https://doi.org/10.47552/ijam.v16i1.5969 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (1), 2025; 184-190

Evaluation and method development for quantification of Piperine in *Balchaturbhadra Churna* by RP- HPLC

Research Article

Adithya Kumar C[1], Bhavya Sree P[2], Suresh Kumar SV[3*], Nageswara Rao R[1], Siva Sanker Reddy L[1], Ragamala KC[4]

1. Department of Pharmaceutical Analysis, 2. Department of Industrial Pharmacy, 3. Department of Pharmacognosy, Santhiram College of Pharmacy, Nerawada, Nandyal, Andhra Pradesh. India.
3. Department of Kaumarabhritya, SV Ayurvedic College & Hospital, Tirupathi, Andhra Pradesh. India.

Abstract

Aim and Objective: This study aimed to prepare *Balchaturbhadra Churna* in the laboratory and evaluate its Pharmacognostic, physicochemical, and phytochemical properties, including developing an RP-HPLC method for estimating Piperine as a marker compound. Methods: The prepared *Balchaturbhadra Churna* was analysed using WHO guidelines for macroscopic, physical, and chemical parameters. The methanolic extract was used for Piperine estimation through RP-HPLC. Results: The macroscopic characteristics, such as colour, odour, and taste were recorded. Physical parameters were determined, including loss on drying, ash value, extractive value, swelling index, foaming index, and powder properties (angle of repose, bulk density, tapped density, and compressibility index). Additionally, total phenolic, flavonoid and preliminary phytochemical screening were performed. The results were compared with a marketed formulation of Balchaturbhadra Churna. The retention time of standard Piperine was 4.50 minutes, while the Piperine in the laboratory and marketed formulations was detected at 4.478 and 4.480 minutes, respectively. The Piperine concentration in the laboratory and marketed formulations was 0.19% w/w and 0.21% w/w, respectively. The developed HPLC method was successfully validated. Conclusion: The laboratory-prepared and marketed formulations of *Balchaturbhadra Churna* were systematically evaluated and compared. The findings contribute to the standardization of Balchaturbhadra Churna, an Ayurvedic formulation.

Keywords: *Balchaturbhadra Churna,* Total phenolic content, Total flavonoid content, Preliminary phytochemical screening, Marker compound, RP-HPLC.

Introduction

Balchaturbhadra churna is an Ayurvedic formulation reported in The Ayurvedic Pharmacopoeia of India, 2007 (1). The ingredients of *Balchaturbhadra churna* (BC) include Mustaka (*Cyperus rotundus* Linn.), Ativisa (*Aconitum heterophyllum* Wall), Pippali (*Piper longum* Linn.), and Karkatshringi (*Pistacia integerrima* Stew). The Ayurvedic Pharmacopoeia of India mentions usefulness in treating diarrhoea, asthma, fever, emaciation in children (1).

The manufacturing of Ayurvedic formulations faces several challenges, including sourcing and verifying high-quality raw materials, establishing proper standards, implementing effective standardization methods for individual herbs and formulations, and maintaining quality control. Due to high demand and limited resources, manufacturers may resort to substitutes or adulterants, leading to lower-quality products. Additionally, product quality varies between manufacturers, and there is a lack of consistency in batch-to-batch production. This study aims to address these concerns.

A literature review revealed a lack of standardization data for *Balchaturbhadra Churna* concerning marker compound estimation using HPLC. While some studies reported the HPTLC estimation of Piperine in the formulation, comprehensive data remains limited. Therefore, this study focuses on formulating *Balchaturbhadra Churna* in a laboratory and standardizing it through organoleptic, physical, and chemical evaluations. Additionally, it aims to develop and validate a simple, rapid RP-HPLC method for estimating Piperine. The findings were then compared with those of a commercially available formulation to assess formulation similarity in terms of preparation and evaluation, as well as to validate the suitability of the developed method for Piperine estimation.

*** Corresponding Author:**
Suresh Kumar SV
Professor and Head, Department of Pharmacognosy, Santhiram College of Pharmacy, Nerawada, Nandyal 518112. Andhra Pradesh
Email Id: sureshsolleti@gmail.com

Materials and methods

Preparation of *Balchaturbhadra churna*

Balchaturbhadra churna consists of 4 ingredients: *Cyperus rotundus L.* (Mustaka), *Piper longum L.* (Pippali), *Aconitum heterophyllum Wall*

(Ativisa), *and Pistacia integerrima stew* (Karkatshringi).

All the ingredients were sourced from the local market. The identity of Mustaka, Karkatshringi, and Ativisa was verified by Dr. Sunita Garg, former chief scientist at the National Institute of Science Communication and Policy Research (CSIR-NIScPR), New Delhi. Pippali was authenticated at the Captain Srinivas Murthy Central Ayurvedic Research Institute in Arumbakkam, Chennai.

All the ingredients were thoroughly cleaned, individually powdered, and passed through sieve number 80. Each ingredient was weighed separately and blended in equal proportions to achieve a homogeneous mixture. The marketed formulation of Balchaturbhadra Churna, sourced online under the brand name Tansukh Herbals (Lucknow), was also collected. The compositions of both laboratory-prepared and marketed formulations are presented in Table 1.

Table 1: Ingredients of laboratory and marketed formulations of *Balchaturbhadra churna*

S. No	Drug	Lab Formulation	Marketed Formulation	Part	Quantity
1	Mustaka	*Cyperus rotundus* Linn	*Cyperus scariosus* R.Br	Rhizome	1 Part
2	Pippali	*Piper longum* Linn	*Piper longum* Linn	Fruit	1 Part
3	Ativisa	*Aconitum heterophyllum* Wall	*Aconitum heterophyllum* Wall	Root	1 Part
4	Karkatashringi	*Pistacia integerrima* Stew	*Pistacia integerrima* Stew	Gall	1 Part

Organoleptic evaluation

The organoleptic characteristics, such as the formulations' colour, odour, taste, and fineness, were recorded.

Physical evaluation

The physical parameters of the formulation, including moisture content, extractive values, ash values, loss on drying, and pH, were assessed following the procedures outlined in the Ayurvedic Pharmacopoeia of India (2). The swelling index, foaming index, and pH were evaluated using methods specified in the World Health Organization guidelines (3). Additionally, various powder characteristics of the Churna formulation, such as the angle of repose, bulk density, tapped density, and compressibility index, were determined using established methods (4).

Chemical evaluation

Estimation of total phenolic and flavonoid content

The total phenolic content in the ethanolic extracts of both laboratory prepared and marketed formulations was determined using the Folin-Ciocalteu method (5). The absorbance of the resulting blue complex was measured at 765 nm. A calibration curve for standard gallic acid was generated using solutions ranging from 50 to 250 µg/ml.

The total flavones and flavonol content in the ethanol extracts was assessed using the aluminium chloride colorimetric method, with quercetin as the reference standard, and absorbance was recorded at 415 nm (6). Flavanone content was estimated using the 2,4-dinitrophenylhydrazine method (5), with naringenin as the standard (250–2000 µg/ml), and absorbance was measured at 495 nm. The total flavonoid content was calculated as the sum of the values obtained from these two methods.

Preliminary phytochemical screening

The literature survey revealed the lack of standardization data related to The successive solvent extracts, obtained by sequentially extracting Balchaturbhadra Churna with solvents of increasing polarity, were subjected to various qualitative tests to identify the presence of phytoconstituents using established methods (7). The presence of volatile oils was determined through the hydro-distillation process.

Alkaloids were identified using Dragendorff's, Wagner's, and Mayer's tests, while cardenolides were detected through Kedde's, Legal's, and Raymond's tests. Phenolics and flavonoids were analysed using Ferric chloride, Shinoda, and Lead acetate tests. Chlorosulphonic acid and Salkowski tests were employed to detect triterpenoid saponins. Terpenoids and steroids were identified using Vanillin-sulfuric acid and Liebermann-Burchard tests. Phytosterols in the unsaponifiable fraction of the petroleum ether extract were also confirmed using the Liebermann-Burchard test.

Fixed oils and fats were detected through spot and saponification tests, while carbohydrates were identified using Molisch's and Fehling's tests. Amino acids were analysed using the Ninhydrin test.

Thin layer chromatography of *Balchaturbhadra churna*

Thin-layer chromatography (TLC) supported the findings of the qualitative tests conducted during the preliminary phytochemical screening of *Balchaturbhadra churna*. TLC analysis was performed to confirm the presence of various phytoconstituents identified through qualitative tests. Different formulation extracts were analyzed using TLC on Silica gel 60F254 pre-coated plates to verify the presence of specific compounds. The results obtained were then compared with those of the qualitative tests for validation.

Identification of Piperine in *Balchaturbhadra churna* by TLC

For sample preparation, 1 g of each formulation (both laboratory-prepared and marketed) was refluxed with 20 mL of methanol for 30 minutes. The mixture was then allowed to cool, filtered, and concentrated using a rotary film evaporator. The obtained extract was partitioned with a chloroform-water mixture (3:7), and the chloroform layer was evaporated to yield a residue, which was subsequently dissolved in 1 mL of methanol for further analysis.

https://doi.org/10.47552/ijam.v16i1.5969 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (1), 2025; 184-190

For standard preparation, Piperine was dissolved in methanol at a concentration of 1 mg/mL for thin-layer chromatography (TLC) analysis. TLC was performed using silica gel GF 254 as the stationary phase, with a mobile phase consisting of a toluene: ethyl acetate mixture (70:30). Both sample and standard solutions were applied using a capillary tube, then developed, dried, and observed under UV light at 254 nm. The presence of Piperine in the extracts was confirmed by comparing the Rf values with those of the standard.

Estimation of Piperine content in formulation by HPLC

A simple method was developed using RP-HPLC and validated for the estimation of marker compound Piperine in the laboratory and marketed formulations of *Balchaturbhadra churna*.

Instrument and experimental conditions

HPLC system (LC-20AD prominence equipment, Shimadzu, Japan) consisting of SPD-M20A PDA detector, LC- 20AD pump, operated through Shimadzu LC solution software (version 1.25). Phenomenex C18 column and 30°C of column temperature was used for separation. The mobile phase consists of Acetonitrile: 0.1% Formic acid [75:25] and pumped at a flow rate of 1.2 mL/min. The solvents were degassed and filtered through 0.45 µm syringe filter (Millipore). Sample injection volume was kept 20 µL and detection of Piperine was done at 340 nm.

Preparation of standard and sample solutions

A methanolic stock solution of Piperine (1 mg/ mL) was prepared and diluted with the mobile phase to obtain calibration standards in the concentration range of 1 to 5 µg/mL. Each formulation, both laboratory-prepared and marketed, was refluxed with methanol in triplicate. The resulting extracts were combined, filtered, concentrated using a rotary evaporator, and subsequently dissolved in the mobile phase for HPLC analysis.

Validation of developed RP-HPLC method

The developed method was validated by ICH guidelines, assessing system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy.

Assay of Piperine

Each 1 g formulation sample (lab-prepared and marketed) was refluxed with 30 mL of methanol for 1 hour, followed by a second reflux with fresh methanol for 30 minutes. The combined methanolic extracts were filtered and concentrated using a rotary film evaporator. The resulting residue was dissolved in 2 mL of the mobile phase (Acetonitrile: 0.1% Formic acid, 75:25). A 0.2 mL aliquot of this stock solution was diluted further to 10 mL with the mobile phase and injected 20 µL of the final solution into the HPLC for chromatographic analysis. Experimented triplicate and analyzed peak areas to quantify the Piperine content.

Statistical analysis

Conducted all validation studies in triplicate or six replicates and expressed the results as mean ± SD. Used Microsoft Excel to calculate the experimental data's mean, standard deviation, % relative standard deviation (%RSD), slope, and correlation coefficient.

Results and Discussion

The formulation was prepared in the laboratory as per Ayurvedic Pharmacopoeia of India as mentioned in Table 1, and the marketed formulation was procured from the market. Both the formulations were subjected to comparative evaluation.

Organoleptic characters

Both the lab and marketed formulations appear Pale brown in colour, with characteristic odour of pippali. Taste was slightly pungent followed by a tingling sensation.

Physical evaluation

Determination of various physical parameters like moisture content, ash value, extractive values, pH value, swelling index, foaming index and various powder parameters (angle of repose, bulk density, tapped density, compressibility index etc.) was carried out and the results were represented in Table 2. The results fall within the range of specifications as mentioned in Ayurvedic Pharmacopoeia of India. The physical parameter values for plant drugs are more or less constant and hence used for the evaluation of crude drugs and herbal formulations.

The loss on drying equal to moisture content is an indicative of moisture present which can be controlled and determined always. The ash values represent the amount of inorganic matter present in the formulation which is adhering to it and added deliberately. The extractive values are indicative of the number of soluble constituents for the solvent selected, present in the formulation. Swelling index denotes the presence of mucilaginous matter in the formulation. The information related to triterpenoid saponins will be provided by foaming index. The powder parameters provide the various powder properties like flow, compressibility etc. of the powder particles of the formulation.

The loss on drying, water and ethanol soluble extractives, ash value, and pH of the aqueous solution for the laboratory and marketed formulations comply with the specifications outlined in the Ayurvedic Pharmacopoeia of India.

Chemical evaluation

Determination of total phenolic content, total flavonoid content

The Folin-Ciocalteu reagent was used to determine the total phenolic content, with gallic acid as the standard. The phenolic content of the laboratory-prepared and marketed formulations of Balchaturbhadra Churna was found to be 1.723±0.049 % w/w and 2.726±0.052 % w/w, respectively, indicating the

Table 2: Physical parameters of the laboratory and marketed formulations of *Balachaturbhadra churna*

Parameters	Lab formulation	Marketed formulation	Specifications as per API
Loss on drying (% w/w)	9.03±0.33	8.5±0.12	⩾ 9 %
Water soluble Extractive value (% w/v)	37.66±3.78	29.33±5.68	⩽ 14%
Ethanol soluble extractive value (% w/v)	29.66±2.30	24.33±0.57	⩽ 16%
Total ash value (% w/w)	0.054± 0.005	0.055± 0.005	⩾ 7 %
pH (10% aqueous solution)	5.3	5.2	5-5.3
Swelling index	1.4±0.03	3±0.01	--
Foaming index	<100	<100	--
Bulk density (gm/cc)	0.41±0	0.42±0.006	--
Tapped density (gm/cc)	0.58±0	0.62±0	--
Angle of repose (°)	31.25±0.959	30.85±0.95	--
Compressibility index	29.1±0	29.9±0.44	--

presence of various phenolic compounds, including polyphenols, flavonoids, and phenolic acids.

Total flavonoid content was determined using the Aluminium chloride and 2,4-Dinitrophenylhydrazine methods, with quercetin and naringenin as markers, respectively. The flavonoid content of the laboratory-prepared and marketed formulations was recorded as 0.765±0.0001 % w/w and 0.452±0.001 % w/w, respectively, signifying the presence of flavonols, flavones, and flavanones. The phenolic content was more with marketed formulation and the total flavonoid content with laboratory prepared formulation, indicating more amount of flavonoids in the laboratory prepared formulation.

Preliminary phytochemical screening

The phytochemical screening results of various successive extracts from the laboratory and marketed formulations are presented in Table 3. The analysis confirmed the presence of several secondary metabolites, including alkaloids, phenolics, flavonoids, terpenoids, sterols, and triterpenoids in both the formulations.

Thin layer chromatographic studies of Balchaturbhadra churna

Thin layer chromatography (TLC) was performed to confirm the presence of various phytoconstituents identified through qualitative tests. Alkaloids, developed with Toluene: Chloroform: Ethanol (14:23:7.5) as the mobile phase, showed three Dragendorff's reagent-positive spots in the laboratory formulation with Rf values of 0.67, 0.79, and 0.89,

Table 3: Qualitative evaluation of successive extracts

Constituents	Laboratory formulation				Marketed formulation			
	P	C	E	W	P	C	E	W
Alkaloids	-	+	-	-	-	+	-	-
Cardenolides	-	-	-	-	-	-	-	-
Flavonoids	-	-	+	-	-	-	+	-
Anthracene glycosides	-	-	+	-	-	-	+	-
Terpenoids	+	-	-	-	+	+	-	-
Sterols	+	-	-	-	+	-	-	-
Triterpenoids	+	+	-	-	-	+	-	-
Amino acids	-	-	-	+	-	-	-	+
Carbohydrates	-	-	+	+	-	-	+	+
Fixed oils and fats	+	-	-	-	+	-	-	-
Volatile oils	Absent				Absent			

P: Petroleum ether, C: Chloroform, E: Ethanol, W: Chloroform water, -: Negative, +: Positive

while the marketed formulation exhibited three spots at 0.09, 0.81, and 0.86.

Terpenoids, developed with Ethyl acetate: Toluene (0.7:9.3), showed six Vanillin-Sulphuric acid-positive spots in the laboratory formulation with Rf values of 0.09, 0.23, 0.38, 0.44, 0.73, and 0.96, whereas the marketed formulation revealed five spots at 0.15, 0.23, 0.28, 0.53, and 0.88.

Flavonoids, using Toluene: Ethyl formate: Formic acid (20:12.5:17.5) as the mobile phase, produced four NP-PEG reagent-positive spots in the laboratory formulation with Rf values of 0.05, 0.24, 0.43, and 0.54, while the marketed formulation showed three spots at 0.05, 0.25, and 0.45. Phenolics, developed with Toluene: Ethyl acetate: Acetic acid (2.25:2.5:0.25), exhibited three Ferric chloride-positive spots in the laboratory formulation at Rf values of 0.66, 0.84, and 0.92, whereas the marketed formulation revealed three spots at 0.13, 0.28, and 0.54.

Anthracene glycosides showed four positive spots in the laboratory formulation with Rf values of 0.32, 0.76, 0.78, and 0.96, while the marketed formulation exhibited four spots at 0.08, 0.56, 0.72, and 0.91.

These studies confirmed the presence of alkaloids, terpenoids, phenolics, flavonoids and anthracene glycosides in both the formulations.

Identification of Piperine content in *Balachaturbhadra churna* by TLC

Piperine was identified using thin layer chromatography (TLC) with Toluene: Ethyl acetate (70:30) as the mobile phase and silica gel GF254 as the stationary phase, comparing the results with standard Piperine. The standard solution showed a single spot with an Rf value of 0.40. The lab formulation exhibited three spots with Rf values of 0.40, 0.44, and 0.55, while the marketed formulation displayed four spots with Rf values of 0.40, 0.46, 0.55, and 0.63.

The Rf value of standard Piperine (0.40) matched the first spot in both the lab and marketed formulations, and all showed a similar UV response at 254 nm,

https://doi.org/10.47552/ijam.v16i1.5969 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (1), 2025; 184-190

confirming the presence of Piperine. The TLC chromatogram of standard Piperine, lab, and marketed formulations is presented in Figure 1.

Figure 1: Chromatogram of the Piperine in laboratory and marketed formulations

The study confirms the presence of marker compound Piperine in laboratory prepared and marketed formulations.

Method development and validation for estimation of Piperine by RP-HPLC

The Piperine solution exhibited maximum absorbance at 340 nm in the UV region. Based on multiple trials, the optimized HPLC mobile phase was determined to be Acetonitrile: 0.1% Formic acid (75:25). The HPLC chromatogram of Piperine is shown in Figure 2. The optimal experimental conditions are detailed in Table 4.

Figure 2: Optimized HPLC chromatogram of standard Piperine (3 µg/ml)

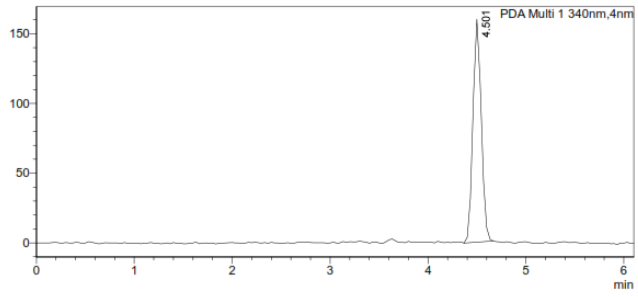


Table 4: HPLC optimized conditions for Piperine estimation

S. No	Parameters	Conclusion
1	Mobile Phase	Acetonitrile:0.1% Formic acid (75:25)
2	Column	C-18
3	Wavelength	340 nm
4	Column temperature	30^0 C
5	Run time	07 min
6	Retention time	4.5
7	Flow rate	1.2 ml
8	Injection volume	20 µl
9	Concentration	3 µg/ml
10	Theoretical plates	9464

Method development

In this study, a RP-HPLC method was successfully developed for the estimation of Piperine in laboratory and marketed formulations of Balchaturbhadra Churna. The method was optimized based on various chromatographic parameters, including mobile phase composition, flow rate, and column oven temperature, to achieve sharp and well-defined peaks.

Peak sharpness and intensity were assessed using peak height, peak area, peak width, and tailing, ensuring minimal peak broadening, shoulder peaks, or splitting. The optimized mobile phase of Acetonitrile: 0.1% Formic acid (75:25) produced a well-resolved peak with a retention time of 4.50 minutes (Figure 2). The most suitable flow rate was determined to be 1.2 mL/min, ensuring optimal peak characteristics. A column oven temperature of 30°C further enhanced peak sharpness while minimizing retention time.

As part of the analytical method validation, system suitability, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were carried out.

System suitability

The HPLC system's suitability confirms the developed method's reliability and acceptability. Various system suitability parameters were within the acceptable range, including peak area, retention time (Rt), tailing factor, and plate count (Table 5).

The system's suitability was assessed through six replicate analyses of the analyte. The acceptance criterion required a ±2% relative standard deviation (%RSD) for both peak area and retention time. The results confirmed that the method was appropriate for further Piperine analysis in various formulations.

Table 5: Data representing system suitability

Piperine	Peak area	Ret. time	Plate count	Peak height	Tailing factor
Mean	875296	4.494	9386	259718.17	1.061
SD	2248.165	0.001	41.2	0.008	2248.165
% RSD	0.25	0.02	0.4	0.7	0.25
Limits: Theoretical plates, >2000; Tailing factor, ≤ 2; % RSD, <2					

Linearity, Limit of quantification (LOQ) and Limit of detection (LOD):

The linearity of the developed HPLC method was established by assessing the relationship between peak areas and corresponding concentrations of Piperine. Regression analysis confirmed that the method was linear across the concentration range of 1–5 µg/mL, with a correlation coefficient ($R^2 > 0.996$), indicating acceptable linearity (Table 6; Figure 2).

The limit of detection (LOD) and limit of quantification (LOQ) represent the lowest detectable and quantifiable concentrations of the analyte. A signal-to-noise (S/N) ratio of 3 and 10 was used to determine LOD and LOQ, respectively. At 340 nm, the LOD and LOQ for Piperine were found to be 0.015 µg/mL and

0.044 µg/mL, respectively. These values confirm the high sensitivity of the developed HPLC method for Piperine estimation in Balchaturbhadra Churna.

Table 6: Linear regression analysis parameters

Linearity range	1-5 µg/ml
Regression coefficient (r^2)	0.9961
Slope	32516
Intercept	285524
LOD	0.015
LOQ	0.046

Precision and accuracy

Precision refers to the degree of agreement between multiple measurements of the same sample under identical conditions. It was evaluated through intra-day (same-day) and inter-day (three consecutive days) analyses at low, medium, and high concentrations. The relative standard deviation (%RSD) values ranged from 1.21% to 1.90% for intra-day precision and 0.67% to 1.63% for inter-day precision, remaining below 2%. These results confirm that the method meets the acceptance criteria and exhibits high precision.

Accuracy was assessed by comparing experimental values with actual values. Known concentrations of Piperine were spiked into a pre-analysed sample (3 µg/mL) at different levels (50%, 100%, and 150%). The mean percentage recoveries ranged from 100.6% to 101.4%, demonstrating the high accuracy of the developed HPLC method.

Robustness

Robustness refers to the ability of the developed HPLC method to remain unaffected by slight intentional variations in chromatographic parameters. The method's robustness was evaluated by introducing minor changes in mobile phase flow rate (±0.2 mL/min), wavelength (±2 nm), and oven temperature (±5°C). The results showed that the percent RSD (<1%) and system suitability parameters remained stable and unaffected, confirming that the developed HPLC method is robust and reliable.

Assay of Piperine the Balchaturbhadra churna

Methanolic extracts of Laboratory and marketed formulations were injected into HPLC and peak areas were noted. The concertation of the Piperine was obtained from the calibration curve using regression equation. The amount of Piperine in laboratory and marketed formulations were found to be 0.194 ± 0.0058 % w/w and 0.212 ± 0.003 % w/w respectively.

The HPLC chromatograms of the methanolic extract of laboratory and marketed formulations were represented in Figure 4 and Figure 5

Figure 4: RP-HPLC chromatogram of the laboratory formulation of *Balchaturbhadra churna*

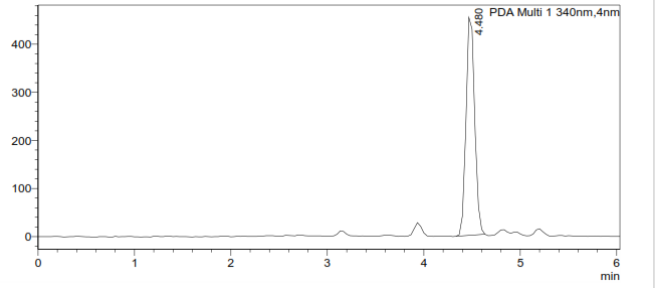
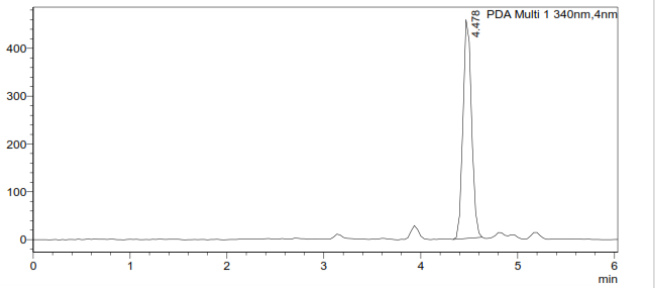


Figure 5: RP-HPLC chromatogram of the marketed formulation of *Balchaturbhadra churna*



Conclusion

This study successfully formulated Balchaturbhadra Churna (BC) in the laboratory following the guidelines of the Ayurvedic Pharmacopoeia of India and compared it with a commercially available formulation. Organoleptic evaluation indicated similarities in colour, odour, and taste between both formulations. Physical parameters, including moisture content, ash values, extractive values, and pH, were found to be within the acceptable limits set by the Ayurvedic Pharmacopoeia, ensuring compliance with quality standards.

Chemical analysis confirmed the presence of key phytoconstituents such as phenolics, flavonoids, alkaloids, terpenoids, and sterols. Notably, the marketed formulation exhibited a higher total phenolic content, whereas the laboratory-prepared formulation contained a greater total flavonoid content, indicating variations in bioactive compound concentrations.

TLC analysis confirmed the presence of piperine, a significant bioactive marker, in both formulations. Furthermore, an RP-HPLC method was successfully developed and validated for the quantification of piperine, demonstrating good linearity, precision, and accuracy. This ensures reliable and reproducible estimation, making it a valuable tool for standardizing Ayurvedic formulations containing piperine.

Overall, this study underscores the importance of standardization in Ayurvedic formulations to maintain consistency, efficacy, and quality. The findings indicate that while both the laboratory-prepared and marketed formulations adhere to the required standards. The validated RP-HPLC method for piperine estimation can serve as a robust tool for quality control in herbal formulations, contributing to the scientific validation and broader acceptance of Ayurvedic medicine worldwide.

References

1. The Ayurvedic Pharmacopoeia of India, Part II, Vol I, 1st edition, Ministry of health and family welfare, Department of Ayurveda, Yoga & Naturopathy,

Unani, Siddha, Homoeopathy, The Controller of Publications, New Delhi; 2007. 44p.
2. The Ayurvedic Pharmacopoeia of India, Part II, Vol II, 1st edition, Ministry of health and family welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha, Homoeopathy, The Controller of Publications, New Delhi; 2007; 156p.
3. WHO, Quality control methods for medicinal plant materials. Geneva; World Health Organization; 1998. 51p
4. Subramanyam C.V.S, Text book of Physical Pharmaceutics, 5 ed. Delhi; Vallabh Prakasan; 201. 275p.
5. Singleton VL and Rossi JA: Colorimetry of total phenolics with phosphor molybdic-phospho tungstic acid reagent Am J Enol Vitic 1965,16, 144-148.
6. Chang CC, Yang M, Wen H and Chern J: Estimation of flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002, 10 (3), 178-182.
7. Kokate C.K, Practical Pharmacognosy. 5 ed. Delhi; Vallabh Prakasan; 2014. 108p.
8. Wagner G, Blatt S, Plant Drug Analysis. 2 ed. Berlin; Springer Verlag; 1996. 352p
9. Validation of analytical procedure: Methodology International Conference on Harmonization. "ICH Harmonised tripartite guideline - Validation of analytical procedures: Text and methodology Q2(R1). 2005.
10. Jagwani S, Jalalpure S, Dhamecha D, Hua GS, Jadhav K. Development and Validation of Reverse-Phase High-Performance Liquid Chromatographic Method for Determination of Resveratrol in Human and Rat Plasma for Preclinical and Clinical Studies. Indian J of Pharm Edu and Res. 2020;54(1):187-93.
11. Jagwani S, Jalalpure S. A Stability Indicating Reversed Phase HPLC Method for Estimation of trans -Resveratrol in Oral Capsules and Nanoliposomes. TACL. 2019;9(5):711-26.

*****