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A VALIDATED STABILITY-INDICATING HPLC-DAD METHOD FOR THE ESTIMATION OF BACOSIDE- A FROM *BACOPA MONNIERI* L. PLANT AND TABLETS

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ABSTRACT

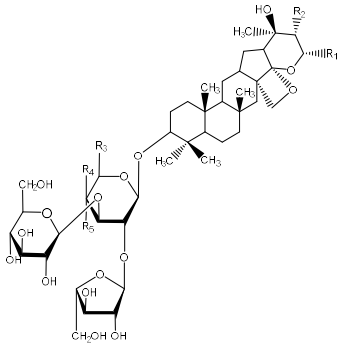
Bacoside-A is a common biomarker of *Bacopa Monnieri*, a commonly used herb in Ayurvedic formulations for the treatment of various CNS disorders. Stability indicating RP-HPLC method for the estimation of bacoside-A (mixture of bacoside A_3, bacopaside-II, jujubogenin (isomer of bacopasaponin-C) and bacopasaponin-C) from *Bacopa Monnieri* plant extract and its marketed formulation (*tablets*) was developed. The marker compound bacoside–A was resolved using mobile phase acetonitrile:phosphoric acid (30:70 v/v) with gradient elution on a C_{18} column at ambient temperature. The detection was performed at 205 nm. The pure bacoside-A, plant extract, and marketed formulation were subjected to forced degradation and accelerated stability studies. The developed RP-HPLC method was further validated as per ICH guidelines.The retention time was found to be 17.98,18.56,20.27,21.20min for four different peaks of bacoside A_3, bacopaside-II, jujubogenin (isomer of bacopasaponin-C) and bacopasaponin-C respectively in standard bacoside-A, plant extracts, and tablets. The developed method can be used satisfactorily for the quantification of all four components in the presence of degradation products. Forced degradation study revealed that all components of bacoside-A was found to follow first-order kinetics during acidic and alkaline hydrolysis. The contents of all four components was reduced considerably at the end of accelerated stability studies in standard bacoside-A, plant extract, and tablets.It was concluded that moisture and pH play a pivotal role in maintaining the stability of *Bacopa Monnieri* formulations. The developed

method can serve as a quality control tool to determines the effect of various environmental factors on the content of Bacoside-A in herbal and polyherbal formulations.

Keywords: ***Bacopa Monnieri,*** **Stability, Forced Degradation, Accelerated Stability, bacoside-A**

INTRODUCTION

The Central nervous system (CNS) is a highly complex system that controls the functioning of the body. Iis estimated that 60% of the world population suffers from depression/anxiety or related CNS disorders at one or the other stage of their life **[1]**. Bacopa monnieri (Brahmi), a most precious CNS stimulating herb used by Ayurvedic medical practitioner recognised as 'medhyarasayana', a drug used to improve memory and intellect (medhya). The herb has been mentioned in several ancient Ayurvedic treatises including the 'Charak Samhita' since sixth century AD for the management of diverse mental conditions including anxiety, poor cognition, and lack of concentration as a diuretic and as an energiser for the nervous system and the heart **[2]**. The constituents present in Brahmi responsible for its pharmacological effects include alkaloids, saponins, and sterols. The constituents that are responsible for Bacopa's cognitive effects are bacoside A **(Figure 1)** and B **[3-9]**. The medicinal herbal formulations having Brahmi include brahmi vati, brahmi powder, brahmi churna etc.



	R_1	R_2	R_3	R_4	R_5
Bacoside – A_3	**H**	**$CH=C(CH_3)_2$**	**CH_2OH**	**H**	**OH**
Bacopaside-II	**$CH=C(CH_3)_2$**	**H**	**CH_2OH**	**H**	**OH**
Jujubogenin isomer of bacopasaponin C	**H**	**$CH=C(CH_3)_2$**	**H**	**OH**	**H**
Bacopasaponin C	**$CH=C(CH_3)_2$**	**H**	**H**	**OH**	**H**

Figure 1: Chemical Structure of Bacoside-A* (C_{47} H_{76} O_{18}, MW:929.09 g.mol^{-1})

Currently, plant-based drugs are researched, dispensed, formulated, and manufactured in a modern framework rather than in the form of galenical preparations or conventional dosage forms. Herbal medicines include herbs, herbal

materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients. Their popularity is largely due to their presumed safety, efficacy, cultural acceptability, and lesser side effects compared with prescription medications; perhaps most important, they are viewed as cost-effective and accessible **[2]**.

However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameters. Moreover, the constituents responsible for the claimed therapeutic effects are frequently unknown or partly explained. This is further complicated by the use of a combination of herbal ingredients as being prescribed by traditional practitioners. Due to the lack of stringent guideline, the acceptance of herbal formulations remains questionable. The various parameters to be considered while manufacturing a herbal product may require a thorough understanding of the stability, interaction (drug-drug or drug-excipient), and degradation of the active constituents present in it.

The stability study of any formulation is a critical quality control parameter for determining the quality, safety, and efficacy of the product. Literature review revealed several analytical methods like HPTLC **[11-16]**, HPLC **[17-20]**, LC/MS **[21]** are available for the estimation of bacoside-A from the plant extract and formulations. However, none of the methods discussed the determination of bacoside-A from the marketed formulation after accelerated stability study and prediction of its degradation pathway.

Therefore, it is worth developing a stability-indicating HPLC method for the estimation of bacoside-A and degradants formed during stability study and predicting the probable degradation pathway by recording a mass spectrum of bacoside-A after accelerated stability study.

MATERIALS AND METHODS

MATERIALS

Bacoside-A (96.9% pure, mixture of bacoside A3, bacopaside-II, jujubogenin (isomer of bacopasaponin C) and bacopasaponin C) reference standard was purchased from Natural Remedies Pvt. Ltd (Bangalore, India). The raw plant material was purchased from a local market and authenticated by a botanist at the Department of Botany (J&J College of Science, Nadiad, India) The Brahmi tablets containing 250 mg of B.Monerrie extract purchased from local market. Acetonitrile (HPLC grade), phosphoric acid (HPLC grade) , methanol, hexane and ethyl acetate (AR grade) were purchased from Loba Chemie, Mumbai, India.

INSTRUMENTS

The digital analytical balance (Shimadzu, Kyoto, Japan), Vacuum filtration

assembly(Mumbai, India), pH meter (Elico, Hyderabad, India), stability chamber (Thermolab, Mumbai, India), Melting point apparatus(Veego instruments, Mumbai, India), Hot air oven (NOVA Instruments ,Ahmadabad, India), Rota evaporator (Heidolph Instruments, Hyderabad, India), UV double-beam spectrophotometer UV-1800 (Shimadzu) and Dipping chamber and TLC aluminum pre-coated plates with silica gel $G60F_{254}$ (10x10cm), 0.2 mm thickness (Merck, India) were used throughout the study to carry out experiments.

SOLUTIONS

Standard stock solution of bacoside-A

Bacoside-A (20 mg) was accurately weighed and transferred to 10 mL volumetric flask and volume were made up to mark with methanol to obtain a stock solution containing 2000 µg/mL.

Sample solutions

(a) Plant extract: —Plant powder (15 g) was weighed and extracted with 95% methanol (400 mL) using soxhlet extraction (50°C for 3 hr). The resultant marc was filtered, and the filtrate was dried under vacuum. Ten mg of plant extract (PE) was dissolved in 10 mL of methanol, and the solution was filtered through a 0.45 µm syringe filter and used further for HPLC analysis.

(b) Marketed Formulation (Tablets): — Twenty tablets were weighed accurately and powdered using mortar and pestle. Tablet powder (13 mg) equivalent to 10 mg of Brahmi tablet extract (BTE) was taken and soaked in 10 mL methanol for 24 hr. The solution was then filtered through a 0.45 µm syringe filter and used further for HPLC analysis.

OPTIMIZED STABILITY INDICATING HPLC METHOD

The analysis of standard bacoside-A was carried out using a gradient HPLC system (Shimadzu, LC 2010) comprising of a binary pump (LC20AT), Injection (Rheodyne, 20µL loop) with photodiode array detector (SPD-M20A). The chromatograms were recorded using the LC solution software. Analysis of Bacoside-A was performed at ambient temperature. The HPLC gradient method was developed by modifying the reported method.[17] The separation was achieved using Phenomenex Luna C_{18} column (250 x 4.6 mm, 5µ) using gradient mobile phase acetonitrile: 0.5% phosphoric acid (30:70 v/v, 0 min; 40:60 v/v, 25 min; 30:70 v/v, 30 min). The total run time was kept 30 min. The flow rate was kept at 1.5 mL/min. The developed method was validated as per ICH guidelines **[22]**.

VALIDATION OF HPLC METHOD

The developed RP-HPLC method was further validated as per ICH guidelines for selectivity, specificity, linearity and range, precision, accuracy, robustness, and

sensitivity. For determining the selectivity of the RP-HPLC method, the overlay spectra of standard bacoside-A (mixture of bacoside A_3, bacopaside-II, jujubogenin (isomer of bacopasaponin C) and bacopasaponin C) and BTE were recorded. The peak purity of spectra was determined for standard bacoside-A and BTE at three different levels, the start, the middle, and the end of the peak. The linearity was performed using suitable aliquots of bacoside-A (2000 µg/mL) ranging from 0.5- 2.5 mL from solution in 10 ml volumetric flask to get 100-500 µg/mL of Bacoside-A using methanol. As Bacoside-A is a mixture of four components as mentioned above, the mean peak areas (n=6) for each individual peaks were calculated and plotted against concentration to obtain four different calibration curves. The calibration curve equation y = mx+c was obtained using MS Excel for each component of Bacoside-A, where y= concentration of bacoside-A (µg/mL), m=slope and c=intercept. The value of the correlation coefficient (r^2) was also determined. The precision was performed injecting bacoside-A (300 µg/mL) for six times a day and on six consecutive days using optimised HPLC conditions for each component individually. The peak areas were recorded for each measurement, and the percentage of RSD values was calculated.

The %RSD was calculated using the following equation:

% RSD= Standard deviation/mean x 100...(1)

Due to unavailability of the individual standards of all four components of bacoside-A, accuracy was determined by comparing the developed method with already established method reported by Watoo P *et al* (2008). The authors have used standards of two components (Co. Bacopaside I and bacoside A3) for quantification of these components for their quantification during stability study of dried ethanolic extract of *Bacopa Monnieri* plant material. Hence, both the methods were performed using 500 µg/mL of Bacoside-A, the contents of all four components were determined. The peak areas were recorded for all four components using both HPLC methods and paired t-test was applied for determination of difference between the two methods for establishment of accuracy of the method. For the determination of accuracy, the standard addition method was used. The BTE (150 µg/mL) was spiked with a standard bacoside-A solution at three different concentration levels (270, 300, and 330 µg/mL) of bacoside-A (n=3). The robustness of the developed method was determined by deliberately changing flow rate and detection wavelength. The sensitivity of the method was determined

by calculating LOD and LOQ values for each component using following equations:

LOD = 3.3xSD/slope of calibration curve...(2)

LOQ = 10xSD/slope of calibration curve…(3)

Where, SD= the standard deviation of intercepts *(n=6)*.

FORCED DEGRADATION STUDY FOR STANDARD BACOSIDE-A, PLANT EXTRACT AND TABLETS

The aliquots 10 mL of stock solution of bacoside-A (200 µg/mL) were subjected to different stress conditions such as acidic, alkaline, oxidative, and photolytic degradation. The aliquots were reacted individually with 10 mL of 0.1N hydrochloric acid and 0.1N sodium hydroxide and refluxed at 50ºC, 60ºC, and 70ºC for 80 min in different Round Bottom Flask (RBF). From each RBF, one mL aliquot was withdrawn at different time intervals, i.e., 10, 20, 30, 40, 50, 60, 70, and 80 min. in each case. Oxidative degradation was carried out by reacting to 10 mL 3% hydrogen peroxide solution with 10 mL (200 µg/mL) bacoside-A. The samples were kept aside at room temperature for 9 hr. After every one interval, two mL aliquot was withdrawn and analysed. 10 mL (200 µg/mL) bacoside-A was further exposed to UV light (254nm) for 8h, and every hour, one mL aliquot was withdrawn for analysis **[6, 10]**. The degradation kinetics was established for above-mentioned conditions. The aliquots of 10 mL of stock solution of bacoside-A (200 µg/mL) extracted from crude plant extract [60 g of plant powder gave 9.8 mg of bacoside-A] of *Bacopa Monnieri* was reacted in a similar manner. For performing forced degradation of tablets containing *Bacopa Monnieri* extract [402.36 ± 0.38 mg of tablet powder has 217 µg of bacoside-A], was suitably weighed and diluted to get 200 µg/mL and proceeded further as mentioned above. The whole study was performed in triplicate.

ACCELERATED STABILITY STUDY OF STANDARD BACOSIDE-A, PLANT EXTRACT AND TABLETS

The standard bacoside-A, dried methanolic plant extract of *Bacopa Monerrie* and the Brahmi tablets were kept separately in polythene terephthalate (PET) packaging containers that were procured from a local market. The formulations were stored for six months under test conditions mentioned in ICH guidelines at 40 ± 2ºC with 75 ± 5 %RH (10). Sampling was done at 0 day, 15 days, 1 month, 2 months, 4 months and 6 months. The samples underwent to physicochemical stability study. The following parameters were evaluated during accelerated stability study for dried PE: the content of bacoside-A (using the developed RP-HPLC method) and different flowability parameters. Further, the standard of bacoside-A was subjected to mass analysis initially and at the end of the

accelerated stability study with a thought to predict the degradation pathway of bacoside-A. The tablets were evaluated for the content of bacoside-A, hardness, friability, disintegration time and weight variation test during accelerated stability study **[23]**.

RESULTS AND DISCUSSION

DEVELOPMENT OF STABILITY INDICATING HPLC-DAD METHOD

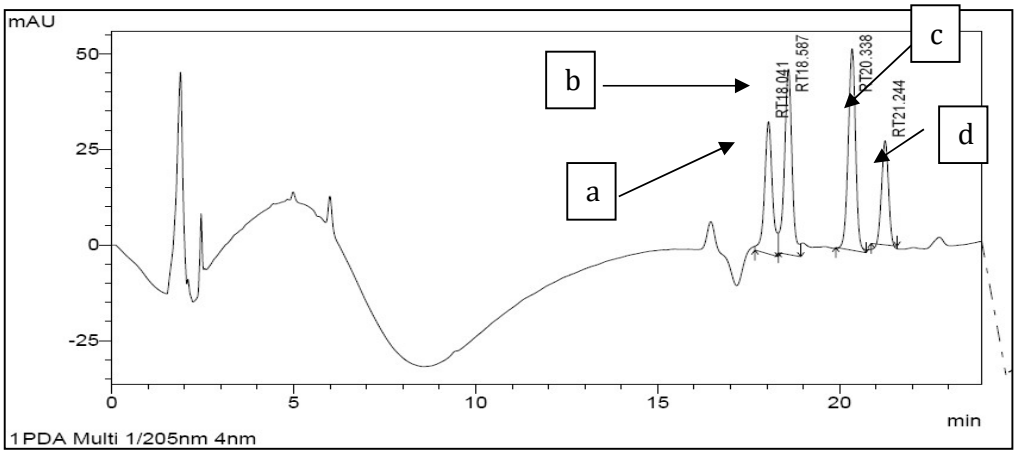
The prepared solution of standard bacoside-A (200 µg/mL) was scanned in the range of 200-800 nm, and a wavelength maximum was found to be 205 nm. For resolving peaks of bacoside-A, the combination of acetonitrile and phosphoric acid were tried in isocratic and gradient mode. The conditions for HPLC analysis in gradient model were the same as aforementioned to give resolved peaks of bacoside-A. The bacoside-A is a mixture of bacoside-A_3, bacopaside II, jujubogenin (isomer of bacopa saponin C) and bacopa saponin C **[24-28]**. The chromatograms of standard bacoside, PE and TE **(Figure 2)** were resolved for all four peaks along with average run time; bacoside-A_3 (18.01min), bacopaside II (18.58 min), jujubogenin (20.33 min) and bacopa saponin C (21.34 min). The method was further applied to determine bacoside-A from samples collected during accelerated stability testing. The optimised mobile phase could practically resolve bacoside-A from other constituents of the market formulation as well as plant extract and degradants.

VALIDATION OF THE DEVELOPED HPLC-DAD METHOD

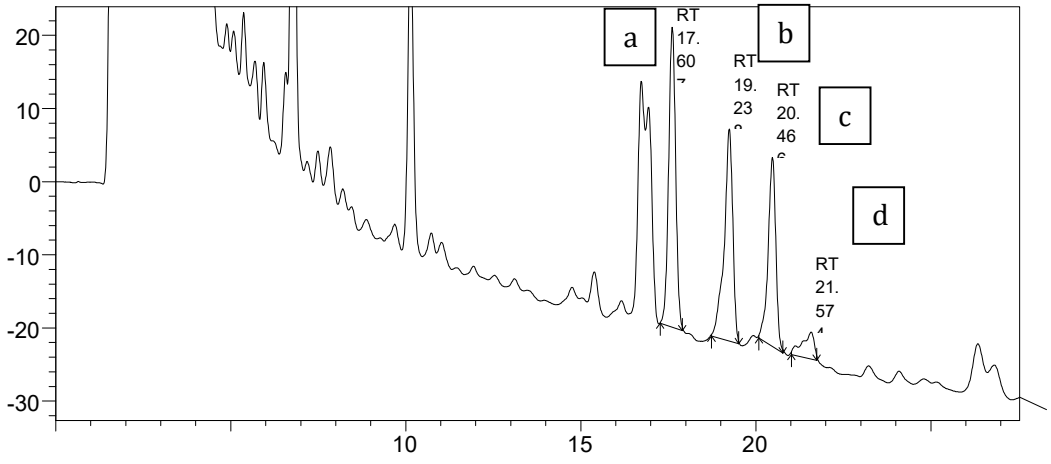
The overlain spectra of pure bacoside-A and BTE were recorded for the determination of peak purity. The purity of the bacoside-A peak was determined in the overlain spectra by measuring correlation coefficients at different positions; at the start (s), middle (m), and end (e) of the peak. The values of coefficients were found to be r (s, m) 0.997 and r (m, e) 0.999 for standard bacoside-A whereas, for BTE, they were r (s, m) 0.999 and r (m, e) 0.999. Hence, it can be put forward that other components present in the sample solution were not interfering with the resolution of bacoside-A in case of the marketed formulation. Moreover, the run time of peaks recorded in PE was also matching with that of bacoposide-A. The linear regression analysis for the calibration curves showed a linear relationship over the concentration range of 100-500 µg/mL for bacoside-A with respect to the mean peak area for all four individual peaks.The data for method validation is mentioned in **Table 1**. The developed method was found to be linear and the result of regression analysis are shown in **Table 1**.

The precision is performed to determine the degree of reproducibility or repeatability of the analytical method. It indicates an

indiscriminate error. The precision of the analytical method is generally expressed as % relative standard deviation (%RSD) or coefficient of variance (CV) of successive measurements made. For determining repeatability, the mean area was calculated after injecting 300 µg/mL concentration of bacoside-A six times during a day and reproducibility was established by injecting three different concentrations 200 µg/mL, 300 µg/mL and 400 µg/mL (n=3) on six consecutive days. The peak areas were recorded after each injection. Then mean peak areas were calculated, and % RSD of peak areas were determined for both the studies. The % RSD values were indicative of precise method. Accuracy is an indication of the systematic error of an analytical method.



(I)



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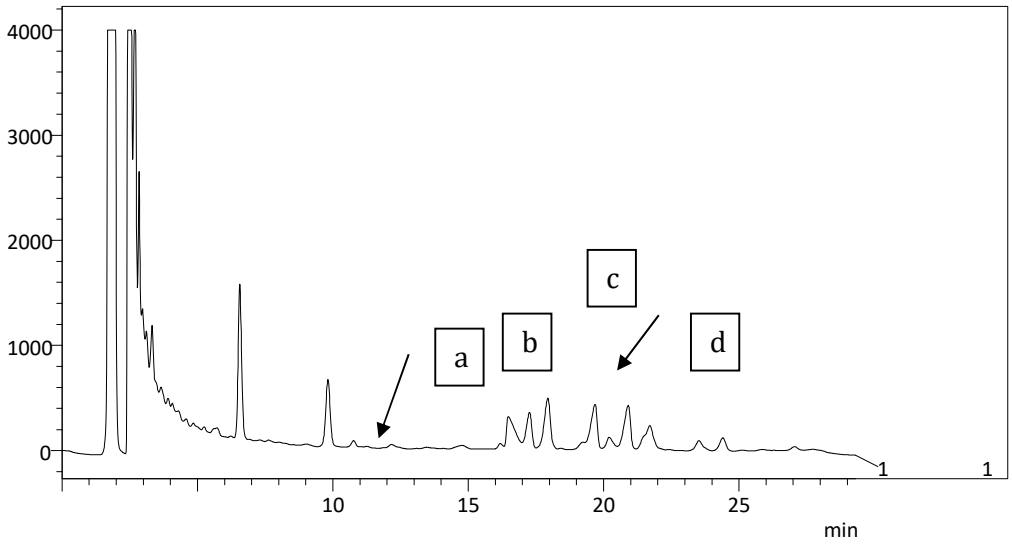

(III)

Figure 2: Chromatograms obtained by LC-DAD of (I)Standard Bacoside-A, (II) Bacopa Monnerie Extract and (III)Tablet,(a) Bacoside-A_3, (b) Bacopaside-II, (c) Jujubogenin isomer of bacopasaponin C and (d) Bacopasaponin C

Table 1: Validation of developed stability indicating HPLC method

Parameters	bacoside-A_3	bacopaside-II	Jujubogenin (isomer of bacopa saponin C)	bacopa saponin C
Linearity*	y=1013x- 44925, r^2=0.999	y= 1213.3x-63338, r^2=0.992	y=1467.2x-49640, r^2=0.991	y=801.29x-27588, r^2=0.995
Precision (Repeatability)	1.71	2.58	2.85	2.51
Inter day Precision	1.34	2.54	2.70	2.04
Intraday Precision	1.11	2.13	2.52	2.11
Limit of Detection (LOD) (µg/mL)	48.35	125.89	134.96	104.67
Limit of Quantitation (LOQ) (µg/mL)	146.52	381.48	408.98	317.19

***y= mean peak area (n=6) and x= concentration of respective components and r^2= regression coefficient.**

For determination of accuracy, the peak areas of all four peaks (n=3) were considered after injecting 500 µg/mL of bacoside-A using both the methods; and paired t-test was applied to the data. The p-value was found to be 0.614 suggesting no significant difference between available and developed stability indicating RP-HPLC method for estimation of bacoside-A. Hence, the developed method can be considered as accurate.

Accuracy of analysis is determined by calculating the systematic error involved. It was determined by calculating the recovery of the bacoside-A. The amount of bacoside-A was calculated using the corresponding calibration curve equation, and the recovery was found to be in the range of 98.3-101.8% for plant extract and 98.5-102.67% for marketed formulation (n = 3).

Robustness of the method was checked by recording peak areas of bacoside-A under slightly modified experimental conditions i.e., flow rate (1.5± 0.2 mL/min) and wavelength of detection (205 ± 2 nm). The % RSD was calculated for each factor and

found to be 1.02 and 0.53, respectively. The lower % RSD values indicated that the developed method is robust. The obtained LOD and LOQ values for bacoside-A_3 were 48.35 and 146.25, bacopaside-II were 125.89 and 381.48, Jujubogenin (isomer of bacopa saponin C) were 134.96 and 408.98 and bacopa saponin C were 104.67 and 317.19 respectively.The values of LOD and LOQ denoted the sensitivity of the method to efficiently detect and quantify lower amount of bacoside-A in the sample.

FORCED DEGRADATION STUDY FOR PURE BACOSIDE-A, PLANT EXTRACT AND MARKETED FORMULATION

The substantial amount of bacoside-A was degraded in acidic and alkaline medium. The amount of bacoside at the different temperatures was determined at regular intervals from 10 min to 80 min in both the cases.Bacoside-A belongs to O-glycoside linkage and its rapid degradation leads to the fragmentation of aglycone and sugar moieties **[29]**. It was found that the rate of degradation was increased(30-80%) as the temperature was increased in both conditions. The oxidative degradation study revealed 8.12%, 9.2%, and 7.98% degradation of bacoside-A in pure bacoside-A, plant extract, and tablets. The photolytic degradation was found to be <5% for all three samples.

DETERMINING THE RATE OF REACTION FOR DEGRADATION

The value of activation energy for acid degradation of bacoside-A was found to be 32.84 and 33.45 J/mole, respectively when calculated by equation and graph method. The value of activation energy for acid degradation of tablets of bacoside-A was found to be 22.94 and 23.43 J/mole respectively when calculated by equation and

graph method. The value of activation energy for alkaline degradation of bacoside-A was found to be 34.69 and 44.98 J/mole, respectively, while calculated by equation and graph method. The value of activation energy for alkaline degradation of tablets of bacoside-A was found to be 24.48 and 32.46 J/mole, respectively, while calculated by equation and graph method. The activation energy was used to predict degradation constants at different temperatures. The graph of the logarithm of percentage drug remaining versus time (min) was plotted. This data may used to predict the stability of the drug at different temperatures, provided the rate of reaction remains unaltered. As it showed a straight line, it can be concluded that the degradation reaction followed first-order kinetics **[30-31]**.

Table 2: Accelerated stability study of standard bacoside-A, plant extract and marketed formulation (Tablets)

Parameters	Sampling time (n=3)					
	0 Day			After 6 months		
	Standard of bacoside-A	Plant extract of *B. Monnerei*	Tablets of *B. Monnerei*	Standard of bacoside-A	Plant extract of *B. Monnerei*	Tablets of *B. Monnerei*
Description	Brown in colour	Blackish brown in colour	Brown uncoated tablets	Light brown in colour	Light brown in colour	Light brown uncoated tablets
% Friability	Not applicable	Not applicable	0.98±0.26	Not applicable	Not applicable	0.8±0.51
% Moisture content	7.48±0.79	10.69±0.41	8.98±1.71	9.65±0.94	14.12±1.71	11.36±1.53
Disintegration time (min)	Not applicable	Not applicable	11.2±0.86	Not applicable	Not applicable	10.3±0.59
Content of bacoside-A (mg)	200±0.71	200.3±0.88	200±1.91	159.64±0.79	119.54±0.86	135.74±2.03

ACCELERATED STABILITY STUDY OF PURE BACOSIDE-A, PLANT EXTRACT AND MARKETED FORMULATION

The standard bacoside-A, dried methanolic plant extract, and marketed formulation *(tablets)* were tested for their stability as per ICH guidelines. They were evaluated for change in appearance, moisture content, and content of bacoside-A, whereas friability, hardness, and disintegration time were evaluated only for tablets. The colour of the PE and tablets fed upon storage, as mentioned in **Table 2**.

The moisture content was determined using the gravimetry method wherein the increase in weight of tablets and the dried extract was measured initially and after 6 months. But it was observed that a gain of 50% weight in dried

methanolic extract and 32% weight in tablets within a short period of a week. The results were in synchronisation with the study reported by Watto Phrompittayarat and co-authors **[32]**. The probable reason behind the increase in moisture content at high temperatures could be the presence of a high amount of sugar moieties in Brahmi plant extract. Moreover, the tablets showed a reduction in disintegration time, decreased friability, and hardness due to increased moisture content **(Table 2)**.

Henceforth, we also concluded that the Brahmi extract and tablets are hygroscopic in nature and should be stored at low temperature and dry conditions or low humidity. The content of bacoside-A was estimated from pure standard bacoside-A, dried methanolic plant extract, and Brahmi tablets using the developed HPLC method. The content of bacoside-A in standard bacoside-A, plant extract, and tablets was reduced after 6 months. The bacoside-A was resolved into four different peaks: bacoside-A3, bacopaside II, jujubogenin (an isomer of bacopa saponin C), and

bacopa saponin C. We were able to draw a conclusion that in all three samples, all four components were susceptible to moisture degradation at 40° C and 75% RH. The bacopa saponin C was reduced in the highest amount in comparison with the other three components of bacoside-A. The probable reasons could be either due to interaction of bacopa saponin C with some other components of extract, or it may interact to a greater extent with excipients of tablets and may lead to the formation of newer complex/structures which may be non-absorbing in nature.

It was found that upon degradation, three sugar moieties were separated from bacoside-A, which is considered to be responsible for degradation by hydrolysis. This may lead to the generation of unwanted/undesirable effect in patients as the biological activity of saponins are closely related to their structure **[33-34]**.

Overall, the reduction in the amount of bacoside-A in tablets was less as compared to whole-plant extract and standard of bacoside-A. This could probably be due to the compression of plant extract, the addition of stabilisers/anti-oxidants while formulating a tablet under a controlled environment. Further, the selection of excipient can also have a remarkable impact on the amount of bacoside-A.

Hence, in order to get a stable formulation of Brahmi, the choice of dosage form could be a coated tablet or capsule, as both of them may prevent moisture absorption and subsequent degradation of the biomarkers of Brahmi. The formulations should be manufactured at ambient temperature and low humidity for preserving biomarkers to exhibit the desired pharmacological action of *B. Monneiri.*

CONCLUSIONS

The bacoside-A is an important triterpenoid saponin of *Bacopa Monnieri* responsible for cognition enhancement. The developed method was validated as per ICH guidelines and found to be effective in resolving the peak of bacoside-A from other degradation products of the plant extract and formulation. Bacoside-A was found to be more sensitive towards acidic and alkaline degradation than photolytic and oxidative degradation conditions. The degradation of bacoside-A was observed to follow first-order kinetics during acidic and alkaline hydrolysis. It was concluded from the accelerated stability study that, on plant extract and tablets of Bacopa *Monnieri* that the bacoside- A content was reduced substantially after a period of 6 months. The mass spectrum of bacoside-A revealed that it contains a high amount of sugar moieties responsible for moisture absorption and ultimately its degradation. Henceforth, the accelerated and forced degradation studies revealed that pH, temperature, and moisture content are

critical process parameters while designing of herbal products containing *Bacopa Monneiri.*

CONFLICT OF INTEREST

There are no conflicts to declare.

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