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RESEARCH ARTICLE

Comparative Evaluation of Protecting Affordability of Flexible Laminates to *Bacopa monnieri* Powder Extract

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Abstract

Background: *Bacopa monnieri*, a medicinal herb, is widely used for its cognitive-enhancing properties. The stability of its active component, Bacoside-A, in commercially available formulations, is influenced by packaging materials. High-Performance Thin-Layer Chromatography (HPTLC) is a reliable technique for the quantification of Bacoside-A. **Aim:** This study aims to validate the HPTLC method for the quantification of Bacoside-A in commercially available formulations and to evaluate the impact of packaging materials on the stability of these formulations. **Methods:** The HPTLC method was validated following the ICH Q2 (R1) guidelines. Validation parameters, including linearity (100–500 µg/ml), precision, accuracy, robustness, and sensitivity, were assessed. Stability studies were conducted on commercially available *B. monnieri* formulations (tablets and powdered extracts) under specific conditions using six different packaging materials. Graphical analysis was used to evaluate degradation trends over time. **Results:** The validated HPTLC method demonstrated reliability for Bacoside-A quantification. Stability studies revealed degradation over time in both tablet and extract formulations. For powdered extracts, PET-12µ + MET.PET-12µ + MET.CPP (cast polypropylene) and PET-12µ + AL.FOIL-9µ (aluminium foil) was the most effective in preventing degradation. In tablet formulations, PET-12µ + MET.PET-12µ + POLY-85µ and PET-12µ + AL.FOIL-9µ + POLY-62µ provided superior stability. **Conclusion:** The validated HPTLC method is suitable for the quantification of Bacoside-A in *B. monnieri* formulations.

Major Findings: Stability studies highlight the essential role of suitable packaging materials in reducing degradation and preserving the long-term effectiveness of formulations. Packaging combinations such as PET-12µ + MET.PET-12µ + MET.CPP, PET-12µ + MET.PET-12µ + POLY-85µ, and PET-12µ + AL.FOIL-9µ + POLY-62µ provide enhanced product stability and protection.

Keywords: Aluminum Foil, *Bacopa monnieri*, Bacoside-A, Packaging Material

1. Introduction

Today, herbal formulations are extensively utilised worldwide, with a growing shift from allopathic medicines[1]. The widespread use of these herbal formulations has led to a substantial demand. However, many countries lack stringent guidelines, making reliance on these formulations questionable. The absence of specific guidelines for the extraction, formulation, and packaging of herbal materials raises concerns about potential degradation during storage[2]. While Low-Density Polyethylene (LDP) and Poly Vinyl Pyrrolidone (PVP) are commonly used as packaging materials for storing herbal products, there is a lack of

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data on the degradation of active constituents in the presence of these materials. Therefore, our objective is to identify an effective packaging material that ensures the stability of herbal formulations during storage. In the field of phytochemistry, it has been observed that constituents present in the drug may react with each other, posing challenges to the stability of such formulations[3]. Testing herbal products is challenging due to their classification as active substances, with defined therapeutic activities.

Furthermore, the conditions for storage and retest intervals, as well as recommended shelf life are crucial considerations. Chemical, physical, microbiological, and toxicological studies play a vital role in stability studies[4-6]. Herbal and Ayurvedic formulations are inherently poly-herbal, containing drugs/plants in various forms such as crude extracts. The stability of plant drugs poses a significant challenge, and predicting the shelf life of these drugs is difficult. In light of these challenges, the aim of this study is to identify a packaging material that effectively ensures the stability of herbal formulations during storage[7].

Numerous factors can impact the stability of drugs and the measurement framework, encompassing pH, temperature, solubility, light, air, carbon dioxide, humidity, and particle size. pH stands out as a crucial factor affecting product stability, with certain solutions being susceptible to pH alterations when in contact with glass. Solubility becomes a significant consideration for liquid or semisolid solutions, with certain plastics allowing solvent permeation[8-10].

The initiation of degradation reactions may occur with exposure to light, and some light-induced reactions follow zero-order kinetics. To safeguard light-sensitive substances, light-resistant packaging, particularly with aluminium, is recommended. Air exposure can induce degradation through oxidation[11]. Minimising headspace in containers, filling them as much as possible, or replacing headspace with nitrogen are strategies to mitigate degradation. Alternatively, incorporating antioxidants into the formulation is an additional option. Solid dosage forms may experience issues when carbon dioxide leads to the formation of insoluble carbonates, diminishing the dissolution and disintegration properties of the product. Tight packaging and maximising container fill reduce this condition[12-15].

Bacopa monnieri, extensively employed in Ayurved, an ancient Indian system of medication, has a history spanning many years. Referred to as *Brahmi*, the name is derived from the Hindu pantheon's mythical 'creator,' Lord Brahma[16-18]. This herb falls under the category of *medhyarasayana*, a substance utilised to enhance memory and intellect (*medhya*). It finds mention in various ancient Ayurvedic texts, including the 'Charak Samhita' dating back to the sixth century AD. In this text, *B.monnieri* is recommended in formulations designed for managing various mental conditions, Including issues like anxiety, impaired cognitive function, and difficulty in concentration. It is moreover suggested used for its diuretic properties and as a stimulant for the nervous system and the heart[19]. A general description of *Bacoside* is shown in Table 1 and the chemical structure of the mixture saponin (Bacoside- A) is shown in Figure 1[20-22].

2. Materials and Methods

The Bacoside-A (Purity 96.9%) reference standard used throughout the experiment was purchased from A Natural Remedies Pvt Ltd, Bangalore. Powder and *Brahmi* tablets were purchased from the market.

2.1 Chemical and Solvent

Methanol as Analytical Reagent (AR) grade made by Loba Chemicals Pvt. Ltd, Mumbai India, Ethyl acetate (as AR grade made by Loba Chemicals Pvt. Ltd, Mumbai India, Water (Millipore),

2.2 Instruments

Digital critical balance: Model number- AUX 220 made by Shimadzu Corporation, Japan,

Table 1. General description of Bacoside-A

Combination of Saponin	Bacoside A_3, Jujubogenin isomer of Bacopasaponin C, Bacopasaponin C, Bacopaside- II
Description	White amorphous powder
Solubility	Readily dissolves in methanol and ethanol, but exhibits insolubility in water.Top of Form
Melting Indicate	250.6˚C
Molecular Formula	$C_{41}H_{68}O_{13}$
Molecular Weight	768.9708
HPLC and TLC	In HPLC bacoside A gives four different peaks and in TLC gives a single spot.

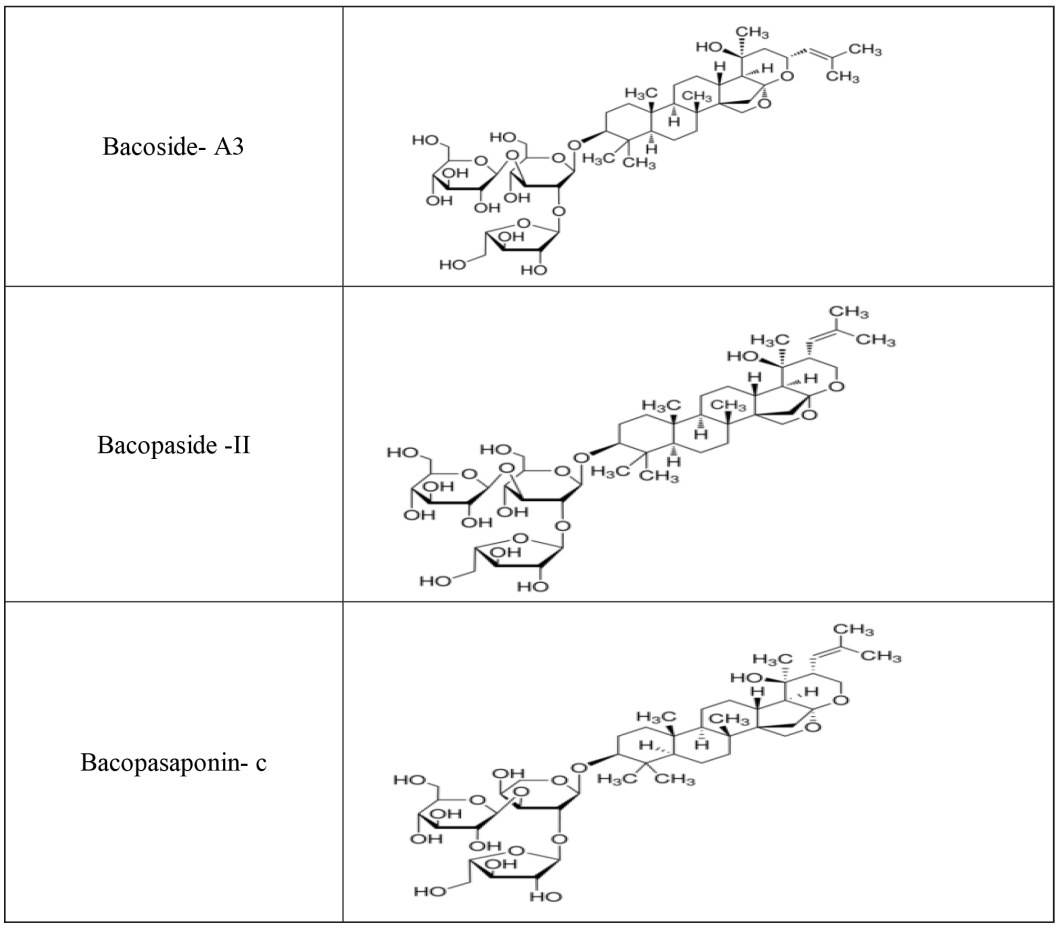


Figure 1. Chemical structure of mixture saponing (Bacoside-A).

UV- visible spectrophotometer: Model number- UV 1800 manufactured by Shimadzu Corporation, Japan, HPTLC system: Utilised instrumentation includes the Camag Linomat V for semi-automated application with band application through spray technique (2-500µL), a Hamilton Syringe (100µL), Camag Thin Layer Chromatography (TLC) Scanner IV with scanning speed up to 100 mm/s and a spectral range of 190-800nm, Camag twin trough chamber in 10 × 10cm and 20 × 10cm dimensions, a dual wavelength UV Cabinet offering wavelengths of 254nm and 366nm, and Camag win CATS software I.4.7.

2.3 Establishment and Validation of an HPTLC Technique for *Bacopa monnieri*

2.3.1 Preparation of Standard Stock Solutions

Bacopa monnieri standard stock solution concentration was 1000 µg/ml: perfectly weighed 1mg of the standard is dissolved into 1ml methanol which contains a final concentration of 1mg/ml of Bacoside A.

2.4 Development and Optimisation of HPTLC Method

2.4.1 Selection of Mobile Phase

- Choosing and optimising an appropriate mobile phase poses a formidable challenge in the development of HPTLC methods. Various factors influence the selection of the mobile phase, including the drugs' polarity, desired R_f values, and practical issues such as spot diffusion, tailing and achieving the right peak shape upon scanning.
- Various mobile phases were tested for the separation of Bacoside-A from *B. monnieri* powder. The optimal mobile phase determined for this purpose was a blend of ethyl acetate, methanol and water in the proportion of 7:2:1 v/v/v, respectively, which

resulted in improved separation of Bacoside-A from *B. monnieri*.

- Due to the weak chromophoric group in *B. monnieri*, derivatisation was employed using the Anisaldehyde sulfuric acid reagent for accurate visualisation of bands. The process involved immersing the plate in the derivatising reagent and heating it for 5 minutes at 110°C in a hot air oven.
- Various mobile phases with different compositions were experimented with for the quantification of Bacoside-A.

2.4.2 Selection of Detection Wavelength

The spots that were developed underwent scanning across a range of 200-700nm, and the spectrum of Bacoside-A was analysed to determine the appropriate detection wavelength.

2.4.3 Chromatographic Separation

A 10µl standard solution of Bacoside-A was applied to a TLC plate using a semi-automatic Apply the sample using a spotter while being exposed to a stream of nitrogen. The plate was then dry and placed in a twin trough chamber that had been pre-saturated for 10 minutes through the mobile phase of ethyl acetate: water: methanol, 7:1:2 v/v/v. Following improvement. The plate was allowed to dry, and Photometric readings were taken conducted at 540nm in absorbance mode using a TLC scanner-IV with win CATS Version 1.4.7 software, integrating the track optimisation feature.

2.4.4 Validation of Developed HPTLC Method

The validation of an analytical approach is the process through which, during laboratory assessments. It is confirmed that the method's performance characteristics satisfy the criteria for the designated analytical purpose.

2.5 Quality Test for Plastic Material

2.5.1 Tensile Strength Equipment used

Universal testing machine. Unit: kg/cm^2, Definition: maximum tensile stress sustained by test piece during strength of material to tensile loading. Significance t represents a mechanical characteristic in high-speed operations. Tensile properties serve as indicators of the strength of polymers and gauge the quality of a product as well as its design.

2.5.2 Coefficient of Friction Equipment used

Buchel Vander (Pendulum method) Unit: kg Definition: ratio of friction force to gravitational force acting perpendicular to the two surfaces in contact. Significance: Co-Coefficient of Coefficient of friction (COF) is defined as the highest value of the frictional force divided by the normal force.

2.5.3 Dart Impact Test

Tools utilized: Various weighted darts with accompanying stand. Significance: To evaluate impact resistance this examination assesses the resilience of the plastic film, employing a single dart configuration and drop height, with the dart weight as the variable.

2.5.4 Puncher Test: Equipment used

Puncher tester Unit: kg Significance**:** It quantifies the maximum force or energy necessary for material penetration. Puncture testing is employed to evaluate the strength of materials like film, rubber, or membranes.

2.5.5 Heat seal strength: Equipment used

Universal testing machine Unit: g/15mm width, **Significance**: It refers to the force needed to break a seal, crucial for ensuring the integrity of the packaging. Heat sealability is a critical property, especially when applied to packaging machinery.

2.6 Stability Study

An accelerated stability study was conducted on six chosen packaging laminates measuring 5*10 cm, filled with tablets and herbal extracts of *B. Monnieri*, following the conditions specified in the ICH Q1A(R2) guideline at 40°C and 75% RH. Sampling intervals were set at 7, 14, 20, 30, 60, 120 and 180 days. The HPTLC method developed was used for sample analysis, and the percentage degradation was calculated. A comparative analysis of the packages was performed to identify a suitable package for *B. monnieri*. Table 2 mentions the different types of packages with their compositions.

3. Results

3.1 Development with Optimisation of HPTLC Technique for Bacoside-A

3.1.1 Conclusion

The optimal polarity for the migration, separation, and resolution of Bacoside-A was achieved by utilising a blend of ethyl acetate, methanol, and water in the ratio of 7:2:1 (v/v/v) at room temperature with a 20-minute saturation time. This resulted in an R_f value of 0.65 ± 0.02.

3.2 Selection of Detection Wavelength

In the initial phase, the plates under development were scanned at various wavelengths. It was observed that at 540nm, the area corresponding to Bacoside-A was greater compared to that obtained at other wavelengths. Consequently, 540nm was chosen as the detection wavelength.

Table 2. Different types of packages

Package 1	12PET(polyethylene terephthalate)+50POLY
Package 2	12PET+12MET(metalized)PET+85POLY
Package 3	12 PET+12MET.PET + 25MET. CPP(cast polypropylene)
Package 4	12 PET+9AL.FOIL +62POLY
Package 5	12PET+25CPP
Package 6	PET+NYLONEFILM

3.3 HPTLC Condition

(i) Stationary phase: Pre-coated Silica gel G60 F254 aluminium sheets layers width 0.2mm, (ii) Mobile phase: ethyl acetate: methanol: water (7: 2: 1 v/v/v), Saturation duration in the chamber: 20 minutes Temperature: Ambient temperature Distance of migration: 80 millimetres.

3.3.1 Spotting Conditions

Band Width: 6 millimetres Gap between two bands: 10 millimetres Distance between two tracks: 12 millimetres spraying rate: 159 nanoliters per second.

3.4 Validation of HPTLC Method

3.4.1 Linearity

Bacoside demonstrated a strong correlation coefficient within the concentration series of 100-500 ng/spot. The analysis of linear regression, achieved by graphing the peak areas of the five analytes against concentration, exhibited excellent correlation coefficients (greater than 0.995), as depicted in Figure 2 (3D chromatogram view of different concentrations of the standard of Bacoside-A). The linearity data is shown in Table 3. Additionally, Figure 3 shows the calibration curve of Bacoside-A, Figure 4 displays the overlain linearity chromatogram and Figure 5 shows the correlation spectra of Bacoside-A- A and the sample.

Data for the repeatability of scanner peak area measurement is shown in Table 4. Data on the repeatability of sample application is shown in Table 5

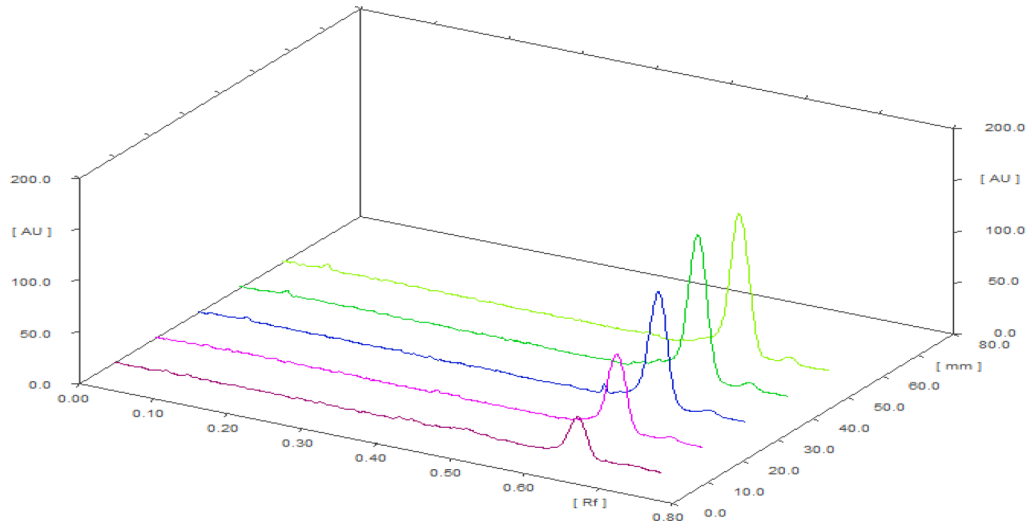


Figure 2. 3D chromatogram view of different concentrations of standard Bacoside-A.

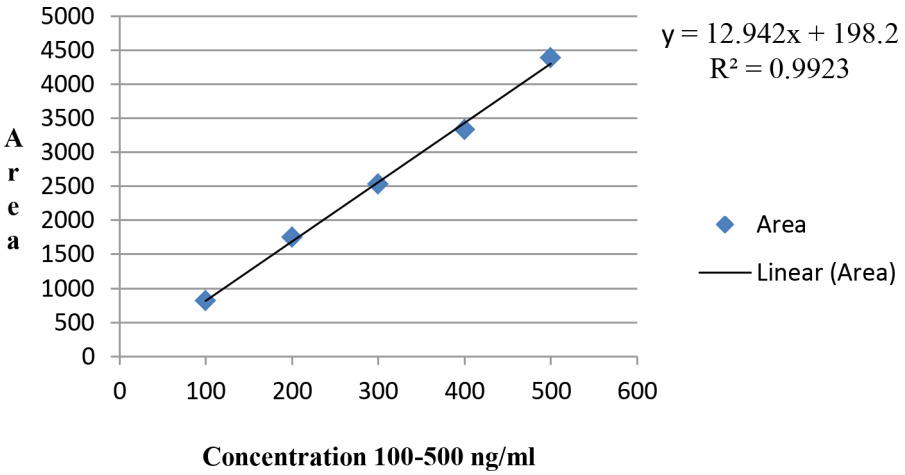


Figure 3. Calibration curve of Bacoside-A.

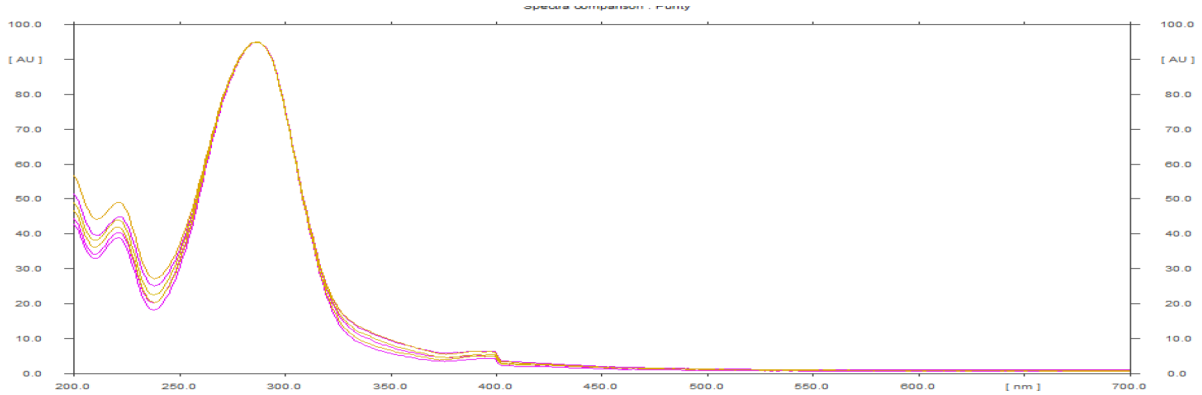


Figure 4. Overlain absorbance/reflectance spectra of Bacoside-A measured from 540nm.

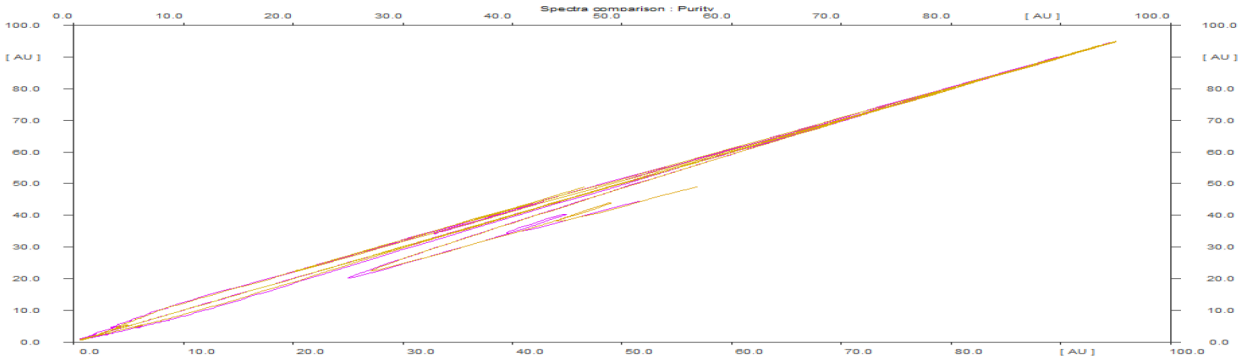


Figure 5. Correlation spectra of Bacoside-A and sample.

and the data on reproducibility for Bacoside-A is shown in Table 6.

From data on the repeatability and reproducibility of *B. monneri* it was found that the developed method was precise.

Inference: The % Reflex Sympathetic Dystrophy (RSD) was less than two hence the method is repeatable.

3.4.2 Selectivity and Specificity

The Overlay spectra of standard and tablet Bacoside A are shown in Figure 6.

3.4.3 LOD and LOQ Results

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) results for Bacoside-A are exposed in Table 7.

Table 3. Linearity data of Bacoside-A (n=5)

Bacoside-A		
Concentration (ng/spot)	**Peak Area Mean±S.D**	**% RSD**
100	823±9.67	1.17
200	1743±32.17	1.84
300	2527±79.19	1.21
400	3325±59.95	1.80
500	4389±39.25	0.89

Table 4. Data for repeatability of scanner peak area measurement

Conc.(ng/spot)	Peak Area	Mean± SD	% R.S.D
200	1790.0 1786.8		
	1785.7 1787.9	1771.85± 20.73	1.16
	1727.7		
	1740.4 1768.7		

Table 5. Data of repeatability of sample application

Conc. (ng/spot)	Spot No	Peak Area	Mean± SD	% R.S.D
200	1 2 3 4 5 6 7	1620 1640 1641 1597 1642 1625 1613	1625.42± 16.93	1.04

Table 6. Data of reproducibility for Bacoside-A

Concentration. (ng/spot)	Interday Precision		Intraday Precision	
	Mean area ± SD	% RSD	Mean area ± SD	%RSD
200	1432.66 ± 16.74	1.16	1226.33 ±21.82	1.77
300	2069.66±21.22	1.05	2910.33 ±64.01	2.19
400	3763 ± 44.19	1.17	3535 ± 31.27	0.84

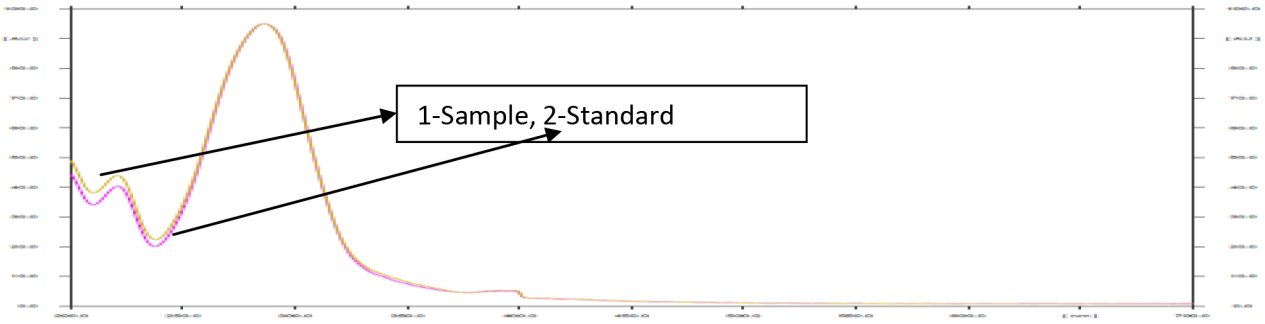


Figure 6. Overlay spectra of standard and tablet Bacoside-A.

Inference: LOD and LOQ results for Bacoside-A are exposed in Table 7 indicating sufficient sensitivity of the technique.

3.4.4 Accuracy Result for Bacoside-A

The result is shown in Table 8.

3.4.5 Robustness Study Data Result for Bacoside-A

The result is shown in Table 9.

4. Discussion

The proposed HPTLC method for the quantification of Bacoside-A was validated by the International Council for Harmonisation (ICH) Q2 (R1) guidelines. The method demonstrated linearity within the concentration range of 100–500 µg/mL. Additionally, validation parameters confirmed the method's precision, accuracy, robustness and sensitivity. The applicability of the method was successfully demonstrated across different commercially available formulations, including tablets and extracts.

5. Plastic Material

A range of flexible packages composed of different laminates was employed to assess their respective capacities in maintaining the chemical integrity of

selected herbal drugs under accelerated conditions. The investigations disclosed that packages constructed from the following materials [details to be added] exhibited noteworthy findings.

- PET-12µ (poly propylene terephthalate) + POLY-50µ (polyethylene).
- PET-12µ (polypropylene terephthalate) + MET-12µ(metalized).CPP (Cast Polypropylene) + POLY-55µ (polyethylene).
- PET12µ (polypropylene terephthalate) + 12MET12µ (metalized). MET (metalized). PET12µ (poly propylene terephthalate) + 25MET (metalized). CPP(Cast Polypropylene).
- PET-12µ (poly propylene terephthalate) + 9AL. FOIL-9µ
- (Aluminum foil) + POLY62µ(polyethylene).
- PET-12µ (poly propylene terephthalate) + CPP-25µ (Cast Polypropylene).
- PET (poly propylene terephthalate) + NYLONE FILM.

Table 7. LOD and LOQ

LOD (nanogram/spot)	0.62
LOQ (nanogram /spot)	2

Table 9. Robustness study data (n=6)

Parameters		Mean area ± SD	% RSD
Mobile Phase Ratio (±10%v/v) (Ethyl acetate: Water) (7:1)	Methanol 2.2	866±9.67	1.11
	Methanol 2	850±8.21	0.96
	Methanol 1.8	874±9.25	1.05
Saturation Tme (15 ±5 min)	20 min	821±9.22	1.12
	10 min	830±7.49	0.90
Dipping Time	30 sec	810±9.75	1.20
	1 min	820±8.95	1.09
Plate Heating	109^0C	815±7.85	0.96
	110^0C	850±8.59	1.22

Table 8. Accuracy result for bacoside-A (n=3)

Name of Compound	Sample Concentration	Std Concentration	Total Concentration	Amount Recovered	%Recovery	%RSD
Bacoside-A	100	50	150	148.23 147.48 140.48	98.82 98.32 93.65	2.14
	100	100	200	197.49 195.20 198.44	98.74 97.6 99.22	0.84
	100	150	250	248.11 240.35 247.48	99.24 96.14 98.99	1.74

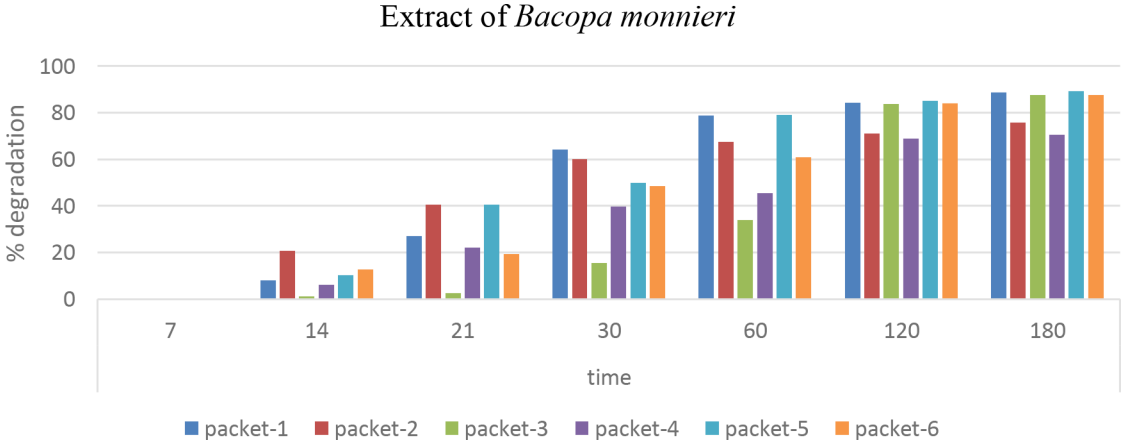


Figure 7. Time V/S % degradation of extract.

Table 10. Accelerated stability studies for extract

Area						
Duration	**Packet-1**	**% Degradation**	**Packet-2**	**% Degradation**	**Packet-3**	**% Degradation**
7 days	3131	0	2846	0	2612	0
14 days	2880	8.01	2256	20.73	2542	2.67
21days	2286	26.98	1695	40.44	2206	15.54
1 month	1121	64.19	1133	60.17	1836	29.70
2 month	665	78.76	927	67.42	1220	52.20
4 month	493	84.25	821	71.15	930	60.65
6 month	351	88.78	691	75.72	520	80.55
Area						
Duration	**Packet-4**	**% Degradation**	**Packet-5**	**% Degradation**	**Packet-6**	**% Degradation**
7 days	3643	0	2613	0	2951	0
14 days	3419	6.14	2345	10.25	2574	12.77
21days	2836	22.15	1553	40.56	2377	19.45
1 month	2199	39.63	1311	49.82	1520	48.49
2 month	1990	45.37	544	79.18	1166	60.82
4 month	1134	68.87	388	85.15	471	84.03
6 month	1074	70.51	278	89.36	364	87.66

Table 11. Accelerated stability studies for tablet

Area						
Time	**Packet-1**	**% Degradation**	**Packet-2**	**% Degradation**	**Packet-3**	**% Degradation**
7 days	14969	0	14059	0	16347	0
14 days	12464	16.73	13227	5.91	11903	27.18
21days	7108	52.81	12914	8.14	10703	34.52
30 days	6883	54.01	11538	17.93	10482	35.87
60 days	5513	63.17	10330	26.52	9964	39.04
120 days	5439	63.66	9356	33.45	6381	60.96
180 days	5222	65.11	8954	36.31	6221	45.22
Area						
Time	**Packet-4**	**% Degradation**	**Packet-5**	**% Degradation**	**Packet-6**	**% Degradation**
7 days	16823	0	16545	0	14166	0
14 days	16112	4.22	10788	34.79	13084	7.63
21days	15502	7.85	7459	54.91	11134	21.40
30 days	12706	24.47	6953	57.97	7843	44.63
60 days	11112	33.94	6384	61.41	5952	57.98
120 days	10329	38.94	5142	68.92	3209	77.34
180 days	10112	39.89	4892	70.43	2110	85.10

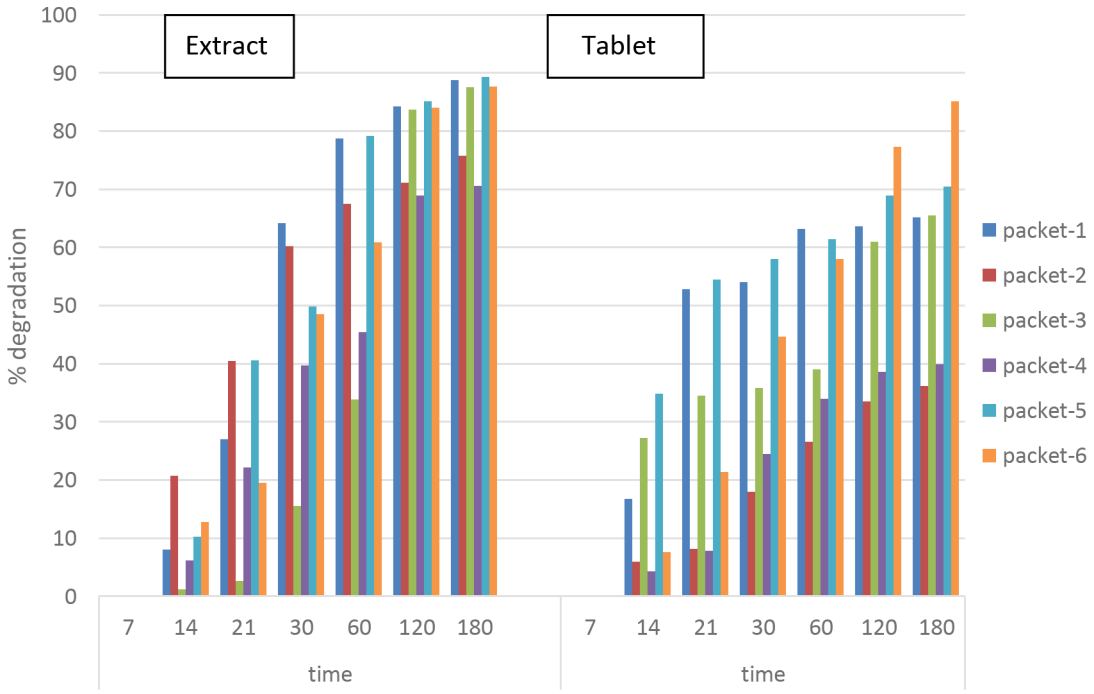


Figure 8. Overall degradation of different packaging at different times.

Table 12. Summary parameter for validation

No.	Validation Parameter	Result
		Bacoside -A
1	Linearity Range(nanogram/spot)	100- 500
2	Correlation Coefficient	0.9923
3	Scanner Repeatability	1.17%
4	Sample application(%R.S.D) Reproducibility (a) Intraday Precision (b) Interday Precision	1.04 (a)1.77 – 3.09% (b) 4.42 – 6.59%
5	Accuracy (% Recovery) Bacoside-A	93.82-98.99%
6	LOD (nanogram/spot)	0.62
7	LOQ (nanogram/spot)	2

There is no such reference value for laminates. The values depend only upon the micron size of the thickness and having different grades of plastics.

6. Stability Study Results

6.1 Accelerated Stability Studies for Extract and Tablet

Time V/S % Degradation of the extract is shown in Figure 7, Accelerated stability studies for the extract are shown in Table 10, Accelerated stability studies for the tablet are shown in Table 11, Overall degradation of different packaging in different times are shown in Figure 8 and the summary parameter for validation was shown in Table 12.

7. Conclusion

The suggested HPTLC technique for quantification of Bacoside-A The validation of the HPTLC method for analysing the commercially available formulation was conducted in accordance with the ICH Q2 (R1) guideline. The procedure exhibited linearity within the 100-500 µg/ml concentration range. Furthermore, the validation assessments confirmed the precision, accuracy, robustness, and sensitivity of the method. Successful application of the method was demonstrated on various forms of the commercially available marketed formulation, including tablets, and extracts. Formulations like tablets and extracts containing *B. monnieri* were subjected to stability study under specific conditions. Analysis data revealed with time, the degradation was observed in all two formulations. The results have shown that the rate of degradation was increased with respect to time. Six different packets placed for accelerated stability show degradation which is shown in the graph.

The graph shows that as time increases degradation occurs in both formulations but in tablet and extract formulations it was observed that for packaging material 12PET+12MET.12METPET+25MET.CPP and12PET+9AL.FOIL+62POLY were found to be a compatible packaging material for extract. Moreover, for tablet formulation 12PET+12MET.CPP+55POLY and 12PET+9AL.FOIL+62POLY packaging showed results. So concluded that 12PET+12MET.12METPET+25MET. CPP and12PET+9AL.FOIL+62POLY were found best for extract and tablet formulation 12PET+12MET. CPP+55POLY and 12PET+9AL.FOIL+62POLY packaging material shows good results.

8. Acknowledgement

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9. Author Contribution

All authors contributed to the study's conception and design. Mahavir kumar sharma conducted the experiments, analyzed the data, and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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