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Evaluation of various marketed formulations of senna by RP-HPLC and HPTLC

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Abstract
In the present study, an optimized RP-HPLC and HPTLC validated methods was established for quantification of Sennosides, the major constituents of present in selected five marketed Senna herbal formulation with a simple and accuracy. It also carried out dissolution study on Senna herbal tablets and capsules. Sennosides A and B were determined using the external standard calibration method. The solvent impact on the selectivity factor and partition coefficient parameters evaluated and LOD, LOQ were calculated. The reliability of the HPLC and HPTLC methods for analysis of Sennosides A and B was validated through its linearity, reproducibility, repeatability, and recovery. Fina1ly marked herbal formulation standardized by assay of Sennosides A and B through above HPLC and HPTLC validated method. By above method, determination of Sennosides in Senna herbal formulation carried out and the results suggested that all the five selected herbal formulation posses Sennosides with the limits.

Keywords: Sennosides A, B HPLC and HPTLC validated method

Introduction
India has a rich history of use of herbs and herbal formulations, both from traditional wisdom as well as cultural usage. Herbs and herbal products are regulated by various laws to maintain pharmaceutical standards (Indian pharmacopoeia, 2014) [5]. Herbs are plant parts which are not ready to use as such medicines because of their physical nature, hence these collected materials need to be separated and processed using different types of techniques, formulated by using modern pharmaceutical excipients results in development of herbal formulations. Commercial production of herbal medicines and their trade are fast growing sector of industry today, due to increasing demand of medicinal plants; the supply line is adversely affected leading to the adulteration and substitution for genuine drugs (Nasrollah *et al.,* 2014; Kokate *et al.,* 2009) [9, 6]. However, some of the plants used in herbal medicines can also be highly toxic, so assessment of the safety of herbal products, therefore, is the first priority in herbal research (Munira *et al.,* 2005) [8]. Senna is an FDA approved nonprescription laxative which is used to treat constipation. The majority of herbal formulations, pre formulation and post formulations studies are lacking. An extensive literature survey was carried out for analytical evaluation of herbal formulations. Hence an attempt was made to compare Senna formulations using standards, powders, capsules and tablets. With the growing need for safer drugs, attention has been drawn to their quality, efficacy of Ayurvedic formulations (WHO Monographs, 1999) [20]. The quality of herbal products which varies with the time under the influence of environmental factors, such as temperature, humidity, moisture, other ingredient or excipients in the dosage form and microbial contamination. Therefore quantitative evaluation of the herbal drugs can serve as an important tool. In the present study, Sennosides content in five different marketed formulations of Senna are determined by different analytical methods to assure their quality, safety and efficacy.

Material and methods
Herbal Formulations Selected for the Study
The Calcium standard Sennoside A and B were purches from Yucca Enterprises (Mumbai), and five herbal formulations selected for the study were procured from local market they are: Senasof Tablets (60mg) - Wanbury Ltd (Mumbai), Kayam Churan Tablets (500 mg) Sheth Bros (Gujarat), Senna Hills Capsules (450 mg) Herbal Hills (Mumbai), Sonamukhi Choornam (100 gm) Maddi Pharmaceuticals (Secunderabad), and Seena-Patta Herbal Powder (100 gm) - Munnalal Dawasaz (Hyderabad).

Chemicals and instuments

All the chemicals and reagents used in different processes were procured from Merck India and Sigma-Aldrich chemicals private limited-Mumbai. Instruments used were Shimadzu liquid chromatography system, an LC -10 AT model pump, and Diode Array Detector and HPTLC (M/s Anchrome and Camag Ltd.).

Dissolution

Dissolution was carried out as mentioned in the IP monograph (Vijay *et al.,* 2011; Rajashree *et al.,* 2014) [17, 11].

Quantitative evaluation of marketed formulations of Senna by HPLC method

HPLC Method

Shimadzu liquid chromatography system, an LC -10 AT model pump, and Diode Array Detector and Analysis was performed on a reverse phase C_{18} column (300 X 3.9 mm); particle size 10 µm. The composition of the mobile phase was optimized by trial and error. Mobile phase is optimized as methanol:water: acetic acid: tetrahydrofuran (48:50:1:1); flow rate 0.8 ml/min; detector wavelength at 254 nm. (Beat *et al.,* 2014; Mohibbe *et al*., 2002; Douglas *et al.,* 2007; Olusegun 1981) [1, 7, 3, 10].

Preparation of Standard Solutions

Standard solution (1mg/ml) of different concentrations (10-60 µg/ml) was prepared using millipore water. Different concentrations of the standards were injected into the HPLC by using the Mobile phase.

Preparation of Sample Solutions

Samples of different herbal formulations were finely powdered and weighed (1 gm each) and extracted with water. Extract diluted up to 100 ml with water. Samples were filtered through millipore filter (0.2 µm) and a known amount of each extract was injected to HPLC analysis. The content of Sennosides present in extracts was calculated using calibration graph of each compound.

Assay of Senna Tablets

It was determined by liquid chromatography. Senna Tablets contain not less than 85.0 percent and not more than 115.0 percent of the stated amount of Sennosides, calculated as Sennoside B.

Assay of Senna Capsules

It was determined by liquid chromatography. Senna capsules contain not less than 85.0 percent and not more than 115.0 per cent of the stated amount of Sennosides, calculated as Sennoside B.

Assay of Senna Powder

It was determined by liquid chromatography

Test solution: Weigh accurately 1.0 gm of the coarsely powdered substance in a round bottom flask, add about 10 ml of 1 percent v/v acetic acid and 25 ml of methanol and reflux on a water bath for about 30 minutes. Cool to room temperature and make up the volume up to 50 ml with methanol and filter.

Reference solution: A 0.004 per cent w/v solution of Calcium Sennosides in methanol.

Quantitative evaluation of marketed formulations of Senna by HPTLC Method

HPTLC Method

The HPTLC system consisted of a CAMAG Linomat IV-automatic spotting device, a CAMAG twin-trough glass chamber (20 X 10cm), a CAMAG TLC scanner-3, CAMAG WinCATS-4 software version, and a 100ul HPTLC syringe (SGE, Australia).

Chromatographic Parameters

Chromatography was performed on silica gel $60F_{254}$ HPTLC layers (10 x 10 cm; 0.3 mm layer thickness).Samples and standard compounds of known concentrations were applied as 6 mm wide bands using automated TLC applicator with nitrogen flow providing delivery speed 150 ŋl/s from the syringe. These parameters were kept constant throughout the analysis (Wagner *et al.,* 1996) [18].

Preparation of Standard Solutions

Standard solutions of Sennoside A and Sennoside B (1 mg/5 ml) were prepared using millipore water. Standard graph was plotted in the concentration of 200-1000 ng/ml.

Extraction of Samples

Each 4 gm of different herbal branded formulation was sonicated with 70% methanol (3 x 20 ml) for about 45 min. Then the extract was filtered in a Buchner funnel using Whatman No. 1 filter paper and was concentrated under vacuum in a rotary evaporator at 50°C, redissolved in methanol and finally reconstituted in 20 ml methanol prior to HPTLC analysis (Shailesh *et al.,* 2000) [13].

Detection and Quantification of Sennosides

After completion of sample application, the plate was developed in a Camag Twin through glass tank presaturated with mobile phase of 2-propanol: ethyl acetate: water: formic acid (17:19:12:2) for one hour. The TLC was performed under laboratory conditions of 25 ± 2°C and 60% relative humidity. After development the plates were taken off and dried. Sennosides A and B were quantified using a Camag TLC Scanner model 3 equipped with Camag Wincats software applying the following conditions: slit width 6 x 0.45 mm**,** wavelength (λmax) 350 nm**,** and absorption reflection scan mode. The identification of Sennosides A and B in formulations was confirmed by superimposing the UV spectra of samples and standards within the same R_f window (Upadhyay et al., 2011) [15].

Validation Method for HPLC and HPTLC

The methods for HPLC and HPTLC validation according to ICH guidelines are depicted below:

Linearity and Range

The linearity of the method was evaluated by analyzing a series of standard solutions .Five different concentrations of standard solutions in the range of 10-60 and 200-1000 µg/ml were tested in HPLC and HPTLC respectively, and each was injected in triplicate. After both chromatograms value obtained, a standard calibration curve was plotting by using the concentration of standard solutions versus peak area and correlation coefficient and regression line equations were determined (Bernard *et al.*, 1986) [2].

Specificity
The specificity of the method was studied by analyzing standards and formulation by injecting three times. The peaks of Sennoside A and B in formulation were confirmed by comparing retention time (R_t) values with that of reference standard (Shao-Wen *et al.,* 2002) [14].

Accuracy
To check the accuracy of the method, recovery measurements were performed by the addition of the standard drug solution at three different levels (50, 100, and 150%) to the preanalyzed sample solution (10, 20 and 30 µg/ml) in HPLC, Whereas HPTLC pre analyzed sample solution (200,400 and 600 ng/ml) band, so that after the addition of standards, the samples would be in the linear range. Three replicate estimations were carried out for each concentration level in both HPLC and HPTLC. The percentage recovery of standard from the proposed method was calculated.

Precision
To study intraday and interday precision, three different concentrations of standard solutions were prepared (10, 20 and 30 µg/ml) and injected into HPLC. In case HPTLC three different concentrations of standard (200, 400, and 600 ng/band) and applied to the TLC plates. All the solutions were analyzed in triplicate on the same day and on different day to record intraday and interday variations respectively. The percentage recovery of standard from the proposed method was calculated.

Repeatability
The repeatability of HPLC method was assessed by injecting 10, 20 and 30 µl/ml of standard respectively in triplicate (n=3) as per proposed chromatographic conditions. The average, standard deviation (SD) and percentage relative standard deviation (% RSD) of peak area was calculated. The repeatability of sample application was in HPTLC assessed by spotting 10 µL of 200,400 and 600 ng/spots of standard respectively on TLC plate (n=3).The plate was developed and scanned as per proposed chromatographic conditions. The average, standard deviation (SD) and percentage relative standard deviation (% RSD) of peak area was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
To estimate the limits of detection (LOD) and quantification (LOQ), the calibration curve plotted was used for determination of LOD and LOQ. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation. LOD and LOQ were calculated using the equations:

$LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$

Where, σ is the standard deviation of the response taken as a measure of the noise and S is the slope of the corresponding calibration plot.

Results
Dissolution profile of Senna tablets and capsules
The dissolution and percentage release of drug from the tablets and capsule were is depicted table 1.

Assay of Senna tablets, capsules and powder.
The proposed method was successfully applied to the analysis of marketed products (Senna tablets, capsules and powder) by HPLC and HPTLC, and the results obtained are given in Table 2.

HPLC Method Optimization
Different compositions of the mobile phase were attempted from earlier reports and the desired resolution of the Sennosides with symmetrical and reproducible peaks and a stable baseline was achieved by HPLC method performed by (Gupta *et al.,* 1996; Reich *et al*., 2007) [4, 12] with few modifications. In brief the mobile phase used was methanol: water: acetic acid: tetrahydrofuran (60:38:2:2) at UV detection 254nm with a flow rate of 0.8 ml/min. Peaks corresponding to Sennoside A and B were symmetrical, sharp, well resolved and reproducible with retention times as 11.89 and 7.59 min respectively. Linear regression data showed a good linear relationship of Calcium Sennoside A and B. The concentration range of 10-60 µg/ml of Sennoside A with calibration curve of Y= 5795.8x – 5646.1 and correlation coefficient (r^2) was found to be 0.9957 and Sennoside B with calibration curve of Y= 6000.2x – 10035 and correlation coefficient (r^2) was found to be 0.9991.

HPTLC Method Optimization
Different compositions of the solvent systems were attempted from earlier reports and the distinct separation of the Sennosides was achieved by HPTLC method performed by (Wasim *et al.,* 2008) [19] with few modifications. In brief the solvent system used was 2-propanol: ethyl acetate: water: formic acid (17:19:12:2) at UV detection 350nm. Spots corresponding to Sennoside A and B were distinct, well separated with retention factor as 0.84 and 0.63 respectively. Linear regression data showed a good linear relationship of Calcium Sennoside A and B. the concentration range of 200-1000 ng/ml of Sennoside A with calibration curve of Y= 14.623x + 311.49 and correlation coefficient (r^2) was found to be 0.9991 and Sennoside B with calibration curve of Y= 14.726x + 171.97 and correlation coefficient (r^2) was found to be 0.9997.

Validation of HPLC and HPTLC Results
Linearity, Range, LOD, and LOQ.
The calibration curve of Calcium Sennosides A and B were plotted using peak area vs. concentration. The method has shown good linearity for Sennoside A and Sennoside B, in the concentration range of 10 - 60 µg/ml (200 - 1000 ng/ml in HPTLC). The LOD, LOQ, slope and intercept were mentioned in the (Table-3).

Accuracy
Accuracy of the method was ascertained by recovery method. The samples of Sennosides A and B were prepared in triplicate (n = 3) and analysis was carried out by the proposed method in both HPLC and HPTLC. The percentage recoveries along with SD and RSD values were calculated and mentioned in the (Table 4)

Precision
Intra-day and inter-day precision
To determine intra-day and inter-day precision, three different concentrations of Calcium Sennoside A and B were prepared in triplicate (n = 3) and analyzed at specific time intervals in the same day and different day. The SD and RSD were calculated and mentioned in the (Table: 5 and 6).

Discussion

According to Indian Pharmacopoeia (IP), not less than 75% of the labeled amount of Sennosides should dissolve in 120min.The tablets and capsule have shown percentage of drug release more than 75% of the labeled amount of Sennosides within 120min(shown in table 1). Hence the all the selected powder, tablet and capsule of Senna herbal formulations have passed the test.

In the present study, an optimized RP-HPLC and HPTLC validated method was established for quantification of Sennosides, the major constituents of Senna herbal formulations. When see the assay values of herbal formulation of Senna (shown in table2), the test samples is not valid unless the relative standard deviation (RSD) for the replicate injections is not more than 2.0 percent. All five selected powders, tablets and capsule of marketed Senna formulation have passed the test because RSD values found to be less than 2.0%.

Estimation of Sennosides A and B carried out by the HPLC and HPTLC methods. in HPLC analysis mobile phase used as methanol: water: acetic acid: tetrahydrofuran (60:38:2:2) at UV detection 254nm with a flow rate of 0.8 ml/min. Peaks corresponding to Sennosides A and B were symmetrical, sharp, well resolved and reproducible with retention times as 11.89 and 7.59 min respectively. By this view we can say method is optimized. Similarly in HPTLC also shows the symmetrical, sharp, well resolved and reproducible with retention times of Calcium Sennosides A and B.

In both optimized RP-HPLC and HPTLC methods, the %RSD of regression coefficient was found to be very less (<2%), which is within in the limits of ICH guidelines and the method's linearity was proved to be reproducible. The SD and RSD of Sennosides A and B were less than 2% which is the limit as per ICH guidelines, which suggested that the present developed method is accurate.

All the three different concentrations of Calcium Sennosides A and B were shown (table 5 and 6) insignificant RSD and SD which indicates that the both optimized RP-HPLC and HPTLC methods has good repeatability and hence the method proved to be precise.

Table 1: Dissolution profile of tablet and capsule of marketed Senna herbal formulation

Time (min)	Name of marketed Senna herbal formulation					
	Senasof	Kayam	SennaHills	Senasof	Kayam	Senna Hills
	Assay (mg)			% release of drug		
5	0.616±0.01154	5.922±0.001	2.954±0.001	1.012±0.001	1.185±0.002	0.435±0.00305
10	1.313±0.0152	29.863±0.001	0.435±0.0005	2.197±0.001	5.977±0.01	0.8136±0.002
15	1.92±0.01	53.816±0.01	0.813±0.001	3.180±0.0283	10.763±0.022	1.013±0.001
30	3.08±0.01	84.93±0.022	1.013±0.001	5.15±0.021	16.986±0.001	1.586±0.002
45	4.743±0.0208	104.4±0.2	1.585±0.001	7.934±0.0483	20.890±0.0072	1.853±0.00208
60	7.13±0.0152	149.63±0.378	1.854±0.001	11.876±0.0194	29.845±0.001	2.075±0.0537
90	8.72±0.01	184.53±0.125	2.074±0.001	14.574±0.01101	36.907±0.01	2.5863±0.0025
120	9.84±0.01	229.6±0.1	2.585±0.001	16.428±0.001	45.985±0.001	2.928±0.0519
Total	10.77±0.01	235.8±1.1	13.275±0.001	17.964±0.007	47.166±0.0577	2.955±0.0294

Table 2: Percentage content of Sennosides in five selected Senna herbal formulation

S.no	Herbal Formulation Name	content of sennosides estimation by HPLC		content of sennosides estimation by HPTLC	
		Mean±SD (n=3)	RSD%	Mean±SD(n=3)	RSD%
1	Seena-Patta	3.436±0.11547	0.3359	3.26±0.057735	1.76739
2	Sonamukhi	5.136±0.11547	0.22481	4.126±0.00577	0.1399
3	Senna Hills	2.955±0.02946	0.9970	2.426±0.005774	0.237919
4	Senasof	17.964±0.007	0.038967	16.293±0.12059	0.74012
5	Kayam	47.166±0.057735	0.122406	46.081±0.07189	0.15602

Table 3: Linearity, LOD, LOQ and Range of Calcium Sennoside A & B

	Sennoside A		Sennoside B	
	HPLC	HPTLC	HPLC	HPTLC
Linear Range (µg/ml)	10-60	200-1000	10-60	200-1000
LOD (µg/ml)	6.72	37.5	1.961	21.16
LOQ (µg/ml)	20.39	113.8	5.94	64.12
Intercept	5646.1	311.49	10035	171.97
r_2	0.9957	0.9991	0.9991	0.9997

Table 4: Accuracy and percentage recovery of Sennoside A and B

Method	components	Concentration	Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)
HPLC	SennosideA	10(µg/ml)	51788.4±1.528 (0.002951)	98.92±0.004 (0.004)
		20(µg/ml)	109165.5±0.985 (0.00090)	98.98±0.008 (0.008)
		30(µg/ml)	16654482.1±1.51 (0.1100)	98.98±0.004 (0.004)
	SennosideB	10(µg/ml)	49877.07±0.79 (0.006)	99.80±0.005 (0.005785)
		20(µg/ml)	109551.46±1.22 (0.001)	99.60±0.005 (0.005796)
		30(µg/ml)	169836.37±1.25 (0.0007)	99.91±0.005 (0.005778)
HPTLC	SennosideA	200(ng/ml)	31796.83±0.99 (0.031)	98.15±0.035 (0.035)
		400(ng/ml)	6112.43±1.02 (0.0016)	98.87±0.5889 (0.595)
		600(ng/ml)	9067.12±0.99 (0.010)	98.78±0.015 (0.0153)
	SennosideB	200(ng/ml)	3092.91±0.99 (0.032)	99.20±0.030 (0.030)
		400(ng/ml)	6015.48±1.0050 (0.0016)	99.21±0.01 (0.015)
		600(ng/ml)	8931.44±1.530 (0.0171)	99.14±0.015 (0.0154)

Table 5: *Intra-day and* inter-day *precision* and percentage recovery of Sennoside A and B

Method	components	Concentration	Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)
HPLC	SennosideA INTRA-DAY	10(µg/ml)	51788.45±1.52 (0.029)	98.92±0.0471 (0.0476)
		20(µg/ml)	109165.52±0.98 (0.0009)	98.98±0.0081 (0.0082)
		30(µg/ml)	16654482.17±1.51 (0.110)	98.98±0.00471 (0.0476)
	SennosideA INTER-DAY	10(µg/ml)	51786.7±1.03 (0.0019)	98.97±0.0057 (0.0058)
		20(µg/ml)	109167.1±1.49 (0.0013)	98.98±0.0057 (0.0058)
		30(µg/ml)	16654487.2±1.50 (0.133)	98.98±0.0057 (0.00583)
	SennosideB INTRA-DAY	10(µg/ml)	49877.07±0.79 (0.0015)	99.80±0.005 (0.0057)
		20(µg/ml)	109551.46±1.22 (0.0011)	99.60±0.005 (0.0057)
		30(µg/ml)	169836.37±1.25 (0.0007)	99.91±0.005 (0.0057)
	SennosideB INTER-DAY	10(µg/ml)	4988.25±1.15 (0.002)	98.81±0.0057 (0.0057)
		20(µg/ml)	109557.13±0.79 (0.0007)	98.60±0.0057 (0.0057)
		30(µg/ml)	169839.14±0.84 (0.0004)	98.90±0.0115 (0.0115)

Table 6: *Intra-day and* inter-day *precision* and percentage recovery of Sennoside A and B

Method	components	Concentration	Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)
HPTLC	SennosideA INTRA-DAY	10(µg/ml)	31796.83±0.99 (0.031)	98.15±0.035 (0.035)
		20(µg/ml)	6112.43±1.02 (0.0016)	98.87±0.5889 (0.595)
		30(µg/ml)	9067.12±0.99 (0.010)	98.78±0.015 (0.0153)
	SennosideA INTER-DAY	10(µg/ml)	3179.22±0.56 (0.017)	98.22±0.020 (0.0211)
		20(µg/ml)	6118.44±0.99 (0.016)	99.29±0.020 (0.020)
		30(µg/ml)	9068.11±1.005 (0.011)	99.79±0.015 (0.0153)
	SennosideB INTRA-DAY	10(µg/ml)	3092.91±0.99 (0.032)	99.20±0.030 (0.030)
		20(µg/ml)	6015.48±1.0050 (0.0016)	99.21±0.01 (0.015)
		30(µg/ml)	8931.44±1.530 (0.0171)	99.14±0.015 (0.0154)
	SennosideB INTER-DAY	10(µg/ml)	3096.95±0.97 (0.031)	99.33±0.030 (0.030)
		20(µg/ml)	6018.48±0.995 (0.016)	99.25±0.020 (0.020)
		30(µg/ml)	8933.15±0.975 (0.010)	99.15±0.015 (0.0154)

Conclusion

Determination of Sennosides in Senna marked herbal formulation carried out and the results suggested that all the five selected herbal formulation possess Sennosides within the limits. The proposed HPLC and HPTLC methods can be used for determination of Sennosides A and B in various commercial samples for quality evaluation. The method is very simple, rapid and reproducible for rapid screening.

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