be used in prevention, mitigation or treatment of many diseases owing to presence of a wide range of bioactive components such as phenolic acids, alkaloids, terpenes, tannins and flavonoids [3-7].

The primary chemical constituents present in plant are catechins which are gallic acid (polyhydroxylated benzoic acid) derivatives and polymers. The predominant catechins that are present in *Acacia catechu* are catechin, epicatechin, epicatechin-3-O-gallate, and epigallocatechin-3-O-gallate. Other chemical constituents which have been reported in A. catechu aqueous extract include rhamnetin, 4-hydroxyphenol, 3,3′,5,5′,7-pentahydroxyflavane, fisetinidol, 5-hydroxy-2-[2-(4-hydroxyphenyl) acetyl]-3-methoxybenzoic acid, and (2S,3S)-3,7,8,3′,4′-pentahydroxyflavane. An extract of

Acacia catechu heartwood was reported to possess approximately 67% catechin and 23% epicatechin, making a total of 90% of the composition comprising of both of these components only [8, 9].

Presence of these many important phytochemicals confirms the broadspectrum activities of plant which include healing of sore throat, antidiarrhoeal activity, hypoglycaemic activity, antioxidant, anti-microbial activity, hepato-protective activity, antipyretic and anti-inflammatory properties, potent wound healing property etc. [10].

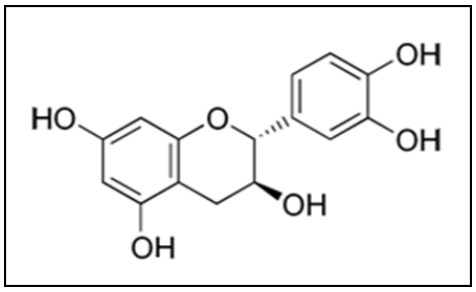

Fig. 1: Chemical structure of Catechin (+)

A survey of literature confirmed that no work has been done till date to standardize *A. catechu* heartwood extract for its main chemical constituent(s) using a simple and economic UV-spectrophotometric method. Thus, the major objective of this study was to develop a simple, rapid and economic validated UV-spectrophotometric method for the estimation of catechin in *Acacia catechu* heartwood extract.

MATERIALS AND METHODS

Materials

Acacia catechu chips were obtained from local market. Methanol (analytical grade) was purchased from Himedia Laboratories Pvt. LTD, India. Catechin marker compound was purchased from Natural Remedies, Banglore. Other chemicals like potassium dihydrogen orthophosphate, sodium hydroxide etc. used in this study were procured from Central drug house (P) Ltd., New Delhi.

Collection and Authentication of *Acacia Catechu* Chips

Acacia catechu chips were purchased from the local market at Jalandhar (Punjab), India and authenticated from Department of Botany, Punjabi University, Patiala, Punjab (India) vide letter no. SPL-112/Bot dated 12-08-2016. The collected chips were cleaned and crushed into small pieces to form powder, weighed and finally stored in desiccators.

Extraction of *Acacia catechu*

Extraction of heartwood was done following a reported procedure with modification. The dried heartwood chips powder weighing about 200 gm was taken and about 400 ml methanol was added to it into round bottom flask (1000 ml). Then it was kept for boiling for around 4 hours and allowed to stand thereafter for cooling. After cooling, the mixture was filtered through whatman filter paper and then filtrate was evaporated at 30ºC on water bath to get the concentrated residue. The residue remained from first maceration was again kept for boiling with methanol (300 ml) for 3 hours, filtered and evaporated as mentioned above to get concentrated extract. Further, it was dried in air and stored in desiccator for further study. The practical yield of extract was calculated to be 36.8% [11].

Development of UV-method

Instrumentation

Spectrophotometric analysis was done using Systronics double beam UV-Visible Spectrophotometer, model UV-2201 (India) equipped with 1 nm spectral bandwidth, ±0.5 nm wavelength accuracy and a pair of 1cm quartz cells.

Method Optimization

Selection and optimization of Solvent

Since the solvents are well known to exert a significant influence on the quality and shape of the spectrophotometric peak [12], different solvents like acetone, methanol, ethanol, ethyl acetate, distilled water and phosphate buffer were tried for UV-method development out of which phosphate buffer pH 6.8 satisfied all the

conditions relative to peak quality & non-interference at the specified wavelength.

Preparation of Phosphate buffer (pH 6.8)

Placed 125 ml of 0.2 M Potassium dihydrogen phosphate (KH_2PO_4) solution in a 500 ml volumetric flask; added 56 ml of 0.2 M sodium hydroxide (NaOH) solution and then made up the volume by adding water [13].

Preparation of Standard stock solution

Accurately weighed quantity of drug (10 mg) was weighed accurately, transferred into a volumetric flask (10 ml) and dissolved by diluting up to the mark with the phosphate buffer pH 6.8 to get a concentration of 1000 µg/ml. Using the buffer solution, further dilutions were made varying in a concentration range of 5-50 µg/ml [14].

Determination of Wavelength (λ_{max})

The stock solution of drug was further diluted with buffer to get a concentration of 100 µg/ml. The reesulting solution was scanned on the UV spectrophotometer in a range from 200-400nm to determine the absorbance maxima (λ_{max}).

Method Validation

Validation of the developed method was done following the guidelines laid down in International Conference on Harmonization (ICH) guidelines Q2 (R1) [15]. The following parameters were evaluated:

Linearity and range

Linearity of any analytical method is its ability, within a given range, to get test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Ten different concentrations (5-50µg/ml) of *Acacia catechu* extract were prepared in phosphate buffer from a fresh stock of 1000µg/ml. The solutions were scanned on UV spectrophotometer in UV-range (i.e. 200-400 nm). The spectrum was recorded. Least square regression analysis was done by constructing the calibration plot between concentration and absorbance [16].

Sensitivity

Sensitivity of the developed method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). A series of varying drug concentrations (5-50 µg/ml) were analyzed to find LOD and LOQ. LOD is the lowest detectable amount of an analyte in a given sample that may or may not be quantified, under the stated experimental conditions whereas LOQ is the lowest quantifiable amount of analyte in any sample. LOD & LOQ were computed by using standard deviation (σ) and slope value (s) obtained from calibration curve [17].

Equations:

$$LOD = 3.3\ \sigma/s$$

$$LOQ = 10\ \sigma/s$$

The LOD and LOQ were calculated according to the 3.3 σ/S and 10σ/S criterions, respectively, where σ is the standard deviation of the y-intercept of the regression line and s is the slope of the calibration curve.

Accuracy

Accuracy is the percentage of analyte recov ered by assay from known added amount. Solutions were prepared at levels 75%, 100% and 125% of 20 µg/ml test concentration of the sample solution using standard working solution as per the test method and absorbance was noted down. The whole procedure was done in triplicate [18].

Precision

Precision of an analytical method is the degree of repeatability under the normal operation conditions. The precision was determined with standard quality control samples prepared in triplicate at different concentration levels co vering the entire linearity range. The precision of assay was determined by intra-day and intermediate i.e. inter-day precision (comparing the assay conducted on 3 different days) and were recorded as % RSD for a statistically significant number of replicate measurements [19].

Repeatability

Repeatability analysis was performed by analyzing samples of same concentrations (six times) of standard catechin (20 µg/ml). From the resulting absorbance, SD (standard deviation) and RSD (relative standard deviation) were calculated.

Robustness

The robustness of any analytical method is the measure of its capacity to remain unaffected by

small, but deliberate variations in method parameters. Robustness is an indicative of reliability of a method during normal usage. Robustness was tested by varying detection wavelength (±2 nm) of optimized conditions from the standard detection wavelength (278.4 nm) [20].

Ruggedness

Ruggedness of the method was confirmed by analyzing repeatedly for six times the standard solution having 20 µg/ml of standard catechin. The analysis was performed by 2 different analysts under the same set of experimental and environmental conditions [21].

RESULTS AND DISCUSSION

Analysis of Drug

The absorbance maximum of the drug was recorded by taking scan of the drug sample solution in the UV region (200-400 nm). It produced a clear peak at a wavelength of 278.4 nm (i.e. λ_{max}).

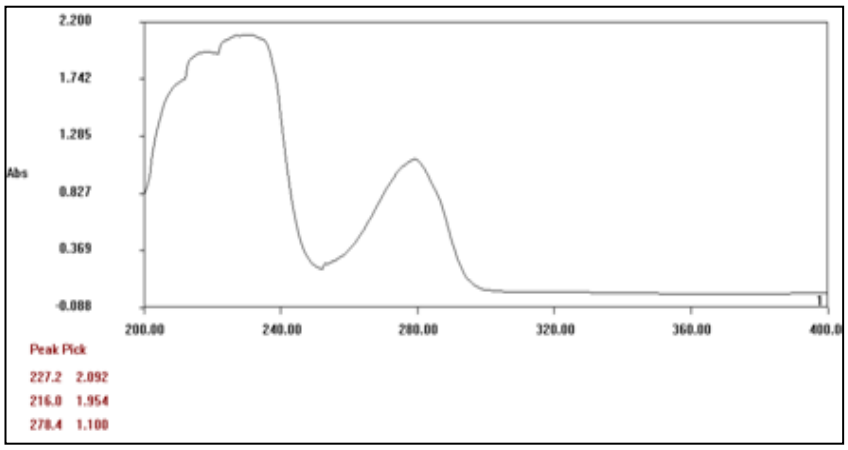

Fig. 2: UV Spectrum of standard catechin (+) in phosphate buffer (pH-6.8)

Validation Parameters of Developed Analytical Method

The method was validated following ICH guidelines (Q2 (R1)).

Linearity and Range

Good linear correlation was observed between absorbance and concentration in the selected concentration range of 5-50 µg/ml. The regression equation was recorded to be y= 0.0121x - 0.0127. The correlation coefficient (r^2) of the standard curve was found to be 0.999. The results are tabulated below.

Table 1: Spectrophotometric data for calibration curve

Concentration (µg/ml)	*Absorbance ± S.D.
5	0.054 ± 0.007
10	0.106 ± 0.027
15	0.165 ± 0.029
20	0.229 ± 0.028
25	0.291 ± 0.026
30	0.353 ± 0.022
35	0.411 ± 0.042
40	0.464 ± 0.020
45	0.544 ± 0.046
50	0.590 ± 0.010

**Each value is the average of three determinations*

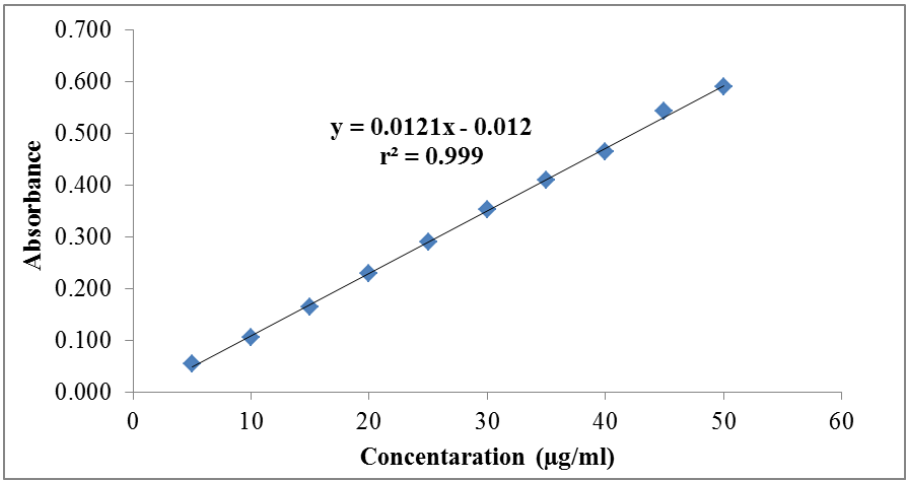

Fig. 3: Calibration curve of Catechin (+) in phosphate buffer (pH 6.8)

Sensitivity

Calculations of LOD and LOQ of method were based on the standard deviation of y-intercept of regression line (σ) and the slope (s) of the calibration curve at levels approximating the LOD and LOQ.

LOD and LOQ were calculated according to the formulae:

LOD = 3.3 σ/S = 7.009 µg/ml

LOQ = 10 σ/S = 21.239 µg/ml

Precision

The intra-day precision was determined by analyzing the drug at particular concentration for three times on the same day taking the time intervals of 2 hrs at 10:15 am, 12:15 pm and 02:15 pm respectively. The Inter day precision was estimated similarly, analyzing the samples daily, for three consecutive days. The results obtained from intra-day and inter-day precision are tabulated below.

Table 2: Results of Intra Day and Inter Day Precision

Results of Intra Day Precision						
Concentration taken	**Absorbance (*n = 3)**			**Mean**	**SD**	**% RSD**
	10:15 am	**12:15 pm**	**2:15 pm**			
20	0.364	0.324	0.322	0.337	0.024	7.12
30	0.327	0.286	0.272	0.295	0.029	9.83
40	0.430	0.402	0.390	0.407	0.021	5.15
Results of Inter Day Precision						
Concentration taken	**Absorbance (*n = 3)**			**Mean**	**SD**	**%RSD**
	Day 1	**Day 2**	**Day 3**			
20	0.358	0.307	0.353	0.339	0.028	8.25
30	0.403	0.299	0.295	0.332	0.061	18.30
40	0.521	0.405	0.402	0.443	0.068	13.99

**Each value is an average of three determinations*

Accuracy

Accuracy of an analytical method is the closeness of practical result to theoretical value. It was determined by spiking the known amount of standard at 75%, 100% and 125% levels of drug concentration in pre-analyzed samples solutions. Observations of recovery experiment were found in desired limits which indicated the absence of any interference from the commonly encountered pharmaceutical additives and excipients. The results for the recovery studies are given in table below.

Table 3: Results of recovery studies

Recovery Level	Initial Conc. (µg/ml)	Concentration of standard drug added (µg/ml) (*n = 3)	% Recovery (*n = 3)	% RSD
75%	20	10	100.08	0.619
100%	20	20	99.91	0.200
125%	20	30	98.80	0.263
Mean			**99.59**	**0.360**

**Each value is the average of three determinations*

Robustness

Robustness studies assumed that the obtained results were insignificantly affected by small variations in any of the variables (table 4). It ensured the reliability of the proposed method during routine analysis.

Table 4: Results of Robustness Studies

276.4 nm			278.4 nm		
Conc. (µg/ml)	**Absorbance**	**Statistical analysis**	**Conc. (µg/ml)**	**Absorbance**	**Statistical analysis**
20	0.245	Mean = 0.250 SD = 0.004 %RSD = 1.6	20	0.354	Mean = 0.355 SD = 0.002 %RSD = 0.563
20	0.248		20	0.355	
20	0.247		20	0.352	
20	0.253		20	0.353	
20	0.254		20	0.356	
20	0.265		20	0.359	

Ruggedness

No statistically significant difference was observed between the 2 operators, suggesting the ruggedness of the developed method (table 5).

Table 5: Results of Ruggedness Studies

Analyst 1			Analyst 2		
Conc. (µg/ml)	**Absorbance**	**Statistical analysis**	**Conc. (µg/ml)**	**Absorbance**	**Statistical analysis**
20	0.344	Mean = 0.343 SD = 0.001 %RSD = 0.291	20	0.336	Mean = 0.338 SD = 0.001 %RSD = 0.295
20	0.343		20	0.338	
20	0.342		20	0.339	
20	0.342		20	0.338	
20	0.343		20	0.338	
20	0.342		20	0.337	

Repeatability

The repeatability of the instrument was validated by taking the absorbance of six samples of the same concentration (20 µg/ml). The mean absorbance was computed to be 0.244 nm. The results are tabulated below.

Table 6: Results of Repeatability Studies

Concentration taken	Absorbance	Statistical Data
20	0.241	Mean = 0.244
20	0.243	S.D. = 0.002
20	0.244	% RSD = 0.819
20	0.243	
20	0.247	
20	0.246	

The summary of all the validation parameters is presented in table 7.

Table 7: Summary of Validation parameters

Validation Parameter	Result
Absorption maxima (λ_{max})	276.8 nm
Linearity Range	5-50 µg/ml
Standard Regression Equation	y = 0.0121x - 0.0127
Slope (m)	0.0121
Correlation Co-efficient (r^2)	0.9991
% Recovery (mean)	99.59
% RSD for Intraday (n = 3) Precision	7.37
% RSD for Inter day (n = 3) Precision	13.51
Repeatability (% RSD)	0.819
LOD	7.009
LOQ	21.23

CONCLUSION

A simple, accurate, precise, robust and rapid UV-visible spectrophotometric method was developed for estimation of catechin in bulk and its subsequent pharmaceutical formulation. The results reveal that the proposed method could be successfully applied in the routine analysis and quality control of pharmaceutical dosage forms containing catechin.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Chander Mohan, Director-Principal, Rayat-Bahra Institute of Pharmacy, Hoshiarpur, Punjab for the providing us the required facilities to perform this analytical study. Authors also wish to thank Mr Mandeep Singh, Ms Maninderjit Kaur and Ms Seema (faculty members) for helping in Pharmacognosy and Analytical part of work.

CONFLICT OF INTEREST

The authors confirmed that there is no conflict of interest for this research paper.

REFERENCES

1. Kumar R, Singh T, Kumar D, Singh M, Kaur S, Garg R. Estimation of Bacoside-A in *Bacopa Monnieri* aerial parts using TLC Densitometry. Int J Pharmacy Pharm Sci 2015; 7: 293-295.
2. Charles MI, Dennis AS, Constantinos VS, Dimitri MU, Christos RS and Vassilios Roussis. Lupane Triterpenoids from Acacia mellifera with Cytotoxic Activity. Molecules 2007; 12: 1035-1044.
3. Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G, Sarma BK and Singh HB. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of Moringa oleifera. Food Chem Toxicol 2009; 47(6): 1109-1116.

4. Clement BA, Goff CM, Forbes TDA. Toxic amines and alkaloids from *Acacia berlandieri*. Phytochem 1999; 46: 249-254.
5. Mujoo K, Haridas V, Hoffmann JJ, Wachter GA and Hutter L. Triterpenoid saponins from Acacia victoriae (Bentham) decrease tumor cell proliferation and induce apoptosis. Cancer Res 2001; 61: 5486-5490.
6. Readel K, Seigler D, Hwang K, Keesy J and Seilheimer S. Tannins from Mimosoid legumes of Texas and Mexico. Economic Botany 2001; 55: 212-222.
7. Fourie TG, Ferreira D and Roux DG. 8-O-methyl- and the first 3-O-methylflavan-3,4-diols from Acacia saxatilis. Phytochem 1974; 13:2573-2587.
8. Shen D, Wu Q, Wang M, Yang Y, Lavoie EJ and Simon JE. Determination of the predominant catechins in *Acacia catechu* by liquid chromatography/ electrospray ionization-mass spectrometry. J Agric Food Chem 2006; 54: 3219–3224.
9. Li XC, Liu C, Yang LX, Chen RY. Phenolic compounds from the aqueous extract of *Acacia catechu*. J Asian Nat Prod Res 2011; 13: 826-830.
10. Hashmat MA and Hussain R. A review on *Acacia catechu* Willd. Interdisciplinary J Contemp Res Business 2013; 5: 293-600.
11. Rajendra P, Titiksh D, Rasika P, Vandana G, Nikhil R. Antiulcer activity of *Acacia catechu* WIlld in Rats. Int J Ayur Res Pharm 2011; 2: 1585-1587.
12. Ahuja S, Scypinsk S. 2001. Handbook of modern pharmaceutical analysis. 5th ed., London: Academic Press, 2001; p 345.
13. Indian Pharmacopoeia, Buffer Solutions, 2007; Volume 1, 240- 242.
14. Patil SK, Salunkhe VR, Mohite SK. Development and Validation of UV Spectrophotometric Method for Estimation of Glycyrrhetinic acid in Hydroalcoholic Extract of *Glycyrrhiza glabra*. Int J Pharm Chem Biol Sci 2012; 2: 617-621.
15. ICH Guideline Q2(R1), Validation of analytical procedures: text and methodology, November 2005.
16. Jain PS, Chaudhari AJ, Patel SA, Patel ZN, Patel DT. Development and validation of the UVspectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. Pharm Methods 2011; 2: 198-202.
17. Revathi E, Thiruvengadam E, Saravanan V, Abdul V, Jithin R. Development and Validation of Stability Indicating Spectroscopic Method for Content Analysis of Ceftriaxone Sodium in Pharmaceuticals. Int Schol Res Notices 2014; 2014: 1-5.
18. Sethuraman S. Analytical Method Development and Validation of Caffeine in Tablet Dosage Form by using UV-Spectroscopy. Int J Novel Trends Pharm Sci 2013; 3: 82-86.
19. Bhavar B, Aher B, Ravindra S, Kakadsachin J and Pekamwar S. Development and Validation of UV Spectrophotometric Method for Estimation of Dolutegravir Sodium in Tablet Dosage Form. Mal J Anal Sci 2015 19: 1156-1163.
20. Desai P, Mori K and Patel M. Development and Validation of UV-Visible Spectrophotometric Method for Simultaneous Estimation of Mometasone Furoate, Hydroquinone and Tretinoin from their Pharmaceutical Dosage Form. Int J Pharm Sci Rev Res 2013; 21: 296-300.
21. Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. J Anal Bioanal Tech 2012; 3: 2-6.

Cite this article as:
Pooja Bhardwaj, Manpreet Kaur, Amit Sharma, Navharman Singh, Manoj Kumar Katual, Rajesh Kumar. Development and Validation of UV Spectrophotometric Method for Estimation of Catechin in Methanolic Extract against Marker Compound. J Pharm Chem Biol Sci 2017; 5(3):238-245