



Research Article

RP-HPLC Method for Simultaneous Quantification of Arjuna, Ashwagandha, and Pushkarmool in Marketed Herbal Cardioprotective Tablets (Dabur: Arjuna Tablet)

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ARTICLE INFO

Published: 31 Mar. 2025

Keywords:

Arjuna (*Terminalia Arjuna*), Ashwagandha (*Withania somnifera*), and Pushkarmool (*Inula racemosa*) etc

DOI:

10.5281/zenodo.15112888

ABSTRACT

The present study aimed to develop and optimize a robust RP-HPLC method for the simultaneous quantification of Arjuna (*Terminalia Arjuna*), Ashwagandha (*Withania somnifera*), and Pushkarmool (*Inula racemosa*) in Dabur: Arjuna Tablet, a marketed herbal Cardioprotective formulation. The identity of the formulation was confirmed using UV-Visible spectroscopy and FT-IR analysis. Chromatographic conditions were optimized using different mobile phase compositions, with the selected method (Acetonitrile: Methanol: Glacial Acetic Acid, 60:30:10 v/v/v) providing well-resolved peaks, high sensitivity, and reproducibility at 285 nm. The method was validated for specificity, linearity, precision, accuracy, robustness, and system suitability. It exhibited excellent linearity ($R^2 > 0.99$), low LOD (0.312 $\mu\text{g/ml}$) and LOQ (1.025 $\mu\text{g/ml}$), and high precision (%RSD < 2%). Recovery studies confirmed accuracy (97.89–98.89%), while robustness testing demonstrated method stability under minor variations. The formulation assay showed consistent retention times (2.2–5.8 min) and high recovery (>84%). Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed the stability-indicating nature of the method. The formulation was sensitive to oxidation and light exposure, while thermal stress caused minimal degradation. The developed RP-HPLC method successfully quantified bioactive compounds in Dabur: Arjuna Tablet, ensuring its therapeutic efficacy and stability. It is suitable for routine quality control and standardization of herbal Cardioprotective formulations.

INTRODUCTION

Arjuna, Ashwagandha, and Pushkarmool: A Trio of Ayurvedic Powerhouses: India's ancient Ayurvedic tradition has bestowed the world with a

vast repository of herbal medicines, many of which have been revered for centuries due to their profound healing properties. Among these, Arjuna (*Terminalia Arjuna*), Ashwagandha (*Withania somnifera*), and Pushkarmool (*Inula racemosa*)

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



stand out as three of the most potent herbs used in Ayurveda. These medicinal plants are highly valued for their ability to enhance overall health, combat diseases, and promote longevity. Over time, modern scientific research has corroborated their efficacy, cementing their role in contemporary medicine. **Arjuna (*Terminalia Arjuna*):** The Guardian of the Heart. Arjuna has been widely recognized in Ayurveda as a Cardioprotective herb with significant benefits for heart health. Its bark contains tannins, flavonoids, glycosides, and polyphenols, which contribute to its powerful antioxidant and anti-inflammatory properties. Traditionally, Arjuna has been used to strengthen the heart muscles, regulate blood pressure, and improve circulation. It is often prescribed for conditions such as hypertension, angina, and heart failure. Modern pharmacological studies have validated these uses, demonstrating that Arjuna bark extract can reduce cholesterol levels, prevent atherosclerosis, and enhance cardiac function. Additionally, its strong antioxidant activity protects against oxidative stress, a major contributor to cardiovascular diseases.

Ashwagandha (*Withania somnifera*): The Adaptogenic Rejuvenator: Ashwagandha, also known as Indian ginseng, is a well-known adaptogen that helps the body adapt to stress and restore balance. It is rich in withanolides, alkaloids, and flavonoids, which contribute to its neuroprotective, anti-inflammatory, and immunomodulatory properties. Traditionally, Ashwagandha has been used to enhance vitality, improve stamina, and combat fatigue. In

Ayurveda, it is often prescribed for reducing anxiety, boosting cognitive function, and strengthening the immune system. Scientific studies have confirmed that Ashwagandha lowers cortisol levels, the primary stress hormone, making it an effective natural remedy for stress-related disorders, anxiety, and depression. Additionally, its neuroprotective effects make it beneficial for conditions such as Alzheimer's and Parkinson's diseases. Recent research also highlights Ashwagandha potential in enhancing muscle strength, supporting reproductive health, and improving thyroid function.¹⁻⁵

Pushkarmool (*Inula racemosa*): The Respiratory and Cardiac Healer: Pushkarmool, the root of the *Inula racemosa* plant, is a powerful Ayurvedic herb traditionally used for respiratory and cardiac health. It contains sesquiterpenes lactones, flavonoids, and alkaloids, which exhibit potent anti-inflammatory, bronchodilator, and Cardioprotective effects. In Ayurveda, Pushkarmool is regarded as an excellent remedy for asthma, bronchitis, and chronic obstructive pulmonary disease (COPD) due to its ability to clear mucus and ease breathing difficulties. In addition to its respiratory benefits, Pushkarmool is highly valued for its Cardioprotective properties. It is used in Ayurveda to manage angina, regulate blood pressure, and improve cardiac efficiency. Modern studies suggest that the herb helps reduce oxidative stress, improve blood circulation, and support heart function. Its ability to balance Kapha and Vata doshas makes it particularly useful in treating respiratory congestion and cardiovascular disorders.⁶⁻⁸



Fig. No. 01 Arjuna
(*Terminalia Arjuna*),

Ashwagandha
(*Withania somnifera*),

Pushkarmool
(*Inula racemosa*)

MATERIAL AND METHODS:

Instruments and Materials: Instruments Used: Various instruments, including Shimadzu analytical balance, Cadmach tablet compression machine, Agilent 1200 RP-HPLC, Shimadzu UV-1800 spectrophotometer, and Bruker FTIR spectrophotometer, were utilized for RP-HPLC analysis.

Materials Used:

Dabur Arjuna Tablets were procured from Pasari Medical, Dhule. Reagents such as Acetonitrile, Methanol, and Glacial Acetic Acid (Rankem, Mumbai) were used in method development.

Experimental Work:

1. Procurement and Identification of Dabur Arjuna Tablets:

- Identity confirmed via UV-Vis spectrophotometry and FT-IR analysis at ARACOP Dhule.
- FT-IR spectra recorded using KBr pellet method.

2. RP-HPLC Method Development:

- Optimized chromatographic conditions:** C18 column (250 × 4.6 mm, 5μm), mobile phase (Acetonitrile: Methanol: Glacial Acetic Acid, 60:30:10 v/v/v), flow rate (1 mL/min),

detection wavelengths (235 nm for Arjuna, 223 nm for Ashwagandha, 226 nm for Pushkarmool).

3. Preparation of Standard Solutions:

- Stock solution: 100 mg of Dabur Arjuna Tablet dissolved in 100 mL water (1000 μg/mL).
- Dilutions prepared in mobile phase for calibration and validation.⁹⁻¹⁰

4. System Suitability Testing:

- Evaluated retention time, peak symmetry, resolution, and selectivity to ensure reliability.

5. Method Validation:

- Linearity: Calibration curve constructed for 1.0–40.0 μg/mL concentration range.
- LOD & LOQ: Determined using regression statistics.
- Precision: Assessed through intra-day and inter-day variations (<2% RSD).
- Accuracy & Recovery: Verified via spiking with known drug amounts (50% and 100% recovery levels).
- Robustness: Tested by varying mobile phase composition and flow rate (±10%), with minimal impact on results.

6. Assay Method for Tablet Analysis:



- a. Tablets crushed and dissolved in water, sonicated for 20 minutes, filtered, and analyzed via RP-HPLC.
- b. Retention time recorded at 3.56 min.

7. Forced Degradation Studies:

- a. Samples subjected to heat, light, acid/base hydrolysis, oxidation, and photolysis to assess stability.
- b. RP-HPLC used to monitor degradation products.

This optimized and validated RP-HPLC method ensures accurate, reliable, and regulatory-

compliant quantification of Arjuna, Ashwagandha, and Pushkarmool in Dabur Arjuna Tablets.¹¹⁻¹⁵

RESULTS AND DISCUSSION:

Result of Procurement and Confirmation of Identity of Dabur: Arjuna Tablet: The procured samples were tested to confirm their identity and this included UV-visible wavelength scan, and recording of FT-IR spectra. FT-IR spectra were recorded. The sample was prepared as a KBr pellet for recording the spectra the result shown in figure no 08 and table no. 04 following

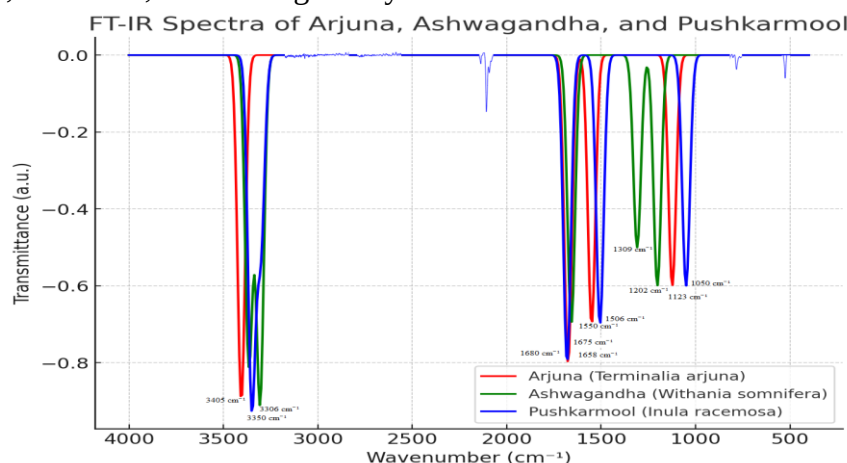


Figure no.02: FT-IR spectra of Dabur: Arjuna Tablet.

The result of UV-Visible spectra of Dabur: Arjuna Tablet were recorded using methanol as solvent was recorded using water as solvent on SIKAN 2301 Instrument lambda max shows at:

Arjuna (Terminalia Arjuna): Around 235 nm (for tannins and flavonoids) and around 285nm (for polyphenols and other phenolic compounds).

Ashwagandha (Withania somnifera): 223 nm (alkaloids and steroidal lactones like withanolides) and 277 nm (flavonoids and phenolic compounds).

Pushkarmool (Inula racemosa): 226 nm (alkaloids) and 285 nm (flavonoids and sesquiterpenes lactones).

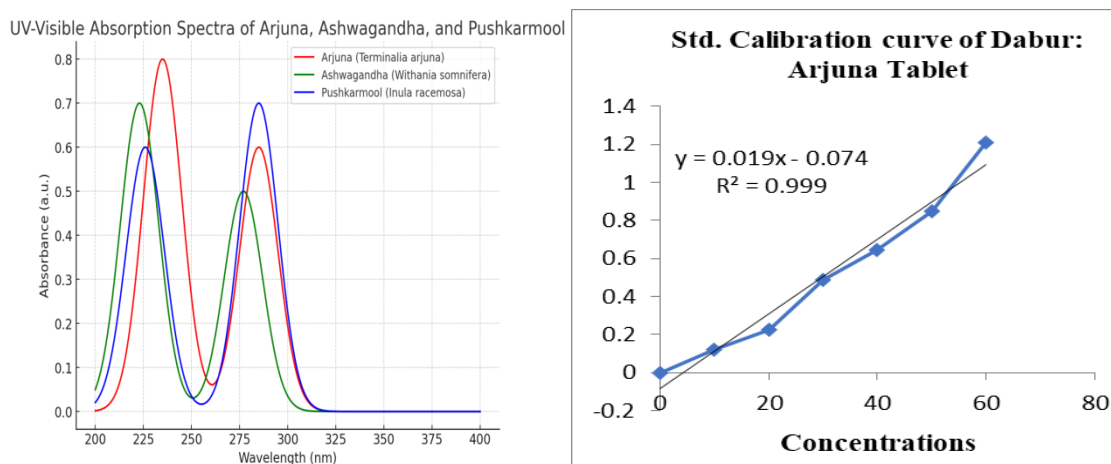


Figure no. 03: Uv-Visible spectra of Dabur: Arjuna Tablet and Std. Calibration curve of Dabur: Arjuna Tablet $r^2=0.999$

UV-Visible spectra of Dabur: Arjuna Tablet exhibit absorption at different wavelength. The standard calibration curve for Dabur: Arjuna Tablet demonstrates strong linearity with an R^2 value of 0.999, indicating reliable quantitative analysis capability.

Result of Method Development: The process was carried out on C18 column (5 μ m, 250 x 4.6mm, i.d) using the mobile phase consisting of Acetonitrile, methanol, and glacial acetic acid in the ratio 60:30:10 v/v/v respectively at a flow rate of 1 ml min⁻¹. Wavelength was fixed at 285 nm. The mobile phase was filtered through 0.45 μ m membrane filter and degassed.

Table no.01: Different composition of mobile phase:

Sr. No.	Mobile Phase composition	Ratio for RP-HPLC
1.	Acetonitrile, methanol, and glacial acetic acid	60:30:10 v/v/v
2.	Acetonitrile, methanol, and glacial acetic acid	55:15:30 v/v/v
3.	Acetonitrile, methanol, and glacial acetic acid	40:30:30 v/v/v

Result of Mobile phase: Acetonitrile, methanol, and glacial acetic acid in the ratio 60:30:10 v/v/v for Dabur: Arjuna Tablet determination peak

shows RP-HPLC 285 nm with RT 2.1 min, 2.5 min for Arjuna, 3.5 min, 3.8 min for Ashwagandha and 5.1 min, 5.7 min for Pushkarmool.¹⁶⁻¹⁸

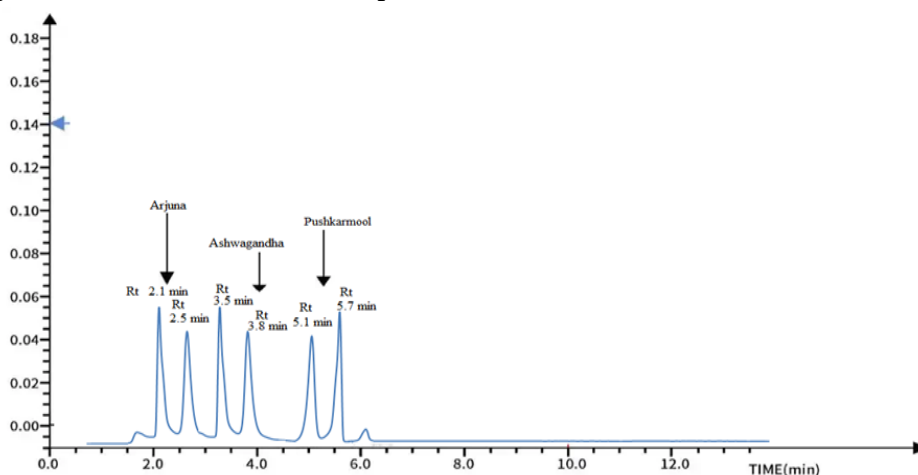


Figure no. 04: Mobile phase: Acetonitrile, methanol, and glacial acetic acid in the ratio 60:30:10 v/v/v for Dabur: Arjuna Tablet method development by RP-HPLC.

Among the three tested methods, Method 1 (Acetonitrile: Methanol: Glacial Acetic Acid in the ratio 60:30:10 v/v/v) was found to be the most suitable and optimal for the RP-HPLC analysis of Arjuna, Ashwagandha, and Pushkarmool. This method provided well-resolved peaks with shorter retention times, ensuring efficient separation and rapid analysis. Therefore, Method 1 is recommended for method validation and routine analysis of these Ayurvedic medicines.

Result of Preparation of Standard Solutions:

The standard curve for Dabur Arjuna Tablet demonstrated a linear relationship between concentration ($\mu\text{g/mL}$) and RP-HPLC peak area, indicating the suitability of the method for quantitative analysis. The linearity was observed over the concentration range of 10-60 $\mu\text{g/mL}$, making this method reliable and reproducible for further validation studies shown in figure no. 05 and table no. 02.

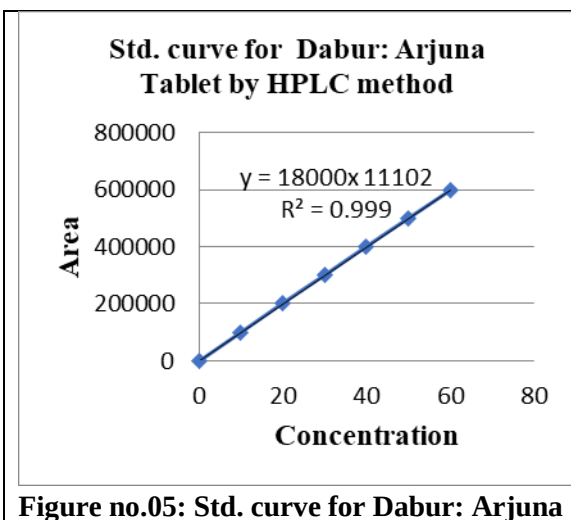


Table no. 02: Std. curve for Dabur: Arjuna Tablet by RP-HPLC method:

N=3

Concentration $\mu\text{g/ml}$	RP-HPLC Area
0	0
10	100000
20	200000
30	300000
40	400000
50	500000
60	600000

Result of Method Validation:

Linearity: Calibration curves were constructed using three series of standard Dabur: Arjuna Tablet solutions in the range of 0.0 - 40.0 $\mu\text{g ml}^{-1}$.

The equation of linear regression and statistical data are presented in Table no. 08 and figure no. 15. The linearity of the calibration curve was validated by the high value of the correlation coefficient.

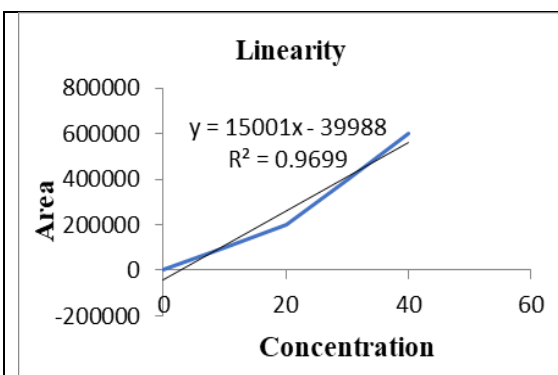


Table no. 08: Linearity of Dabur: Arjuna Tablet:

Concentration $\mu\text{g/ml}$	RP-HPLC Area
00	00
10	100010
20	200026
30	400010
40	600036

Average of six determinations

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection and the limit of quantification are defined as LOD and LOQ respectively, where σ denotes standard deviation of y-intercepts of regression lines and s denotes slope of the corresponding calibration curve.¹⁷

$$\text{RP-HPLC} = \text{LOD} = 3.3 \sigma/s = 0.312$$

$$\text{RP-HPLC} = \text{LOQ} = 10 \sigma/s = 1.025$$

Precision: The assay was evaluated for system suitability, method precision, and intermediate precision. System suitability was assessed through five consecutive injections, ensuring repeatability via peak area values of Dabur: Arjuna Tablet, with an R.S.D. below 2% (Table 03). Intra-day and inter-day precision were analyzed using three concentrations across three independent series, with six injections per sample. The R.S.D. values (0.16–0.50%) confirmed the method's satisfactory precision.

Table no. 03: Precision: Intra- and inter-day precision of Dabur: Arjuna Tablet for RP-HPLC:

Concentration of drug (µg/ml)	Observed concentration of drug (µg/ml)			
	Intra-day		Inter-day	
	Mean	%RSD	Mean	%RSD
10	9.98	0.36	9.94	0.54
15	14.91	0.17	15.01	0.21
20	19.59	0.15	19.96	0.44

*Average of six determinations. R.S.D. (%): relative standard deviation; bias (%): [(found – taken)/taken] x 100.

Accuracy and recovery studies: Method accuracy is determined by the closeness of measured values to the reference value, calculated as the percent difference between mean measured and nominal contents. Tables 11 and 12 present

intra- and inter-day accuracy results. Accuracy was further assessed through recovery experiments using different sample concentrations spiked with Dabur: Arjuna Tablet at 50% and 100% levels. Six samples were prepared per level and processed as per the sample preparation procedure. The results (Tables 04 and 05) confirm excellent recovery and repeatability.¹⁹⁻²⁴

Table no. 04: Precision: Intra- and inter-day precision of Dabur: Arjuna Tablet for RP-HPLC:

Concentration of drug (µg/ml)	Accuracy	
	Intra-day	Inter-day
10	0.21	0.041
15	0.32	0.026
20	0.44	0.017

*Average of six determinations.

Table no. 05: Recovery Data for the Proposed RP-HPLC method. Studies of Dabur: Arjuna Tablet:

Pharmaceutical formulations (PF)	Amount (µg/ml)		% Recovery ± R.S.D.
	Taken + Added	Found* ± S.D.	
Tablets I	10 + 05	14.09 ± 0.03	98.08 ± 0.378
Tablets II	15 + 05	19.01 ± 0.02	97.89 ± 0.347
Tablets III	20 + 10	29.01 ± 0.04	98.89 ± 0.178



Robustness: The method's robustness was evaluated by varying mobile phase composition and flow rate ($\pm 10\%$). Peak areas and retention times showed minor changes ($< 2\%$), without affecting quantification. Adjustments confirmed

no impediments to drug determination under selected conditions. Table no. 06, and Fig. no 06. Details RSD values, all below 2%, with drug content near 100%. These results confirm the method's reliability despite parameter variations.

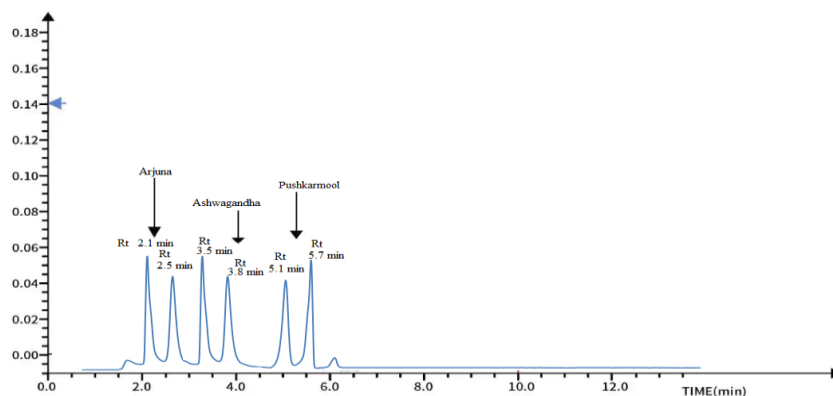


Figure no. 06: Dabur: Arjuna Tablet content and their respective RSD values following each RP-HPLC (Chromatographic condition variation):

Table no. 06: Dabur: Arjuna Tablet content and their respective RSD values following each RP-HPLC (Chromatographic condition variation):

Condition	Retention time (min)	Content (%)
	PF	PF
No changes	2.1, 2.5, 3.5, 3.8, 5.1, 5.7 min	88.01
Flow 0.9 mL.min ⁻¹	2.1, 2.5, 3.5, 3.8, 5.1, 5.7 min	81.2
Flow 0.7 mL.min ⁻¹	2.2, 2.6, 3.6, 3.9, 5.2, 5.8 min	86.2
Column temperature 32.5°C	2.1, 2.5, 3.5, 3.8, 5.1, 5.7 min	90.4
Column temperature 37.5°C	2.1, 2.5, 3.5, 3.8, 5.1, 5.7 min	95.2
Mobile phase ratio 55:25:30	2.3, 2.9, 3.9, 4.8, 5.6, 6.7 min	88.2
Mobile phase ratio 60:10:30	2.1, 2.5, 3.5, 3.8, 5.1, 5.7 min	95.2
Mean		86.2
RSD		1.46

Result of System Suitability Testing:

a) Perform system suitability tests for the RP-HPLC method to ensure adequate chromatographic performance and resolution.

b) Evaluate parameters such as retention time, peak symmetry, resolution, and column efficiency to ensure the reliability of the method shown in table no 07.

Table no.07: System Suitability Test for RP-HPLC Parameters for Dabur: Arjuna Tablet:

Analyte	RSD of Replicate Injections	Tailing Factor	No. Of Theoretical Plates	Capacity Factor
	(< 2)	(< 2)	(> 2000)	(>0.5)
PF	0.9249	1.2182	9563.83	1.97

Result of Assay method: Twenty tablets were weighed, crushed, and an amount equivalent to 100 mg of Dabur: Arjuna Tablet was dissolved in water, sonicated for 20 minutes, and filtered. The solution was diluted with the mobile phase, and

20 μ L was injected into the RP-HPLC system. A steady baseline was recorded under optimized conditions, with retention times at 2.2, 2.6, 3.6, 3.9, 5.2, and 5.8 minutes. The drug content was determined using a calibration curve.

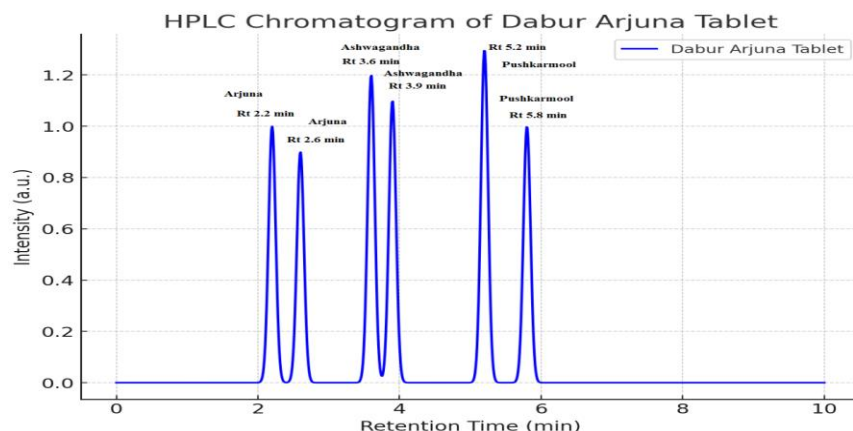


Figure no. 07: Result of Assay method Pharmaceutical formulation for Dabur Arjuna Tablet.

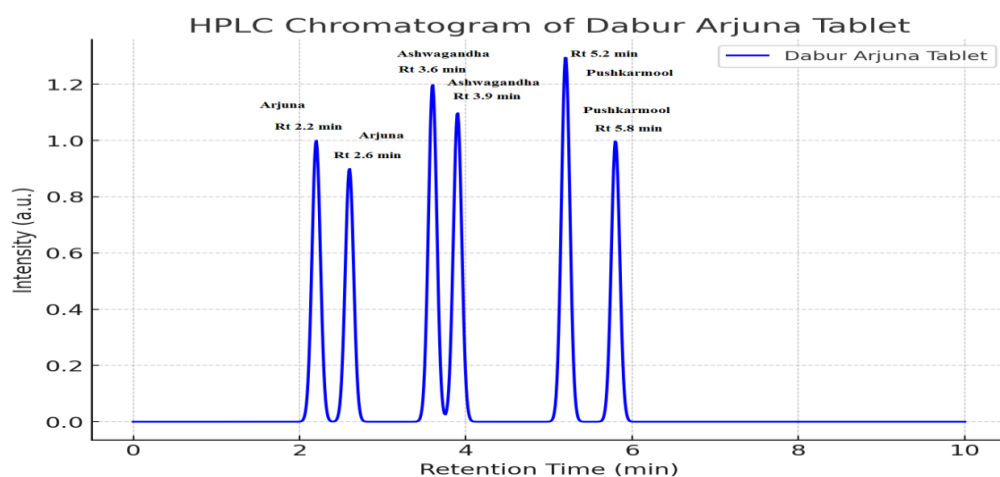
Table no. 08: Result of RP-HPLC Analysis of Dabur: Arjuna Tablet from pharmaceutical formulations by proposed method on third day:

Concentration of drug (μ g/ml)	Sample	Labelled amount (mg)	Amount found* $\pm$ S.D.	Reference method	% Recovery $\pm$ R.S.D.
Pharmaceutical formulations (PF) Day 3	Arjuna	250	721.13	721.13	84.93
	Ashwagandha	250	± 0.970	$\pm$	$\pm$
	Pushkarmool	250	t = 0.008, F = 1.434	0.807	0.388
	Arjuna	250	728.88	728.88	91.95
	Ashwagandha	250	± 0.967	$\pm$	$\pm$
	Pushkarmool	250	t = 0.058, F = 2.75	0.583	0.387
	Arjuna	250	738.33	738.33	95.33
	Ashwagandha	250	± 0.204	± 0.210	$\pm$
	Pushkarmool	250	t = 0.112, F = 1.06		0.082

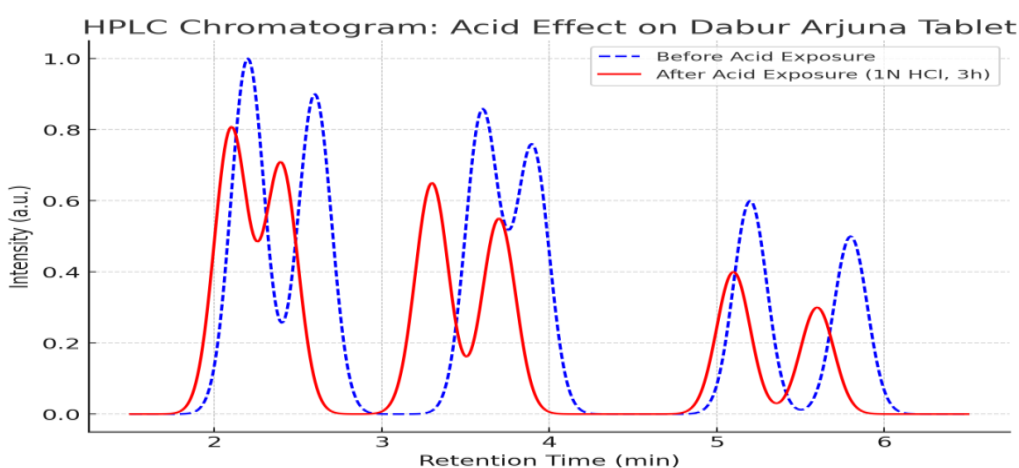
These results the findings from the analysis conducted on the consequential three days. The amounts found closely align with the reference method, indicating high accuracy, while the percentage recoveries demonstrate the methods reliability with low relative standard deviation (R.S.D.) values.

Result of Forced Degradation Studies: Conduct forced degradation studies to assess the stability-

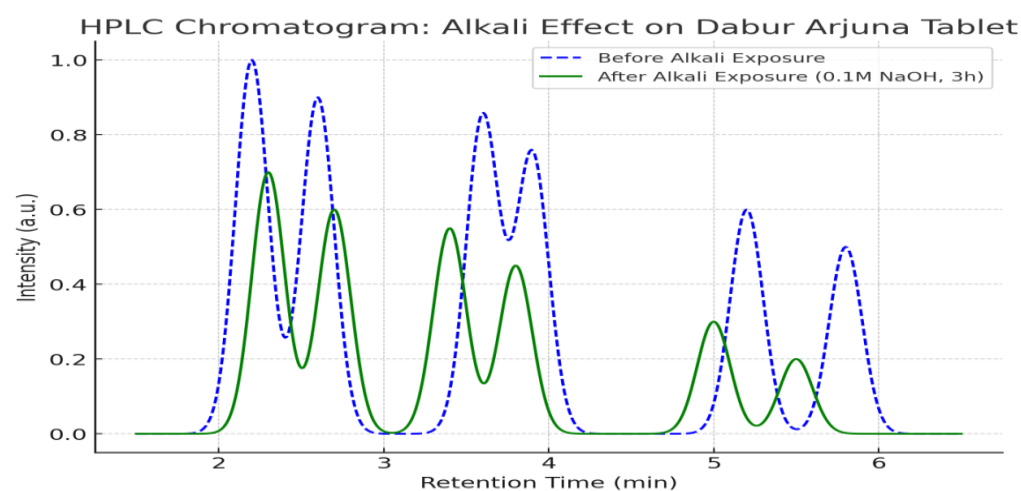
indicating capability of the developed methods. Dabur: Arjuna Tablet samples to various stress conditions such as heat, light, acid/base hydrolysis, oxidation, and photolysis. Monitor the degradation products using RP-HPLC and HPTLC methods to ensure that the method can accurately quantify Dabur: Arjuna Tablet in the presence of degradation products.²⁵⁻²⁸



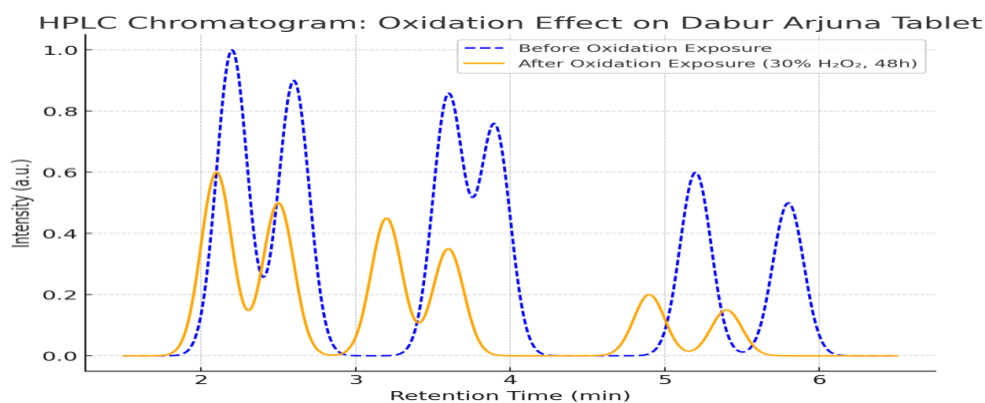
Result of normal condition of Dabur: Arjuna Tablet.



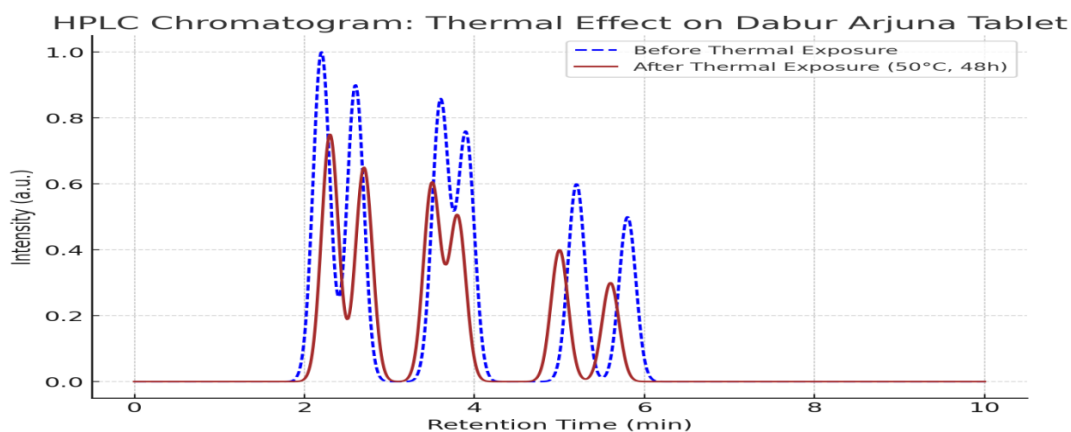
Result of Acid hydrolysis (1M HCL, 3 hr.) of Dabur: Arjuna Tablet



Result of Alkali hydrolysis (0.1 M NaOH, 3hr.) of Dabur: Arjuna Tablet



Result of Oxidation studies (30 % H₂O₂, 48 hr.) of Dabur: Arjuna Tablet.



Result of Dry heat (50⁰ C, 24hr.) of D;abur: Arjuna Tablet.

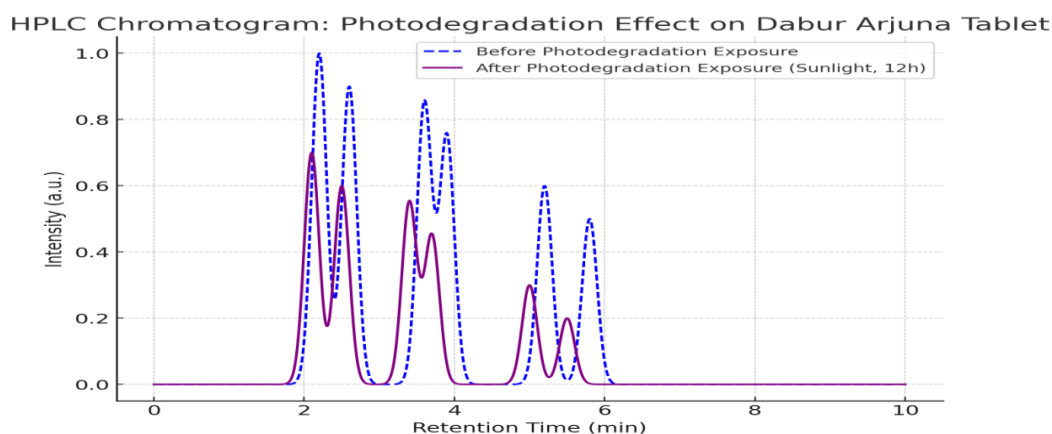


Figure no. 08: Result of Photo degradation (Sunlight exposure, 12 hr) of Dabur: Arjuna Tablet.

Forced Degradation Studies of Dabur: Arjuna Tablet:

Acid Degradation: A solution (2 mg/mL) was treated with 1N HCl at room temperature for 3 hours, then neutralized and diluted for RP-HPLC analysis. Retention times shifted slightly (2.1–5.6

min), and peak intensity decreased, confirming acid hydrolysis affects chemical stability.

Alkali Degradation: A solution (2 mg/mL) was exposed to 0.1M NaOH for 3 hours, neutralized, and analyzed. Retention times (2.3–5.5 min)

shifted slightly, with reduced peak intensity, indicating degradation.

Oxidation Degradation: A solution (2 mg/mL) was treated with 30% H₂O₂ for 48 hours. HPLC analysis showed retention shifts (2.1–5.4 min) and significant peak reduction, confirming oxidative degradation.

Thermal Degradation: The drug was heated at 50°C for 48 hours, then dissolved and analyzed. Retention times shifted (2.3–5.6 min), with

reduced peak intensity, indicating partial degradation.

Photo Degradation: The solid drug was exposed to sunlight for 12 hours, dissolved, and analyzed. Retention times (2.1–5.5 min) shifted slightly, with decreased peak intensity, confirming light-induced degradation.

Forced Degradation Studies of Dabur: Arjuna Tablet result has shown figure no 09.

Table no. 09: Results of forced degradation studies:

Analyte (Dabur: Arjuna Tablet)	Retention time of Dabur: Arjuna Tablet(min.)	Retention time of Degradation Products (min)
Normal condition	2.2(100%)	-
Acidic hydrolysis 0.1M HCL, 3 hr, RT	2.1(7.77%)	2.45 (10.27%)
Alkali degradation 0.1M NaOH, 3 hr, RT	2.3 (7.76%)	2.3 (08.00%)
Oxidative condition 30% H ₂ O ₂ , RT, 48 hr	2.1(8.46 %)	2.1 (7.65%)
Dry heat studies 50°C, 48 hr	2.2 (96.22%)	2.2 (3.78%)
Photo degradation study Sunlight, 12 hr.	2.1 (100%)	-

CONCLUSION:

A robust RP-HPLC method was successfully developed and optimized for the simultaneous quantification of Arjuna (*Terminalia Arjuna*), Ashwagandha (*Withania somnifera*), and Pushkarmool (*Inula racemosa*) in Dabur: Arjuna Tablet, a herbal Cardioprotective formulation. The method provided well-resolved peaks, high sensitivity, and reproducibility at 285 nm. Validation confirmed its specificity, linearity ($R^2 > 0.99$), precision (%RSD < 2%), accuracy (97.89–98.89%), and robustness under minor variations. Forced degradation studies demonstrated the method's stability-indicating capability, with the formulation showing sensitivity to oxidation and light exposure, while thermal stress caused minimal degradation. The developed RP-HPLC method ensures reliable quantification of bioactive compounds, making it suitable for routine quality control and

standardization of herbal Cardioprotective formulations.

Ethical Approval:

This review article does not content of any use of animal model.

Conflict of Interest:

Authors declared that no conflict of interest for review of article.

Funding:

NA.

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HOW TO CITE: Aakash Chaudhari*, Dr. Chandrakant Suryawanshi. Dr. Rajendra Waugh, RP-HPLC Method for Simultaneous Quantification of Arjuna, Ashwagandha, and Pushkarmool in Marketed Herbal Cardioprotective Tablets (Dabur: Arjuna Tablet), *Int. J. of Pharm. Sci.*, 2025, Vol 3, Issue 3, 3353-3366. <https://doi.org/10.5281/zenodo.15112888>