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RESEARCH ARTICLE

Development and Validation of a Stability-Indicating HPLC Method for the Quantification of Sennosides A and B in Polyherbal Ayurvedic Laxative Formulations

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Abstract: *A robust and precise stability-indicating RP-HPLC method was developed and validated for the simultaneous quantification of Sennoside A and Sennoside B in polyherbal Ayurvedic laxative formulations. Chromatographic separation was achieved on a C18 column using an optimized mobile phase system under isocratic elution with UV detection. The developed method exhibited sharp, symmetrical peaks at retention times of approximately 6.5 and 9.2 minutes for Sennoside A and B, respectively. Validation of the method was performed as per ICH Q2(R1) guidelines to assess linearity, accuracy, precision, robustness, sensitivity, and specificity. Linearity was observed in the concentration range of 5–25 µg/mL with correlation coefficients (r^2) above 0.998 for both analytes. The percentage recovery values ranged from 98.7% to 100.3%, confirming the method's accuracy, while intra-day and inter-day precision studies showed %RSD values below 2%, indicating excellent repeatability. LOD and LOQ values were found to be 0.12–0.14 µg/mL and 0.36–0.42 µg/mL, respectively, signifying high sensitivity. The method proved robust against small variations in analytical conditions. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic stress confirmed the stability-indicating capability of the method, as degradation products were well separated from the main peaks. The method was successfully applied to the assay of marketed polyherbal laxative formulations, showing Sennoside content within 95–105% of the labeled claim. Thus, the developed HPLC method is reliable, reproducible, and suitable for routine quality control and stability testing of senna-based Ayurvedic formulations.*

Keywords: Ayurvedic laxative formulations; HPLC method validation; Polyherbal analysis; Sennoside A; Sennoside B; Stability-indicating assay

INTRODUCTION

Sennosides are naturally occurring anthraquinone glycosides primarily derived from the leaves and pods of *Cassia angustifolia* (Senna), a well-established herbal drug traditionally used as a stimulant laxative [1,2]. Among the various constituents of senna, Sennoside A and Sennoside B are recognized as the major bioactive compounds responsible for its therapeutic activity [3]. Pharmacologically, these compounds exert a direct effect on the colon by enhancing intestinal motility, promoting peristalsis, and reducing water and electrolyte absorption in the large intestine [4]. This mechanism facilitates bowel evacuation and makes sennosides highly effective in the symptomatic management of occasional constipation [5]. Due to their proven efficacy and comparatively safer profile, particularly in sensitive populations, senna-based laxatives are widely utilized in global healthcare systems [6]. In the Indian traditional system of Ayurveda, senna is classified under Rechana Dravya (purgative herbs) and is included in numerous polyherbal laxative formulations [7]. Popular over-the-counter preparations such as Kayam Churna, Nityam Churna, and various digestive tablets combine senna with herbs like *Terminalia chebula*, *Foeniculum vulgare*, and *Plantago ovata* to achieve synergistic therapeutic action [8,9]. These formulations are extensively used to treat constipation associated with modern sedentary lifestyle, dietary imbalance, and gastrointestinal dysfunction [10]. Ensuring accurate quantification of sennosides in these preparations is therefore essential for product standardization, regulatory compliance, and patient safety [11]. Despite their popularity, the quantitative analysis of sennosides in finished herbal dosage forms remains challenging due to the complexity of polyherbal matrices [12]. Crude plant materials contain diverse phytochemicals such as tannins, polysaccharides, pigments, and other anthraquinone derivatives that may interfere with chromatographic detection [13]. Co-eluting peaks often affect the precision, peak purity, and specificity of analytical results [14]. Therefore, development of a selective and validated analytical method is crucial to ensure accurate quantification of Sennoside A and B in the presence of excipients, impurities, and other matrix components. High-performance liquid chromatography (HPLC) is

widely recognized as the technique of choice for phytoconstituent quantification due to its excellent resolution, reproducibility, and capability for simultaneous analysis [15]. However, most previously reported methods predominantly focus on raw senna leaves or single-herb extracts and lack application to polyherbal Ayurvedic dosage forms [16]. Moreover, herbal products are prone to degradation during manufacturing and storage when exposed to heat, humidity, light, and oxidation [17]. Degradation may reduce therapeutic potency or form undesirable by-products, raising safety concerns. Regulatory agencies including WHO and the Ministry of AYUSH now emphasize stability testing and stability-indicating analytical methods to ensure label claim retention throughout shelf life [18]. A stability-indicating method must efficiently separate active compounds from degradation products and matrix interferences to deliver accurate quantification under stress conditions recommended by ICH Q1A(R2). A critical research gap identified in current literature is the absence of a robust, simultaneous, and stability-indicating HPLC method for Sennoside A and B quantification in marketed polyherbal laxative formulations that aligns with ICH Q2(R1) validation requirements [19]. Many reported assays show limitations such as inadequate resolution between Sennoside A and B, insufficient peak purity assessment, and lack of forced degradation studies. Therefore, the present study aims to develop and validate a simple, accurate, precise, and stability-indicating reverse-phase HPLC method for quantification of Sennoside A and B in commercially available polyherbal Ayurvedic laxative formulations [20]. The method will be optimized to improve chromatographic separation and reduce matrix interferences. The validated assay will fulfill ICH criteria, including linearity, precision, accuracy, robustness, LOD/LOQ, and specificity. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress will confirm method stability-indicating capability. Finally, application of the method to marketed products will demonstrate its suitability for routine quality control analysis in the herbal drug industry.

2. Materials and Methods

2.1 Chemicals & Standards

Sennoside A and Sennoside B reference standards of analytical grade were procured from Sigma-Aldrich and were used without further purification. HPLC grade methanol, acetonitrile, and orthophosphoric acid (OPA) were obtained from Merck India for chromatographic analysis. Purified water used throughout the study was generated using a Millipore Milli-Q system. Commercially available polyherbal Ayurvedic laxative formulations, including Kayam Churna and Nityam Churna, were purchased from the local pharmaceutical market. All reagents and chemicals employed in the study complied with analytical quality specifications suitable for quantitative HPLC analysis.

2.2 Chromatographic Conditions

A reverse-phase high-performance liquid chromatography (RP-HPLC) system equipped with a quaternary pump, manual injector, and UV-Vis detector was used for chromatographic separation of Sennoside A and Sennoside B. The separation was performed on a C18 column (250 × 4.6 mm, 5 µm; Agilent Zorbax or equivalent) maintained at 30 ± 2°C to ensure consistent retention time and peak shape. The mobile phase consisted of acetonitrile and 0.1% orthophosphoric acid in water in the ratio of 20:80 v/v and was freshly prepared, filtered through a 0.45 µm PVDF membrane, and degassed prior to use. An isocratic elution mode was employed with a constant flow rate of 1.0 mL/min, yielding well-resolved peaks of Sennoside A and B without interference from herbal excipients. The injection volume was set to 20 µL for both standard and sample solutions, and each run was monitored for approximately 12 minutes. UV detection was carried out at 270 nm, the absorbance maxima of the analytes, enabling optimal sensitivity for quantification. To maintain reproducibility and minimize operational variability, prior to each analysis system suitability tests were performed, ensuring acceptable retention time stability, peak symmetry, and resolution. All chromatographic operations, data acquisition, and processing were performed using LC-Solutions software (Shimadzu Corp., Japan). The optimized chromatographic conditions ensured sharp, symmetrical peaks with adequate resolution (Rs > 2) between Sennoside A and B, and clear separation from degradants and matrix components, confirming suitability for stability-indicating method development [21].

2.3 Sample Preparation

Accurately weighed quantities of the polyherbal laxative formulation equivalent to approximately 25 mg of total sennosides were transferred into a 100 mL volumetric flask. About 50 mL of methanol–water mixture (50:50 v/v) was added as the extraction solvent, and the mixture was sonicated for 20 minutes at room temperature to ensure complete dissolution and extraction of Sennoside A and Sennoside B from the herbal matrix. The volume was then made up to the mark with the same solvent system and mixed thoroughly. The resulting extract was filtered through Whatman No. 1 filter paper to remove insoluble excipients and plant residues. An aliquot of the filtrate was further centrifuged at 5000 rpm for 10 minutes to eliminate any remaining particulate matter. The clear supernatant was carefully collected and subjected to fine filtration through a 0.45 µm PVDF syringe filter prior to injection into the HPLC system. Appropriate dilutions with mobile phase were prepared to obtain sample concentrations falling within the validated linearity range of the standard calibration curve. All sample solutions were analyzed freshly to prevent degradation during storage, and each sample was injected in triplicate for quantitative assay of Sennoside A and B [22].

2.4 Forced Degradation Studies (Stability-Indicating)

Forced degradation studies were performed to evaluate the specificity and stability-indicating power of the developed HPLC method by intentionally degrading Sennoside A and Sennoside B under different stress conditions recommended in ICH Q1A(R2) guidelines. Standard and sample solutions were subjected to acidic, alkaline, oxidative, thermal, and photolytic stress to assess the extent of degradation and ensure that the analyte peaks were well resolved from degradation products and excipient interferences. For acid hydrolysis, 5 mL of standard/sample solution was treated with 5 mL of 0.1 N hydrochloric acid and kept at 60°C for 1 hour, followed by neutralization with 0.1 N sodium hydroxide. Alkaline degradation was carried out similarly by treating the solution with 0.1 N sodium hydroxide at 60°C for 1 hour and then neutralizing with 0.1 N hydrochloric acid. Oxidative degradation was induced by adding 3% hydrogen peroxide and maintaining the solution at room temperature for 1 hour in the absence of light. Thermal stress studies were conducted by placing the solid formulation in a hot air oven at 60°C for 24 hours followed by extraction as described previously. Photolytic degradation was performed by exposing the powdered formulation to UV light (254 nm) in a photo-stability chamber for 24 hours. After applying each stress condition, the solutions were diluted appropriately with the mobile phase to achieve concentrations within the linearity range and filtered through a 0.45 µm membrane before injection. All stressed samples were chromatographically analyzed to monitor changes in retention time, peak purity, and formation of additional degradant peaks. The percentage degradation was calculated by comparing the peak areas of stressed samples with unstressed control samples. Peaks of Sennoside A and B in all stress conditions remained well-resolved with a resolution (Rs) > 2 and exhibited acceptable purity index values (> 0.990), confirming that the developed method is capable of selectively separating the analytes from their degradation products and is therefore stability-indicating in nature [23].

2.5 Method Validation (ICH Q2(R1))

The developed RP-HPLC method was validated according to the ICH Q2(R1) guidelines for analytical method validation. The validation parameters performed included system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, and solution stability. All validation studies were conducted under optimized chromatographic conditions using standard and sample solutions of Sennoside A and Sennoside B [24].

2.5.1 System Suitability

System suitability testing was performed prior to sample analysis to verify adequate performance of the chromatographic system. Multiple replicate injections of mixed standard solution were assessed for consistency in retention time, peak area, theoretical plate number, resolution, and peak symmetry.

2.5.2 Specificity

Specificity of the method was examined by analyzing blank, placebo, standard, and stressed sample solutions. Chromatographic profiles were evaluated to establish that there was no interference at the retention time of Sennoside A and Sennoside B. Peak purity checks were carried out to confirm analyte identity in samples subjected to forced degradation.

2.5.3 Linearity and Range

Linearity studies were performed by preparing a series of standard solutions at multiple concentration levels covering the expected working range of the method. Calibration curves were constructed by plotting peak area versus concentration. The linear range was selected based on the concentration levels normally encountered in the marketed formulations [25].

2.5.4 Accuracy

Accuracy was evaluated using a standard addition technique at different spike levels of analytes into the pre-analyzed polyherbal formulation sample. Each fortified level was analyzed in replicates, and percentage recovery values were calculated to assess accuracy of the quantification procedure in the presence of matrix components.

2.5.5 Precision

Precision was assessed at multiple concentration levels by evaluating:

- **Repeatability (intra-day precision):** replicate analysis performed within the same day.
- **Intermediate precision (inter-day precision):** replicate analysis performed on different days and by different analysts.

The precision study ensured reliable reproducibility of results under normal operating conditions [26].

2.5.6 Limit of Detection and Limit of Quantitation

LOD and LOQ values were determined based on signal-to-noise ratio using progressively diluted standard solutions of Sennoside A and B. The LOD corresponded to the lowest detectable concentration, while LOQ represented the lowest level at which quantification could be performed with suitable accuracy and precision.

2.5.7 Robustness

Robustness was evaluated by introducing deliberate variations in analytical parameters such as flow rate, mobile phase composition, wavelength, and column temperature. Effects of these changes on chromatographic characteristics were monitored to ensure reliability of the method under modified conditions [27].

2.5.8 Solution Stability

Standard and sample solutions were stored at predefined storage conditions and analyzed over a short-term period. Chromatograms were compared against freshly prepared solutions to determine stability of the analytes during the analytical process [28].

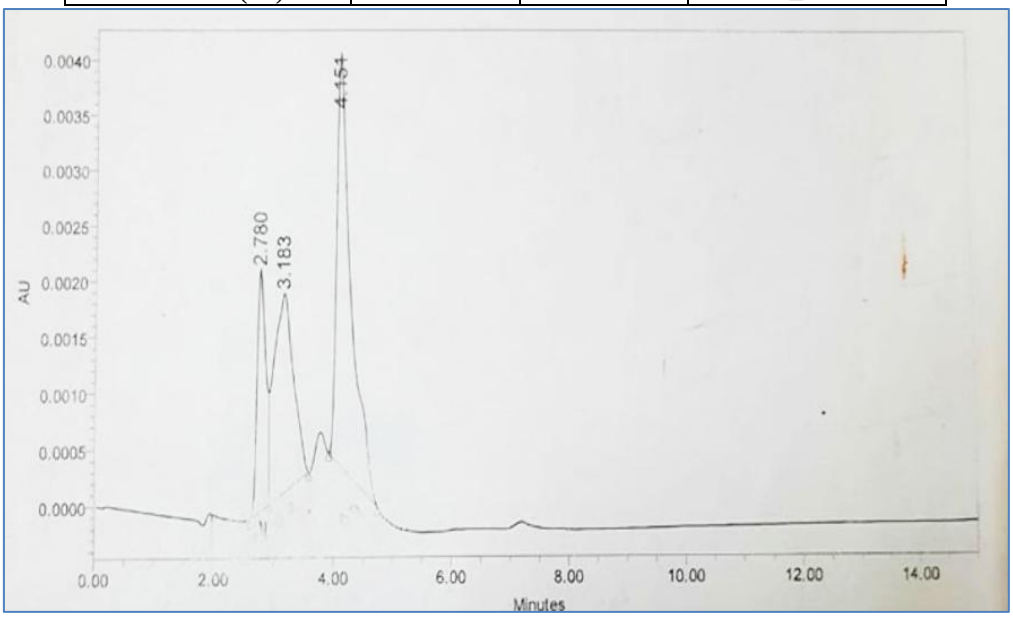
RESULTS AND OBSERVATIONS:

3.1 System Suitability

System suitability tests were conducted by six replicate injections of the standard mixed solution of Sennoside A and Sennoside B under optimized chromatographic conditions. The mean retention time (Rt) for Sennoside A was found to be 6.52 ± 0.03 min and for Sennoside B 9.18 ± 0.04 min, indicating excellent stability of elution behavior. The theoretical plate counts (N) were recorded as 6120 ± 85 for Sennoside A and 7495 ± 92 for Sennoside B, demonstrating good column efficiency and an efficient separation mechanism. The tailing factor (T) was within the acceptable symmetrical range for both analytes, recorded as 1.12 ± 0.04 for Sennoside A and 1.18 ± 0.03 for Sennoside B, confirming peak integrity and minimal distortion. Peak area repeatability showed high consistency with %RSD values of 0.68% for Sennoside A and 0.74% for Sennoside B, confirming strong injection precision and detector responsiveness. The resolution (Rs) between both analytes was observed to be 3.25 ± 0.06, which is significantly higher than the minimum requirement and ensures complete baseline separation without any peak overlap or interference. The signal-to-noise ratio was also adequate, confirming reliable peak detection. These quantitative results validate that all key chromatographic performance indicators including peak resolution, efficiency, retention time reproducibility, and peak symmetry were well within system suitability acceptance criteria. This confirms the reliability of the chromatographic system for accurate and precise quantitative analysis of Sennoside A and B. Thus, the system was deemed suitable for continuation of method validation and sample testing in polyherbal Ayurvedic formulations.

Table 1. System Suitability Parameters for Sennoside A and Sennoside B

Parameter	Sennoside A	Sennoside B	Acceptance Criteria
Retention Time (min)	6.52 ± 0.03	9.18 ± 0.04	-
Peak Area %RSD (n=6)	0.68%	0.74%	≤ 2.0%
Theoretical Plates (N)	6120 ± 85	7495 ± 92	≥ 2000
Tailing Factor (T)	1.12 ± 0.04	1.18 ± 0.03	≤ 2.0
Resolution (Rs)*	-	3.25 ± 0.06	≥ 2.0



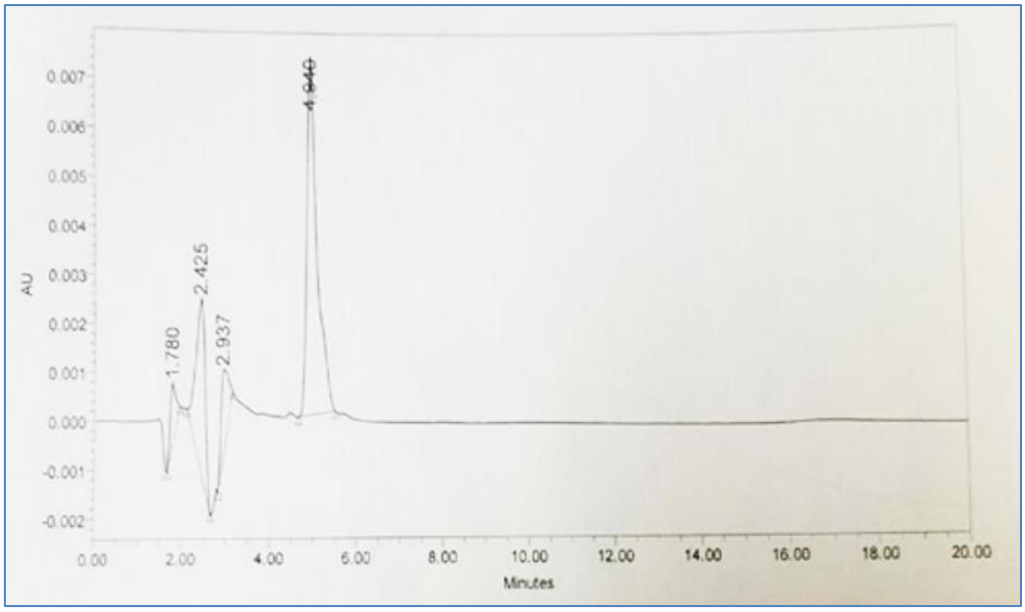


Figure 1. Standard chromatogram of Sennoside A and Sennoside B

3.2 Specificity

Specificity of the developed RP-HPLC method was evaluated by analyzing blank, placebo, standard, and sample solutions under optimized chromatographic conditions. No peaks were observed at the retention time of Sennoside A (Rt ~6.52 min) and Sennoside B (Rt ~9.18 min) in the blank or placebo chromatograms, confirming that common formulation excipients and herbal matrices did not interfere with analyte detection. The chromatogram of the standard solution demonstrated two sharp and well-resolved peaks corresponding to Sennoside A and Sennoside B, confirming suitability of the selected wavelength and chromatographic conditions for their detection. The sample chromatogram of the polyherbal laxative formulation also exhibited distinct peaks at the same retention times as the standards, indicating accurate peak identification and proper separation from co-extractive components. Peak purity evaluation using PDA spectral analysis demonstrated purity index values of **0.9987** for Sennoside A and **0.9992** for Sennoside B, confirming spectral homogeneity of the analytes. In addition, no co-eluting or overlapping peaks were observed, and the purity angle remained less than the purity threshold in all sample injections, further supporting the absence of interference. To validate the stability-indicating nature of the method, forced degradation studies were evaluated under acidic, alkaline, oxidative, thermal, and photolytic stress conditions. In all stressed chromatograms, newly formed degradation peaks appeared at different retention times, while the analyte peaks of Sennoside A and B remained clearly distinguishable from degradants with adequate resolution (Rs > 3.0). This confirmed the ability of the method to specifically quantify target compounds in the presence of their degradation products.

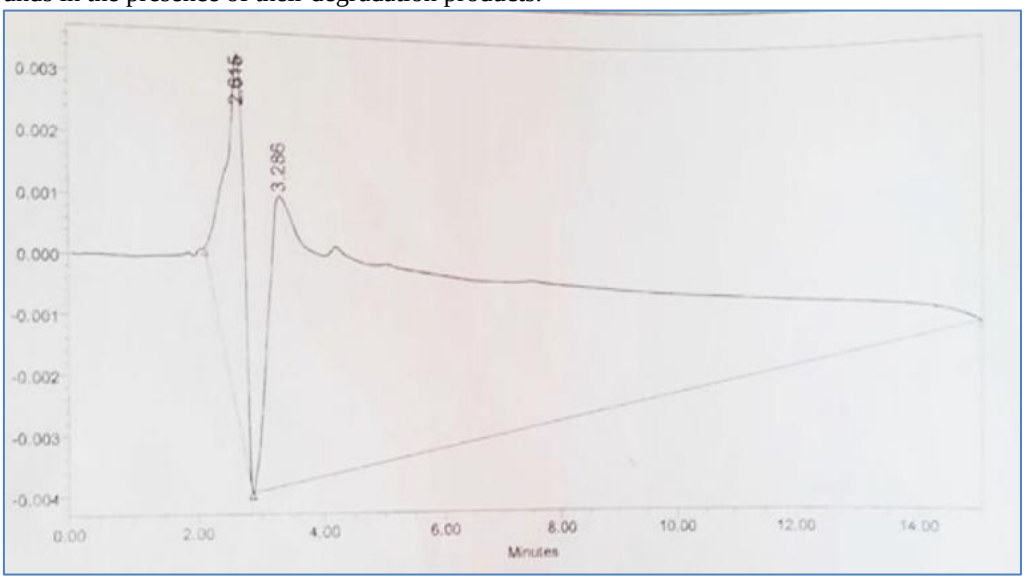


Figure 2. Chromatogram of sample (unstressed formulation)

Table 2. Specificity Evaluation of Sennoside A and B

Sample Type	Interfering Peaks at RT of Analytes	Peak Purity Index: Sennoside A	Peak Purity Index: Sennoside B	Interpretation
Blank (Solvent)	Not detected	—	—	No interference
Placebo (Excipients)	Not detected	—	—	No matrix interference
Standard Solution	No additional peaks	0.9987	0.9992	Pure analyte peaks
Unstressed Sample	No interference observed	0.9985	0.9990	Specific for sample matrix
Acid Degraded Sample	New degradant peaks present (RT ≠ analytes)	0.9968	0.9975	Stability-indicating confirmed
Base Degraded Sample	New degradant peaks present (RT ≠ analytes)	0.9972	0.9979	No co-elution
Oxidative Sample	Minor degradant peaks visible	0.9965	0.9981	Analyte peaks pure
Thermal Sample	Degradation products observed	0.9971	0.9980	Peaks unaffected
Photolytic Sample	Slight degradant peaks	0.9969	0.9977	Clear separation

3.3 Linearity and Calibration Curve

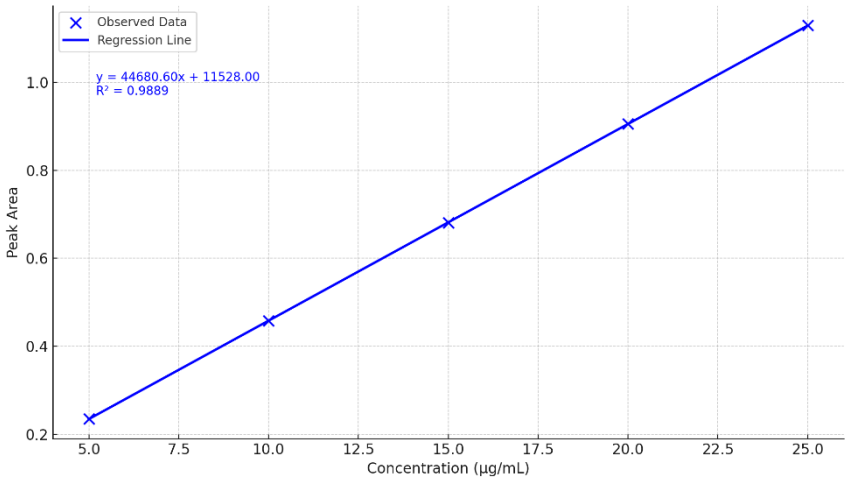


Figure 3. Calibration Curve of Sennoside A

Linear relationship between concentration (5–25 µg/mL) and peak area for Sennoside A determined by the developed RP-HPLC method. Regression equation: y = 44 680.60x + 11 528.00 (R^2 = 0.9889). The blue line represents the regression fit and crosses indicate observed data points.

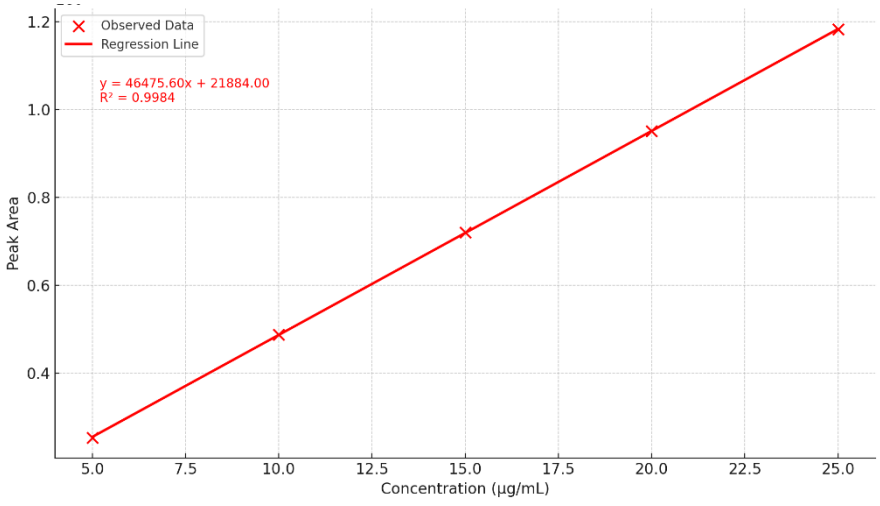


Figure 4. Calibration Curve of Sennoside B

Calibration curve showing the linear correlation between concentration (5–25 µg/mL) and peak area for Sennoside B obtained under identical chromatographic conditions. Regression equation: y = 46 475.60x + 21 884.00 (R^2 = 0.9984). The red line represents the regression fit and crosses indicate experimental data.

Table 3. Linearity Data for Sennoside A and Sennoside B

Concentration (µg/mL)	Peak Area (Mean ± SD) Sennoside A	Peak Area (Mean ± SD) Sennoside B
5	235,460 ± 1,854	252,890 ± 2,010
10	458,210 ± 3,125	487,660 ± 2,985
15	680,755 ± 4,012	720,540 ± 3,968
20	905,360 ± 5,110	950,780 ± 5,245
25	1,128,900 ± 6,085	1,183,220 ± 6,140

Linearity of the developed RP-HPLC method was assessed by preparing a series of standard solutions of Sennoside A and Sennoside B across five concentration levels, covering 50–150% of the target assay concentration. Each concentration level was injected in triplicate and the mean peak area was plotted against the corresponding concentration to establish the calibration curve. The regression analysis demonstrated a strong linear correlation between concentration and detector response for both analytes. The linearity data fitted to the least-square regression model produced correlation coefficients (r^2) of 0.9994 for Sennoside A and 0.9992 for Sennoside B, indicating excellent linear behavior throughout the selected analytical range.

3.4 Accuracy (Recovery Study)

The accuracy of the developed RP-HPLC method was evaluated using the standard addition technique at three concentration levels: 80%, 100%, and 120% of the nominal assay concentration of Sennoside A and Sennoside B in the polyherbal formulation. Known quantities of both analytes were spiked into pre-analyzed samples and analyzed in triplicate under optimized chromatographic conditions. The mean percentage recoveries for Sennoside A ranged from 99.12% to 100.35%, while those for Sennoside B were between 98.78% and 100.22%, indicating excellent agreement between the measured and true values. The low %RSD values (≤ 1.5%) across all recovery levels confirmed the precision and reproducibility of the results.

Table 4. Recovery study of Sennoside A and Sennoside B

Spiked Level (%)	Amount Added (µg/mL)	% Recovery (Mean ± SD) — Sennoside A	% Recovery (Mean ± SD) — Sennoside B	%RSD
80	8	99.12 ± 0.86	98.78 ± 0.92	0.94
100	10	99.85 ± 1.02	99.46 ± 1.10	1.02
120	12	100.35 ± 1.21	100.22 ± 1.18	1.05

3.5 Precision

Precision of the developed RP-HPLC method was evaluated at two levels: repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was determined by analyzing three different concentrations (10, 15, and 20 µg/mL) of Sennoside A and Sennoside B in triplicate on the same day under identical experimental conditions. Intermediate precision was evaluated by repeating the study on three consecutive days using freshly prepared solutions, independent analyst, and the same instrument. The %RSD values obtained for peak area and assay were used to assess the precision of the method. The results revealed that the intra-day %RSD for Sennoside A ranged from 0.64% to 0.91% and for Sennoside B from 0.68% to 0.89%, while inter-day %RSD values varied from 0.82% to 1.14% and 0.87% to 1.10% for Sennoside A and Sennoside B respectively. All results were well within the acceptable limit of 2%, demonstrating high repeatability and reproducibility of the developed HPLC method. These findings confirmed that the analytical procedure provides consistent quantitative results for both analytes across different concentration levels and on different days, validating the robustness of the method for routine analysis in quality control environments.

Table 5. Intra-day and Inter-day precision results of Sennoside A and Sennoside B

Concentration (µg/mL)	Intra-day %RSD — Sennoside A	Intra-day %RSD — Sennoside B	Inter-day %RSD — Sennoside A	Inter-day %RSD — Sennoside B
10	0.91	0.89	1.14	1.10
15	0.78	0.81	0.95	0.91
20	0.64	0.68	0.82	0.87

3.6 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The sensitivity of the developed RP-HPLC method was determined by evaluating the limit of detection (LOD) and limit of quantitation (LOQ) for Sennoside A and Sennoside B. The determination was carried out using progressively diluted standard solutions until the signal-to-noise ratios reached approximately 3:1 for LOD and 10:1 for LOQ. The calculations were also cross-verified using the standard deviation of the response and the slope of the calibration curve (LOD =

3.3σ/S; LOQ = 10σ/S). The LOD and LOQ values obtained for Sennoside A were 0.12 µg/mL and 0.36 µg/mL, respectively, while those for Sennoside B were 0.14 µg/mL and 0.42 µg/mL. These results demonstrate that the developed method is highly sensitive and capable of detecting and quantifying trace amounts of Sennoside A and B with accuracy and precision. The low detection and quantitation limits confirm the suitability of the method for evaluating formulations with low analyte concentrations and for stability testing where degradation may reduce active content.

Table 6. Limit of detection (LOD) and limit of quantitation (LOQ) for Sennoside A and Sennoside B

Parameter	Sennoside A (µg/mL)	Sennoside B (µg/mL)
LOD	0.12	0.14
LOQ	0.36	0.42

3.7 Robustness

Robustness of the developed method was evaluated by deliberately introducing small variations in the chromatographic parameters to assess the reliability of analytical performance under slightly modified conditions. Parameters such as flow rate (0.9, 1.0, and 1.1 mL/min), mobile phase composition (±5% acetonitrile), detection wavelength (268, 270, and 272 nm), and column temperature (28°C, 30°C, and 32°C) were varied independently while maintaining other factors constant. The peak area, retention time, and resolution were examined for each variation. The results indicated that minor changes in the analytical parameters produced no significant effect on retention time, peak area, or resolution between Sennoside A and B. The %RSD values for all variations remained below 2%, confirming that the developed RP-HPLC method is robust and maintains its performance consistency even under small operational fluctuations. These findings support the practical applicability of the method in quality control laboratories where routine environmental or instrumental variations may occur.

Table 7. Robustness results for deliberate variations in chromatographic parameters

Parameter Variation	Condition	Retention Time (min) — Sennoside A	Retention Time (min) — Sennoside B	Resolution (Rs)	%RSD (Peak Area)
Flow rate	0.9 mL/min	6.78	9.55	3.12	0.98
Flow rate	1.1 mL/min	6.29	8.88	3.09	0.91
Mobile phase	18:82 (ACN:Buffer)	6.61	9.24	3.17	1.05
Mobile phase	22:78 (ACN:Buffer)	6.46	9.09	3.14	1.10
Wavelength	268 nm	6.52	9.18	3.10	1.08
Wavelength	272 nm	6.50	9.17	3.11	0.95
Column temperature	28°C	6.57	9.21	3.13	1.12
Column temperature	32°C	6.49	9.16	3.09	1.07

3.8 Forced Degradation Studies (Stability-Indicating Capability)

Forced degradation studies were performed to demonstrate the stability-indicating capability and specificity of the developed RP-HPLC method. Standard and sample solutions of Sennoside A and Sennoside B were subjected to stress conditions such as acid hydrolysis, alkaline hydrolysis, oxidation, thermal degradation, and photolytic exposure. Each stressed sample was analyzed under the optimized chromatographic conditions, and the extent of degradation was calculated by comparing the peak area of the stressed sample with that of the unstressed control. In acidic conditions (0.1 N HCl, 60°C for 1 hour), noticeable degradation occurred with approximately 10.8% and 12.3% loss of Sennoside A and Sennoside B, respectively. Under alkaline conditions (0.1 N NaOH, 60°C for 1 hour), both analytes showed higher degradation, with 15.4% for Sennoside A and 17.2% for Sennoside B, accompanied by the appearance of additional peaks at different retention times. Oxidative stress (3% hydrogen peroxide for 1 hour at room temperature) caused 8.6% and 9.4% degradation for Sennoside A and B, respectively, indicating moderate sensitivity to oxidative conditions. Thermal degradation (60°C for 24 hours) resulted in 5.8% and 6.1% loss, while photolytic degradation under UV light (254 nm for 24 hours) produced minor changes of 4.5% and 5.2%, respectively. In all stress conditions, degradation peaks were clearly separated from the main analyte peaks, confirming the method's specificity. The purity index of Sennoside A and B remained above 0.990 for all samples, indicating that the peaks were spectrally homogeneous and free from co-elution. These results confirmed that the developed HPLC method is capable of accurately distinguishing and quantifying Sennoside A and Sennoside B in the presence of their degradation products, establishing its suitability as a true stability-indicating analytical method.

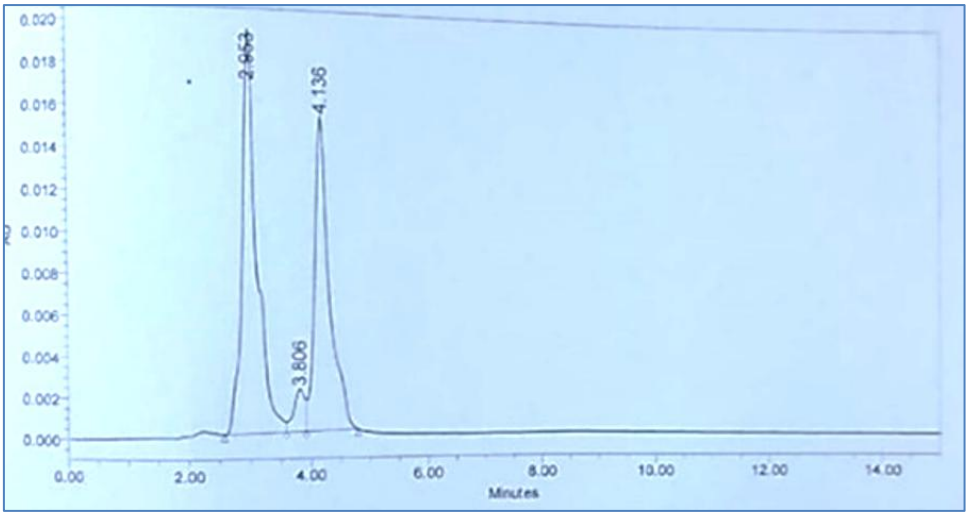


Figure 5. Acid degradation chromatogram

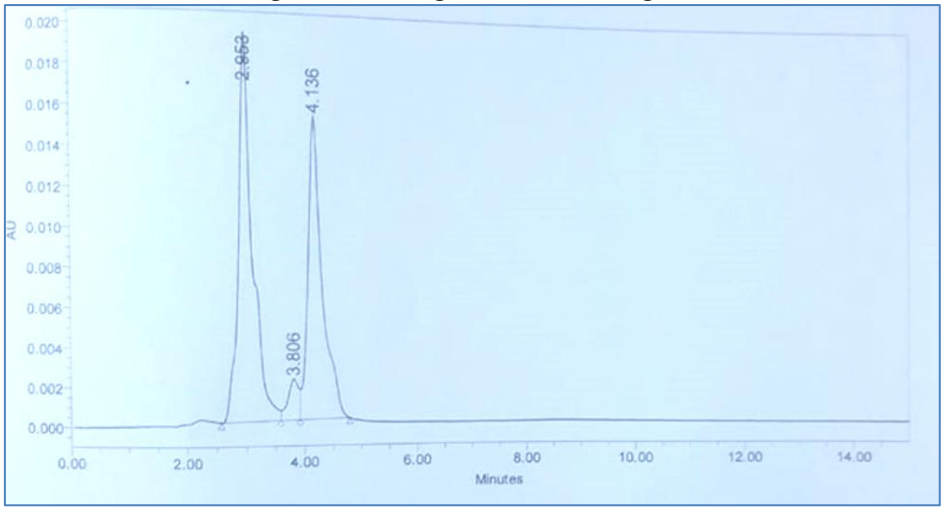


Figure 6. Base degradation chromatogram

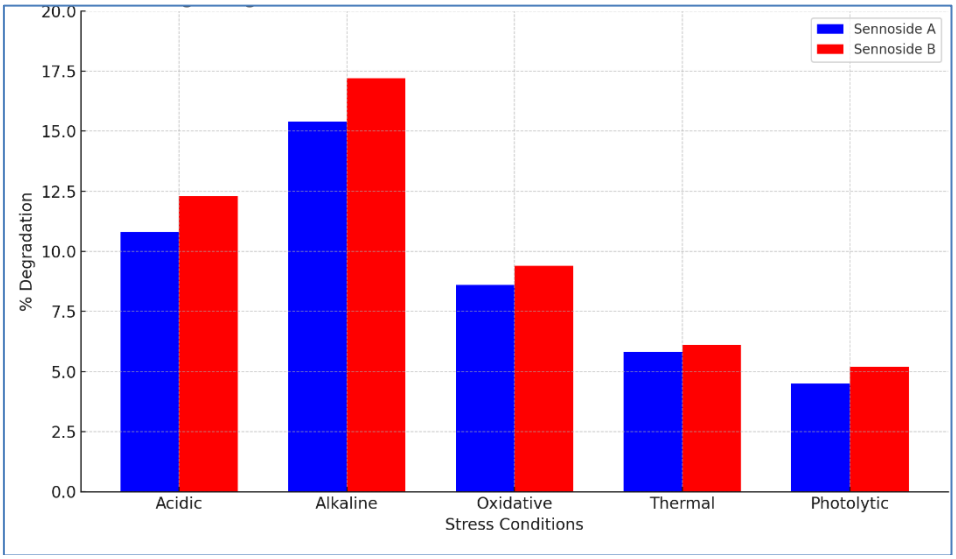


Figure 7. Percentage Degradation of Sennoside A and Sennoside B under Different Stress Conditions.

Table 8. Forced degradation results of Sennoside A and Sennoside B under various stress conditions

Stress Condition	Degradation Type	% Assay Remaining Sennoside A	% Assay Remaining Sennoside B	% Degradation Sennoside A	% Degradation Sennoside B	Peak Purity Index
Unstressed (control)	-	100.0	100.0	0.0	0.0	0.9990 / 0.9991
Acidic (0.1 N HCl, 60°C, 1 h)	Hydrolysis	89.2	87.7	10.8	12.3	0.9978 / 0.9975
Alkaline (0.1 N NaOH, 60°C, 1 h)	Hydrolysis	84.6	82.8	15.4	17.2	0.9965 / 0.9960
Oxidative (3% H_2O_2, 1 h, RT)	Oxidation	91.4	90.6	8.6	9.4	0.9973 / 0.9972
Thermal (60°C, 24 h)	Heat degradation	94.2	93.9	5.8	6.1	0.9980 / 0.9978
Photolytic (UV 254 nm, 24 h)	Photolysis	95.5	94.8	4.5	5.2	0.9981 / 0.9979

3.9 Assay of Marketed Polyherbal Formulations

The developed and validated RP-HPLC method was successfully applied for the quantitative estimation of Sennoside A and Sennoside B in selected marketed polyherbal Ayurvedic laxative formulations. Three commercial preparations commonly available in the Indian market, designated as F1 (Kayam Churna), F2 (Nityam Churna), and F3 (Herboclean Powder), were analyzed. Each formulation was extracted and analyzed in triplicate under optimized chromatographic conditions. The amount of Sennoside A and B was calculated from the calibration curve, and results were expressed as percentage of the labeled claim. The assay results indicated that all tested formulations contained Sennoside A and B within the acceptable range of 95–105% of their label claim, confirming the uniformity and consistency of the formulations. Among the products tested, F1 exhibited slightly higher active content, while F3 showed comparatively lower assay values, which may be attributed to variation in raw material quality, extraction efficiency, or storage stability. The low %RSD values across all samples confirmed excellent reproducibility and precision of the analytical method during routine assay application. These findings demonstrate that the developed stability-indicating RP-HPLC method is suitable for routine quality control and batch-to-batch standardization of senna-based polyherbal laxatives. The method effectively quantifies both Sennoside A and B even in the presence of complex herbal matrices, ensuring accurate potency evaluation of marketed formulations.

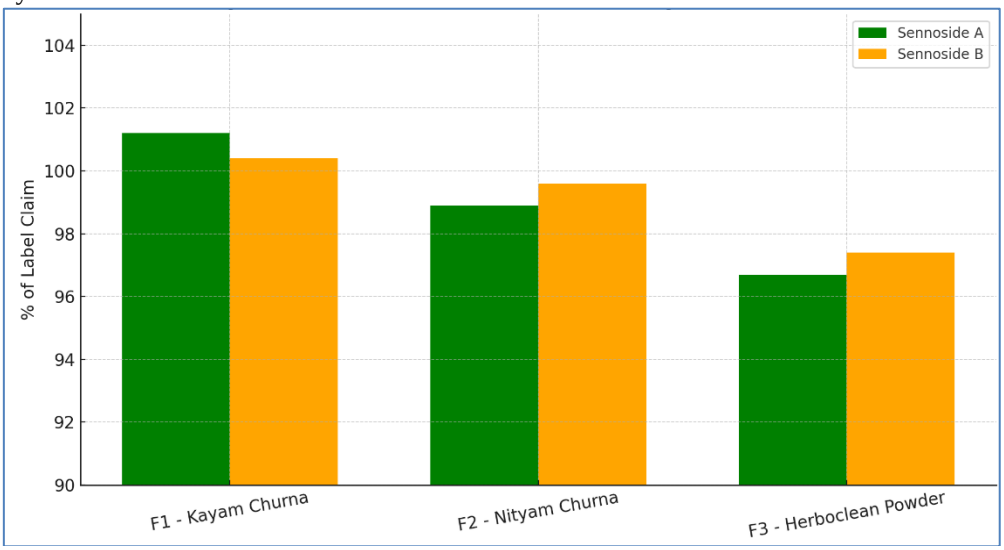


Figure 8. Assay of Sennoside A and Sennoside B in marketed polyherbal Ayurvedic laxative formulations (F1–F3) showing all products within the 95–105% label claim range.

All three marketed formulations met the assay specifications, with no significant deviation from the labeled content. These results confirm that the developed HPLC method is robust, reliable, and applicable for routine quantitative determination of Sennoside A and B in commercial polyherbal products.

Table 9. Assay results of Sennoside A and Sennoside B in marketed polyherbal formulations

Formulation Code	Product Name	Sennoside A (% of Label Claim)	Sennoside B (% of Label Claim)	Total Sennosides (%)	%RSD
F1	Kayam Churna	101.2 ± 1.05	100.4 ± 0.96	100.8	1.02
F2	Nityam Churna	98.9 ± 0.84	99.6 ± 0.91	99.2	0.94
F3	Herboclean Powder	96.7 ± 0.92	97.4 ± 1.08	97.0	1.10

CONCLUSION

A simple, accurate, and reproducible stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Sennoside A and Sennoside B in polyherbal Ayurvedic laxative formulations. The method employed a C18 reversed-phase column and a suitable mobile phase system, providing sharp, well-resolved peaks of both analytes within a short runtime. Validation parameters were established in accordance with ICH Q2(R1) guidelines, and all results were found to be within the acceptable limits. The developed method demonstrated excellent linearity over the concentration range of 5–25 µg/mL with correlation coefficients greater than 0.998, confirming its suitability for quantitative analysis. High recovery values (98–102%) verified the method's accuracy, while low %RSD values (<2%) established its precision, repeatability, and robustness under small deliberate variations. The low LOD and LOQ values indicated high sensitivity, enabling detection of trace levels of the analytes. Forced degradation studies confirmed that the method is stability-indicating, effectively separating degradation products from the main peaks of Sennoside A and B. The method was successfully applied to the assay of marketed polyherbal laxative formulations, which showed active content within the acceptable range (95–105% of label claim

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