

# A Novel Dissolution Method for Simultaneous Estimation of Sennoside A and Sennoside B in Senna Tablet

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## Abstract

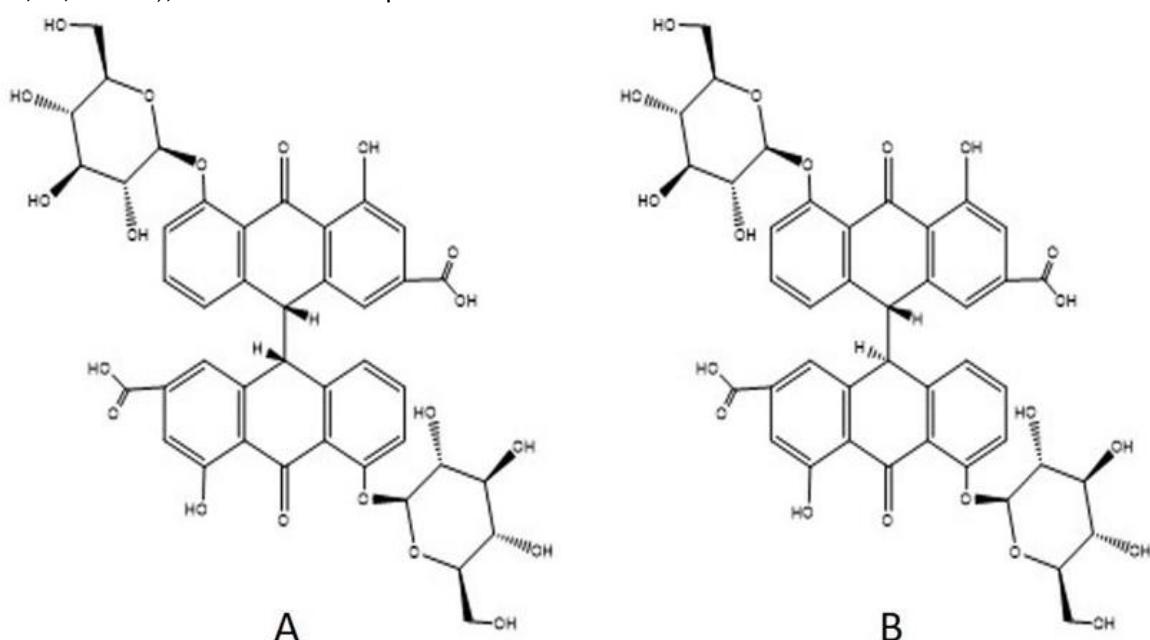
The current method was developed for the small-scale herbal industries established in India or the presented sectors in other countries and for the regulatory interest to amend the Senna tablet monograph stated in Indian Pharmacopoeia. The existing method for the dissolution of Senna tablets was cost-consuming and unsuitable for herbal small-scale industries to assay Senna tablets. The present study indicates a reliable high-performance liquid chromatographic method for quantifying sennoside A and sennoside B in commercial Senna tablets. The separation of sennoside A and sennoside B was conducted on a Hypersil C 18 column (150 × 4.6mm, 3.5µm) at a temperature of 40°C, using a mixture of 75 volumes of 1 percent v/v solution of glacial acetic acid and 25 volumes of acetonitrile containing 10µl tetra n-butyl ammonium hydroxide (ion-pairing reagent) used as mobile phase. The current method was developed and validated. The sennoside A and sennoside B were completely separated from other constituents within 15 minutes. Both run-to-run repeatability (n=10) and day-to-day reproducibility (n=3) of peak area were below 0.5% RSD. The linearity of peak area was tested in the ranges 18-32µg/ml (r>0.9935). Accuracy and recovery were assessed and the recoveries for sennoside A and sennoside B were 100.2% and 104.9% (n=3×6), respectively. Thus, the developed method is simple, accurate, and precise and may be adopted by the pharmacopoeial bodies as an official method. It is also suitable for small-scale herbal industries.

**Key words:** high-pressure liquid chromatography; Sennoside A; Sennoside B; validation; senna.

## Introduction:

The genus Senna includes 500 species, with 26 species of the genus Cassia containing anthracene derivatives within the unfastened shape or glycosides. *Cassia angustifolia* (Indian Senna) and *Cassia acutifolia* (Alexandrian Senna) are official in many pharmacopeias because of their laxative activity and availability in considerable numbers. *C. fistula*, *C. alata*, *C. dentate*, *C. obovata*, *C. podocarpa*, *C. sieberiana*, and *C. sofar* are the other species with documented laxative action. Senna is a medication that is used as a laxative and for treating constipation and bowel evacuation. Senna is used before the surgery to clear the large intestine. The active ingredients of Senna are anthraquinone glycosides (sennoside A, B, C, and D), the free anthraquinones are

rhein, chrysophanol, etc. from the active ingredients present in Senna, sennoside A and sennoside B (Figure 1) were exist in the maximum amount [1]. Senna tablet is generally given by mouth and by rectum. When ingested by mouth, Senna preparation works in twelve hours and within some minutes when introduced through the rectum. Dissolution analysis of herbal dosage forms has become the most crucial test to ensure product quality [2]. The disintegration or dissolution test is generally seen during the analysis of allopathic solid dosage form because it will determine the absorption rate after dissolving in gastric fluid, and ultimately, it will define the bioavailability of the drug [3].



**Figure 1: Structures of sennoside A (A) and sennoside B (B)**

Keeping the same concept in mind, the dissolution analysis for the Senna tablet is to be carried out to assess the release rate and drug bioavailability. While different pharmacopoeias stated in the monograph on Senna tablets like USP, IP, and The Ayurvedic Pharmacopoeia of India to date, the HPLC dissolution method for the estimation of sennoside A and sennoside B in Senna tablets is not formally established and validated in any pharmacopoeias except the United State Pharmacopoeia. Among the various techniques available for the development of the assay method, UV/visible spectrophotometry achieves the advantage of low cost and simplicity with the possibility of achieving high sensitivity and selectivity with reasonable accuracy, precision, and reliability [4] [5] [6].

In India, most herbal industries were established as small-scale industries. These industries cannot implement a method that includes the usage of a cost-consuming detector, such as assaying by fluoroscopy technique. Also,

some need was to be captured for regulatory implantation in the Indian Pharmacopoeia (IP) [4].

The effect of sennoside A and sennoside B is most significant in the Senna drug [7]. Therefore, it is essential to screen out the quantity of sennoside A and sennoside B by dissolving the Senna tablet [8] [9]. To resolve the theory, an accurate, precise method was essential to develop and validate.

The developed method helps to govern the regulatory amendment of dissolution studies of herbal solid dosage forms to be established in the monograph of Senna tablet stated in Indian Pharmacopoeia but also provides a new concept to various herbal small-scale industries by accepting a precise and efficient method for the assay of Senna tablets by HPLC.

## 2. Material and Methods

### 2.1 Chemicals and Reagents:

Sennoside A (>96.0%) and sennoside B (>90.0%) standards were procured from Sigma -Aldrich (Germany), Senna tablets (12 mg) were procured from market, Glacial Acetic Acid, Sodium hydroxide pellets, and HCl were purchased from Avantor, Tetra n-butyl ammonium hydroxide 0.1N aqueous solution LR was purchased from S. D. fine chemical ltd., Acetonitrile of chromatographic grade was purchased from Merck India Ltd. and Hydrogen Peroxide was purchase from Qualikems Pvt. ltd.

## 2.2 Chromatographic equipment and conditions:

For quantification of sennoside A and sennoside B, an Agilent 1260 series HPLC instrument (Agilent Technologies, USA) composed of an online degasser (G1322A), a quaternary pump (G1311C), an auto-Sampler (G1329B), a column temperature controller (G1316A), and a DAD (G1315D) and Dissolution test apparatus (Lab India DS 8000) was used. Analysis was carried out on an Agilent TC-C18 reverse-phase column (150× 4.6 mm, 3.5  $\mu$ m). A mixture of 75 volumes of 1 per cent v/v of glacial acetic acid, 25 volumes of acetonitrile v/v, and 10  $\mu$ l of tetra n-butyl ammonium hydroxide was used as a mobile phase. The method was developed with a flow rate of 0.5 ml/min, injection volume of 10  $\mu$ l, detection wavelength at 350 nm, and column temperature at 40°C.

## 2.3 Preparation of standard solutions:

Sennoside A and sennoside B standard solutions were

prepared by dissolving 12 mg of the respective compounds in 10 ml of water. After filtration through 0.45  $\mu$ m Nylon membranes, the standard solutions were used to make calibration curves.

## 2.4 Sample Preparation:

Twenty tablets from five batches of 12 mg of Senna tablets were taken from the market and ground to make a fine powder. 12 mg of the powder was dissolved in 12 ml of water (0.005 mg in 900 ml). The mixture was sonicated to make sennoside A and sennoside B completely dissolve. After filtration through a 0.45  $\mu$ m Nylon membrane, the solution was used as a working solution in method development.

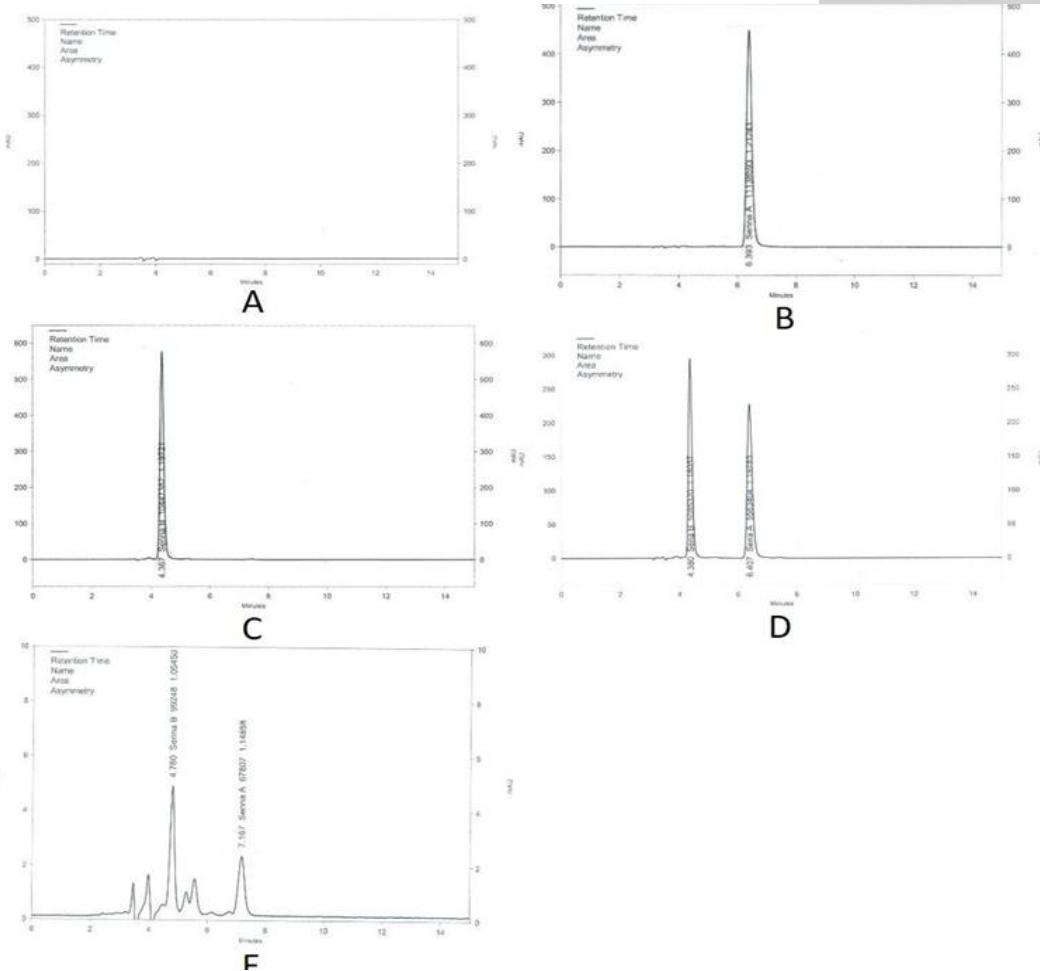
## 2.5 Method validation

The dissolution was validated according to ICH Q2 (R2) guidelines for validating analytical procedures. The method was validated using the isocratic phase, which is simpler and more stable than a gradient system and, thus, more suitable for QC and routine analysis.

## 3. Results

### 3.1 Chromatography and detection

The reverse-phase high-performance liquid chromatography (RP-HPLC) detected sennoside A and sennoside B in standards and samples. Sennoside A and sennoside B were separated within 15 minutes at 350 nm as shown in (Figure 2).



**Figure 2: Chromatogram of Blank (A), sennoside A (B), sennoside B (C), Mix standard A+B (D), and Sample (E)**

### 3.1.1 Specificity and Selectivity:

The developed method was selective for sennoside B and sennoside A. The retention time of sennoside B and sennoside A is 4.3 and 6.2 minutes, respectively. At the retention time of sennoside A and sennoside B, when examined at a wavelength of 350 nm, the chromatogram of the blank (Figure 2A) confirmed the absence of interfering peaks. The result obtained from the chromatogram of sennoside A and sennoside B (Figure 2 B, 2 C) demonstrates no interference from other materials in the developed method and therefore confirms the method's specificity.

### 3.1.2 Accuracy and precision:

Run-to-run repeatability and day-to-day reproducibility were used to assess the precision of repeated injections.

Six injections were made each day and repeated for 3 consecutive days, as shown in Table 1. The relative standard deviation (RSD) of the retention time of sennoside A and sennoside B is 0.55%. The sample solutions were prepared to contain sennoside A and sennoside B in three concentration ranges 80%, 100%, and 120% by spiking the standard solution in the respective range. The recoveries obtained with six repetitive injections are 100.2%, 100.4%, and 104.9%. To test the repeatability and reproducibility of the recoveries, the above procedure was repeated six times a day for three consecutive days. The RSD of recovery of sennoside A and sennoside B were 0.11, 0.10, and 0.03 for concentration 120%, 100%, and 80% respectively, as shown in Table 2.

(sennoside A + sennoside B)			
Injection No.	Area		
	System Precision Data	Method Precision Data	Intermediate Precision Data
1	432845	432845	393544
2	434010	434010	389316
3	427546	427546	394175
4	429588	429588	393839
5	432214	432214	381231
6	430517	430517	390727
Mean	431120	431120	390472
SD	2366.798	2366	4925
RSD	0.55	0.548988	1.26

Table 1: Data of System Precision, Method Precision, Intermediate Precision of sennoside A, and sennoside B

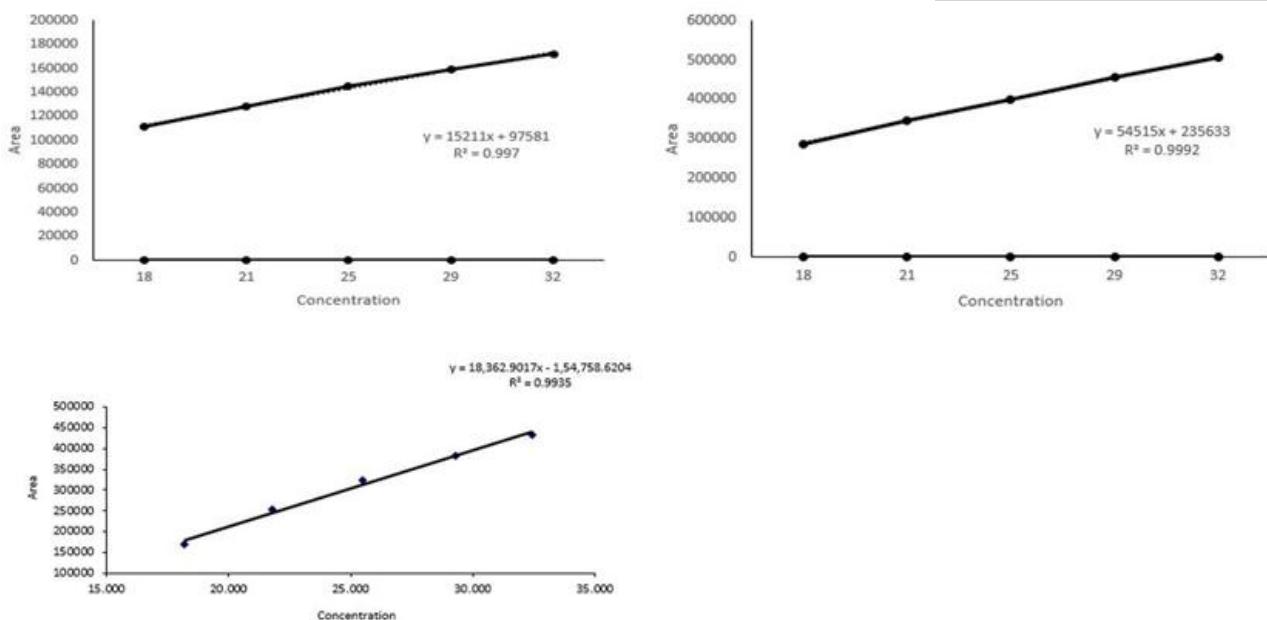
(sennoside A + sennoside B)						
% Concentration	Standard Area	Sample Area	mg/ml	SD	RSD	% Recovery
80	346276	359270	0.02684731	0.029	0.03	104.9
	347208	359222	0.026843721			
	342036	359511	0.026865317			
	343670	359383	0.026855752			
	345771	359378	0.026855378			
	344413	359356	0.026853734			
100	432845	466632	0.034870189	0.284	0.10	100.4
	434010	466787	0.034881772			
	427546	466834	0.034885284			
	429588	464921	0.034742331			
	432214	464582	0.034716998			
	430517	464220	0.034689947			
120	519414	514152	0.038419740	0.112	0.11	100.2
	520812	514628	0.038456805			
	513055	514891	0.038476458			
	515505	514657	0.038458972			
	518656	515592	0.038528842			
	516620	515578	0.038527796			

Table 2: Accuracy Data

### 3.1.3 Linearity

Five standard solutions (18, 21, 25, 29, and 32 µg/ml) were run for the calibration curve. On each day of three consecutive days, the calibration curves were made. Slopes, y-intercepts, and correlation coefficient ( $r^2$ ) were calculated. The calibration curves were found linear over 18-32 µg/ml. Their coefficient of correlation ( $r$ ) was all

above 0.99. The equations were  $y = 18,362.9017x - 1,54,758.6204$  and for sennoside A and sennoside B, respectively [y for peak area and x for concentration in (g/ml) as shown in (Figure 3)]. After passing the statistical test three proved no difference, the three lines were combined to form a new line for quantitative use.



**Figure 3: Linearity of standard sennoside A (A), sennoside B (B), and mix standard A+B (C)**

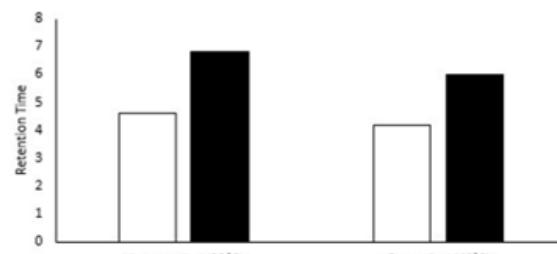
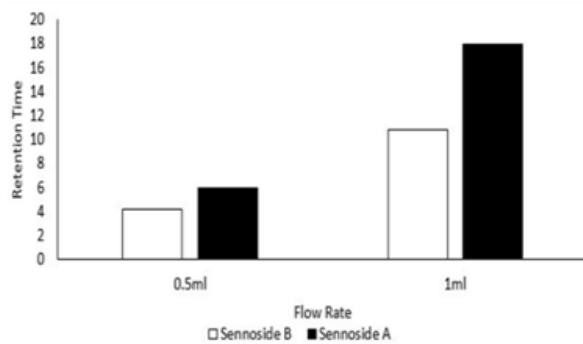
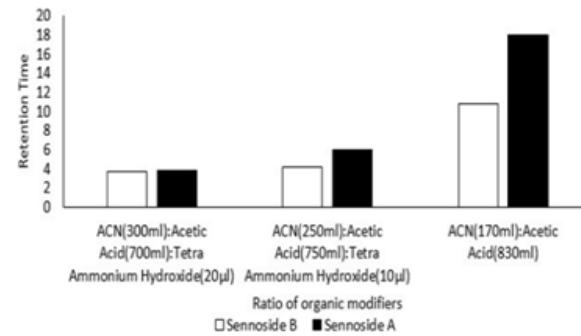
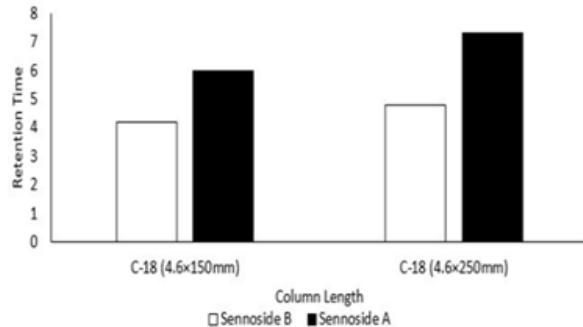
### 3.1.4 Robustness

The effect of each factor was calculated. Based on these data, the effects of the factors were discussed [10] [11].

#### 3.1.4.1 Effect of columns

Resolution was affected by the change of columns, but the effect was unidirectional (Figure 4A); only when the

column was significant. No matter what column was used, the retention time of sennoside A was greatly influenced (Figure 4A). This is in accordance with our previous observation that the symmetry and Luna column retained the sennoside far more than the Hypersil column.



**Figure 4: Bar graph of change in Column Length (A), Ratio of organic modifiers (B), Flow Rate (C), and Column Temperature (D)**

#### 3.1.4.2 Ratio of organic modifiers

The change in the percentage of acetonitrile in the mobile phase greatly influenced both resolution and retention time. Its lessening from (30 to 25% v/v) helped the

resolution to the greatest extent (Figure 5 B), however, at the expense of terrible prolongation of retention time. Because the plate number of sennoside A was affected by the change (Figure 5 B), this might have something to do

with the change in retention time.

#### 3.1.4.3 Effect of flow rate

The peak efficiency of sennoside A was significantly affected by the change in mobile phase flow rate, when the flow rate was slowed from 1 ml/min to 0.5 ml/min. This is because the content of sennoside A lowers the sennoside B for quantifying the sennoside A. The flow rate of the mobile phase shifted to 0.5 ml/min, as shown in (Figure 5 D).

#### 3.1.4.4 Effect of column temperature

The change in column temperature from 20°C to 40°C

shows the change in the retention time of sennoside A and sennoside B. So, the method was developed at 40°C to achieve the desired retention time and high plate numbers.

Limit of detection and Limit of quantification (LOD & LOQ) LOD and LOQ were obtained for sennoside A and sennoside B, which cause a peak height three times and ten times the height of baseline noise, respectively [12] [13]. According to earlier parameters, LOD and LOQ were estimated to be 18.94 ppm and 57.38 ppm, respectively, as shown in Table 3.

Concentration (PPM)	Area
18	169604
21	252546
25	323393
29	382415
32	434010
Slope	18233
SD	104624
LOD	18.94 PPM
LOQ	57.38 PPM
Regression Coefficient( $R^2$ )	0.9935
Y-Intercept	18,362.9017x - 1,54,758.6204

Table 3: Linearity, LOD and LOQ

#### 3.1.6 Stability of solutions

The stability of solutions was tested with standard solutions that were stored at 4°C and room temperature (about 25°C) for 24 hours. After each storage condition,

the concentration of sennoside A and sennoside B was analyzed, and the results were compared to freshly prepared samples, as shown in Table 4.

(sennoside A + sennoside B)			
Hour	Area	SimilarityIndex	% RelativeDifference
Initial	354398	0.985474	-
03	359622	0.999497	0.01
06	359803	1.020579	0.02
09	352548	0.972428	0.01
12	362544	1.012308	0.02
18	358136	0.995148	0.01
24	359882	0.985474	0.02

Table 4: Solution Stability Data

### 3.1.7 Force Degradation

The sample of Senna tablets was provided with an addition of external stress, and the sample was admixed with 1 M HCL (Acid), 1 M NaOH (Alkali), and 10% H<sub>2</sub>O<sub>2</sub> (Oxidation)

separately; the observed degradation was found to be 13.12%, 11.72%, and 16.47% respectively. The sample was also tested for photo stability and kept in light for 24 hours. After 24 hours, the sample was analysed and degraded by 27.09%, as shown in Table 5.

(sennoside A + sennoside B)		
Conditions	% Assay	% Degradation
1M HCL	86.88	13.12
1M NaOH	88.28	11.72
10 % H <sub>2</sub> O <sub>2</sub>	83.53	16.47
Photo Stability	72.91	27.09

Table 5: Forced Degradation Study

### 3.2. Statistical Analysis

Data analysis was performed by using the formula (Average area of sample / average area of standard × weight of standard used / dilution of standard solution × dilution of sample solution / weight of sample × average weight / label claim × potency) and by the implementation of various statistical models (First-order kinetics, Zero-order kinetics, Korsmeyer-Peppas model, and Higuchi model) for assessing the releasing rate of Senna tablet, from which some models were described below as shown in Table 6,

Table 7, and (Figure 5) [14] [15] [16]. The release pattern of the Senna tablet follows the first-order kinetics model that is used to define the elimination and absorption of samples. The elevation of the releasing of the Senna tablet concerning time is shown in (Figure 6), and the obtained records are designed between log cumulative percentage of remaining drug versus time that will provide a straight line, and the slope will be given as -K/2.303 as shown in (Figure 6 A). The rest of the models observed were displayed in (Figure 5).

Dose (mg)	Time (in Hours)	CDR	Qt	$\sqrt{t}$	ln T	ln CDR
10	0	0	12	0	0	0
10	0.25	1.6	10.4	0.5	1.38629	0.470003629
10	0.5	3.4	8.6	0.707107	0.69315	1.223775432
10	0.75	4.3	7.7	0.866025	0.28768	1.458615023
10	1	5.8	6.2	1	0	1.757857918
10	2	8.8	3.2	1.414214	0.693147	2.174751721

Table 6: Calculation of Kinetics

First-order release model		Zero-order release model		Korsmeyer-Peppas model	
Time (hr.)	ln Qt	Time (hr.)	CDR	ln T	ln CDR
0	2.484907	0	0	0	0
0.25	2.341806	0.25	1.6	-1.38629	0.470004
0.5	2.151762	0.5	3.4	-0.69315	1.223775
0.75	2.04122	0.75	4.3	-0.28768	1.458615
1	1.824549	1	5.8	0	1.757858
2	1.163151	2	8.8	0.693147	2.174752

Table 7: First-order release model, Zero-order release model, and Korsmeyer-Peppas model

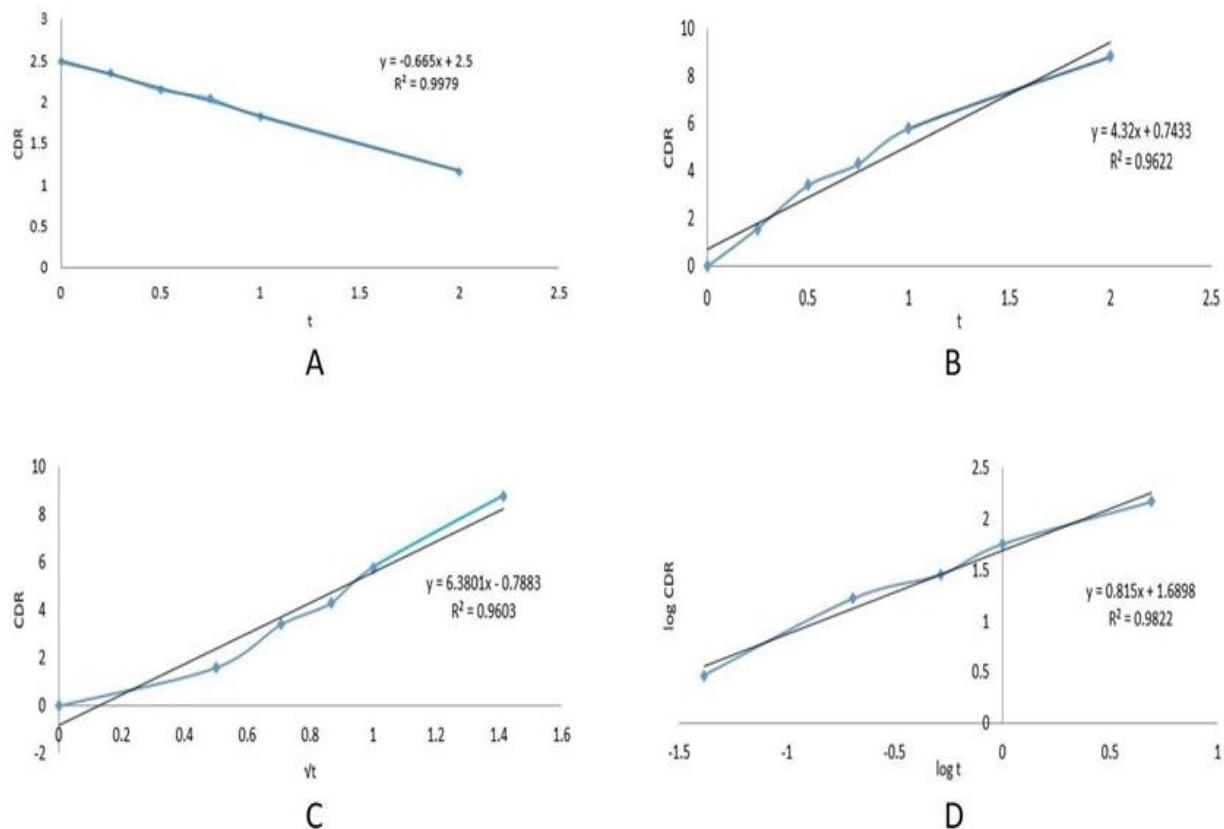
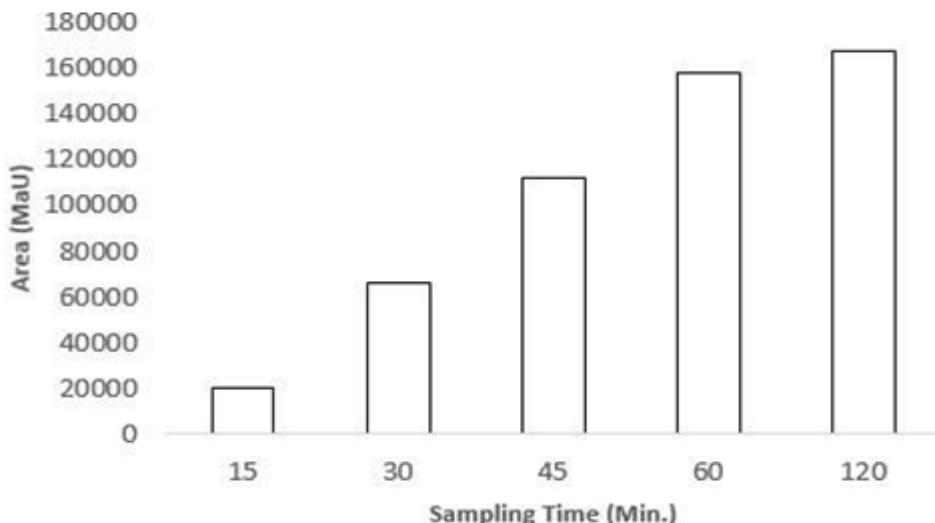


Figure 5: Bar graph of First-order kinetics (A), Zero-order kinetics (B), Higuchi plot (C), Korsemeyer-Peppas plot (D)

**Figure 6: Sample Area**

### 3.3. Assay of the tablets

The average weight of a tablet is  $139.3 \pm 5.0$  mg. The tablet contains  $1.93 \pm 0.01$  mg (n=3) of sennoside A and  $5.44 \pm 0.03$  mg (n=3) of sennoside B. The tablet's combined weight of sennoside A and sennoside B is  $7.37 \pm 0.02$  mg (n=3).

#### Discussion:

The developed method was validated for the estimation of sennoside A and sennoside B in the Senna tablet by the RP-HPLC method. [17] [18] The current method was developed with the help of a Hypersil C-18(150× 4.6 mm, 3.5 $\mu$ m) column, using the mixture of 75 volumes of a 1 percent v/v of glacial acetic acid and 25 volumes of acetonitrile v/v and 10  $\mu$ l of tetra n- butyl ammonium hydroxide was used as a mobile phase. The flow rate was kept at 0.5ml per min. for the optimum analysis condition. An injection volume of 10 $\mu$ l was used to inject the sample. The detection was done using a DAD detector with the  $\lambda_{max}$  of 350 nm. The time of retention of peaks was found to be at 4.3 and 6.2 minutes for sennoside B and sennoside A, respectively.

The linearity range was set at 18-32  $\mu$ g/ml; where a good resolution and peak shape were found with a good correlation coefficient value. The recovery was done at three different levels 80%, 100%, and 120%. The method was found entirely suitable, the precision was carried out by repeated injection of the same sample, and the SD was found to be 104624. The intra-day and inter-day precision was also carried out and the percentage RSD was found to be less than 2%. The robustness of the developed method was countered by variations in column size, ratio of organic modifiers, flow rate, and column temperature [19] [20]. The forced degradation study of the sample was also analyzed by providing some external stress to the sample with 1M HCl (Acid), 1M NaOH (Alkaline), 10 %  $H_2O_2$

(Oxidation), and for photostability, and the results were found in an acceptable range. The sample was analyzed for stability by maintaining the standards at a temperature of 25°C for 24 hours, and no significant variations were found in the concentration of sennoside A and sennoside B. The LOD and LOQ are 18.94 and 57.38  $\mu$ g/ml respectively. The method validation results of all nine factors are satisfactory limits.

This method is fully compatible with resolving both principal compounds sennoside A and sennoside B within 15 minutes of retention time. Further, the developed method was utilized to assay the sennoside A and sennoside B in the Senna tablet. The dissolution apparatus was fully calibrated according to the dissolution conditions for the USP type 1 apparatus. HPLC-grade water with a pH of 7.0 was used as the dissolution media, and the temperature was maintained at  $37 \pm 0.5$  °C with 100 rotations per minute. Samples were withdrawn from the dissolution apparatus at 15, 30, 45, 60, and 120 minutes while maintaining sink conditions, as shown in Figure 6. The retention times of sennoside A and sennoside B were consistent with those obtained during validation. Then, the statistical calculation was done by implantation of the various mathematical models. Based on these models, the drug release rate of the Senna tablet follows first-order kinetics. This is the best condition for any tablet to release the drug concerning the time. Hence, the developed method based on RP-HPLC can be utilized to assay the Senna tablet by dissolution method, and improvement of the quality of the drug during production can be maintained.

#### Conclusion:

The current work describes the developed method for the estimation and determination of sennoside A and sennoside B in standards and Senna tablets on RP-HPLC.

Based on the literature survey, we have decided to develop a new method that would be fast, economical, and reliable. The method was validated leading by the ICH guidelines. The sennoside A and sennoside B both were resolved on Hypersil C-18 (150× 4.6 mm, 3.5 $\mu$ ) column using the mixture of 75 volumes of a 1 percent v/v of glacial acetic acid and 25 volumes of acetonitrile v/v and 10  $\mu$ l of tetra n- butyl ammonium hydroxide as mobile phase and on a flow rate of 0.5ml/min., and the  $\lambda_{max}$  was set at 350 nm. The percentage RSD for the tablet analysis was less than 2%. The percentage RSD of sennoside A and sennoside B for injection reproducibility and interday precision was < 2% indicating a high grade of precision. The outcomes of the robustness study also indicated that the developed method is fully robust and is unaffected by slight variations in the chromatographic conditions. The result of the ruggedness study was found to be acceptable. Hence, the validation results were achieved in a suitable range, and the developed method was found to be fast and economical for the analysis of sennoside A and sennoside B in the Senna tablet. Therefore, we can say that the developed method on RP-HPLC is precise, accurate, and selective, and will successfully work for the immediate

#### Conflict of interests

All authors declare that they have no conflicts of interest.

#### Statement of ethics

Not applicable.

#### Availability of data and materials

Not applicable.

#### Funding

None.

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