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# HPTLC DENSITOMETRIC QUANTIFICATION OF SENNOSIDES FROM *Cassia angustifolia*

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**Summary.** A rapid and simple high performance thin layer chromatographic method has been developed for the quantitative determination of the medicinally useful sennoside A and sennoside B from *Cassia angustifolia*. Senna (*C. angustifolia*) was cultivated as a perennial crop. A different extraction method employing a different solvent was used for the analysis of sennoside A and sennoside B in leaves by HPTLC. The mobile phase was n-propanol: ethyl acetate: water: glacial acetic acid (8: 8: 5.8: 0.2). It was noted that the percentage of active principles varied significantly in the samples procured from different solvent and found to be best in methanol. The method is simple and useful for routine analysis of the samples.

***Key words*:** Densitometric quantification; *Cassia angustifolia*; sennoside A; sennoside B; HPTLC.

## INTRODUCTION

The use of medicinal herbs to relieve and treat diseases is increasing because of their mild features and few side effects (Basgel and Erdemoglu, 2006). These plants are unlicensed and freely available, however, and there is no requirement to demonstrate efficacy, safety or quality (Ernst, 1998). *Cassia angustifolia* Vahl (Family: Caesalpinaceae), popularly known as senna, is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of constipation (Atal et al., 1982; Das et al., 2003; Martindale, 1977; Sharma, 2004). The pods and leaves of senna as well as the pharmaceutical preparations containing sennosides A and B are widely used in medicine because of their laxative properties. An accurate, simple and easy method is needed for estimation of sennosides A and B individually (Anonymous, 1992). Anthraquinone derivatives are the main active constituents of senna, which are responsible for its laxative properties.

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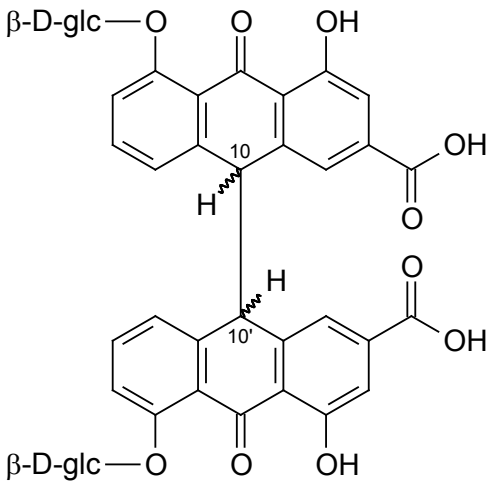
Chemically they contain Sennoside A, B, C and D (Hayashi et al., 1980), kaemferol, phytosterols (Khorana and Sanghvi, 1964), glycosides of rhein and chrysophanic acid. Although a number of synthetic laxatives are available, sennoside-containing preparations are still the most widely used (Atzorn et al., 1981). The structural formula of sennoside A and B is shown in the Fig 1.




| | $C^{10}$ | $C^{10'}$ |
|---|---|---|
| (1) Sennoside A | R | R |
| (2) Sennoside B | R | S |

Fig. 1. Structural formula of Sennoside A and B.

Senna is a strong purgative that should be taken in proper dosage otherwise it may lead to gripping and colon problem (Bhattacharjee, 2004). The leaves containing sennosides are efficient sources of health teas (Kojima et al., 2001) and are considered as astringent, cathartic, depurative, anthelmintic, cholagogue, expectorant and febrifuge, useful for leprosy, leukoderma, jaundice, typhoid fever, tumors (Warier, 1994). Various types of techniques like TLC (Lemmens, 1977), GLC (Baars et al., 1976), radioimmunoassay (Atzorn et al., 1981) and HPLC (Erni and Frei, 1978; Gorler et al., 1979; Ohshima and Takahashi, 1983; Srivastava et al., 1983) have been applied to sennoside analysis in Cassia species.

In recent years there has been greater demand for extraction techniques with less solvent consumption combined with the ability to produce extracts in relatively large quantities in less time. Many of these procedures, however, are time consuming and lack precision. The HPTLC method has also been reported (Shah et al., 2000), but these techniques are tedious and time consuming. In the present study, we investigated the efficiency of a rather simple and rapid HPTLC method for the analysis of sennoside A and B occurring in Senna.

## MATERIALS AND METHODS

### Chemicals

The standard sennoside A and B were purchased from Natural Remedies Pvt. Ltd. (Bangalore, India). Methanol, chloroform and other chemicals were of HPLC-grade (Merck, Germany). Water (Millipore) was of HPLC grade. The solvent was filtered through a 0.45μm filter before use.

### Plant materials

The experiment was conducted in the Department of Crop and Herbal Physiology at the dusty area, Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur, Madhya Pradesh during the Rabi season of 2008-2009. Sennoside A and B were determined in leaf samples collected after the harvesting period from the field. The samples were

dried at room temperature, subsequently milled into powder and stored in air-tight stopped glassware before subjected to physical and chemical evaluation of different properties.

**Preparation of standard solutions**

The standard solutions of sennoside A and sennoside B (1mg/ 5ml) were prepared in methanol. This is the stock solution. It was further diluted for preparing six points calibration curves.

**Extraction Procedure for sample**

***Maceration*:** A dried powder of leaves (2.0 g) was macerated with chloroform, ethyl acetate, ethanol and methanol solvent (10 ml each) and left for 24 h at room temperature. The material was then filtered and the crude extract was collected. The process was repeated 3 times with the solvent, then all the extracts was combined, concentrated under vacuum and the final volume was made up to 10 ml with methanol.

***Reflux*:** Each sample was refluxed and sonicated with chloroform, ethyl acetate, ethanol and methanol (3 x 10 ml) for about 45 min. Then the extract was filtered in a Buchner funnel using Whatman No. 1 filter paper and concentrated under vacuum in a rotary evaporator at 50°C, redissolved in methanol and finally made up to 10 ml with methanol prior to HPTLC analysis.

**Chromatography**

Chromatography was performed on 20 cm x 20 cm aluminium foil plates coated with 0.2 mm thickness silica gel 60 $F_{254}$ HPTLC layers (E. Merck, Darmstadt, Germany). The standard and samples were applied on the plates as 8 mm wide bands. The space between two spots was made 6 mm (10 mm from the bottom and 10 mm from the sides) by means of an automated TLC sample applicator under a flow of nitrogen gas providing delivery speed 150 µL/s from the syringe using Camag Linomat 5 automated TLC applicator ($ATS_5$) (Camag, Multenz, Switzerland). 10 µl of the solution were spotted on the plate. These parameters were kept constant throughout the analysis.

**Detection and Quantification**

After the sample application was completed, the plate was developed in a Camag Twin through glass tank presaturated with a mobile phase of 2-propanol: ethyl acetate: water: formic acid (8: 8: 5.8: 0.2) for 15 min. The TLC runs were performed under laboratory conditions of 25 ± 2°C and 60% relative humidity. After development, the plates were removed from the chamber, dried in air for 5 min and spots were visualized under UV light using a Camag UV viewer cabinet. The densitometric scanning at 366 nm was performed with a Camag TLC Scanner-3 operated by winCATS software1.4.1. The slit width was 6 mm x 0.45 mm and wave length at $\lambda_{max}$ 366 nm. Evaluation was based on the peak area with linear regression. The dried plate was dipped into freshly prepared p-anisaldehyde reagent followed by heating the plate at 110°C for 10 min. The Rf of 0.52 and 0.32 showed the presence of sennoside A and B, respectively in all samples. The identification of sennosides A and B was confirmed by superimposing the UV spectra of samples and standards within the same $R_f$ window.

**Validation**

The linearity of the method was evaluated by analyzing a series of standard solutions. Six different concentrations of standard solutions in the range of 200-1000 µg/ ml were tested and each was applied in duplicate to a plate. After chromatography a standard calibration curve was obtained by plotting the concentration of standard solutions versus peak area. The amount of sennosides A and B present in the samples were determined using the calibration curve. The correlation coefficients for sennoside A and B were found to be 0.993 and 0.998, respectively, thus exhibiting good linearity between concentration and area. The percentage recoveries of sennoside A and B were found to be 98.70 and 98.82, respectively which were highly satisfactory. The method was specific. A peak purity test of sennoside A and B was done by comparing the UV spectra of sennoside A and B in standard and sample tracks. To estimate the limits of detection (LOD) and quantification (LOQ), blank (methanol) was applied, to a plate six times and the signal-to-noise ratio (S/N) was determined. LOD and LOQ were calculated using the equations: LOD = 3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the blank (n = 6), taken as a measure of the noise, and B is the slope of the corresponding calibration plot. LOD were found to be 160 and 180 µg/spot for sennoside A and B and LOQ were found to be 500 and 550 µg/spot, respectively (Table 1).

## RESULTS AND DISCUSSION

Different compositions of the mobile phase for HPTLC were tested and the desired resolution of sennoside together with symmetrical, high resolution and reproducible peaks were obtained by using the mobile phase n-propanol: ethyl acetate: water: glacial acetic acid (8: 8: 5.8:

Table 1. Method validation data for determination of Sennoside A and B.

| Validation of HPTLC | Sennoside A | Sennoside B |
|---|---|---|
| Specificity | Specific | Specific |
| Linear range | 200-1000 µg/ml | 200-1000 µg/ml |
| Linearity (correlation coefficient) | 0.993 | 0.998 |
| Limit of detection (µg) | 160 | 180 |
| Limit of quantification (µg) | 500 | 550 |
| Robustness | Robust | Robust |

Table 2. Effect of the extracting solvent on the estimation of sennoside content.

| Extracting solvent | Sennoside A (%) | Sennoside B (%) |
|---|---|---|
| Chloroform | 0.100 | 0.570 |
| Ethyl acetate | 0.123 | 0.630 |
| Ethanol | 0.153 | 0.750 |
| Methanol | 0.188 | 0.820 |
| Average (n=3) | | |

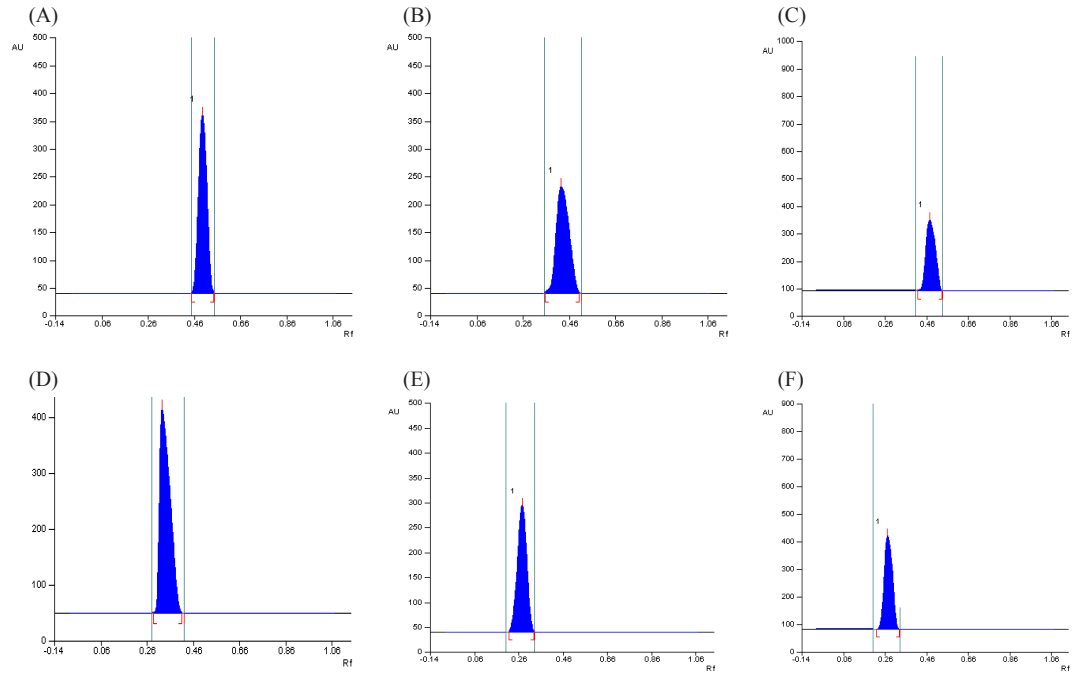



Fig. 2. HPTLC profiles of the extract containing sennosides. Densitograms of the extract of (A) sennoside A standard, (B) methanol extract, (C) chloroform extract, (D) sennoside B standard, (E) methanol extract, (F) chloroform extract.

0.2). Linearity of sennoside A and B was detected in the concentration range 200-1000 µg/ml. For the recovery rates known amounts of the stock solution were added to plant extracts and quantitative analysis replicated three times. After spraying the plate with a detecting agent the presence of sennoside A and B with Rf values of 0.52 and 0.32, respectively was visualized in all samples (Fig 2). Sennoside A and B when qualitatively estimated were found to be present in all samples though their quantity

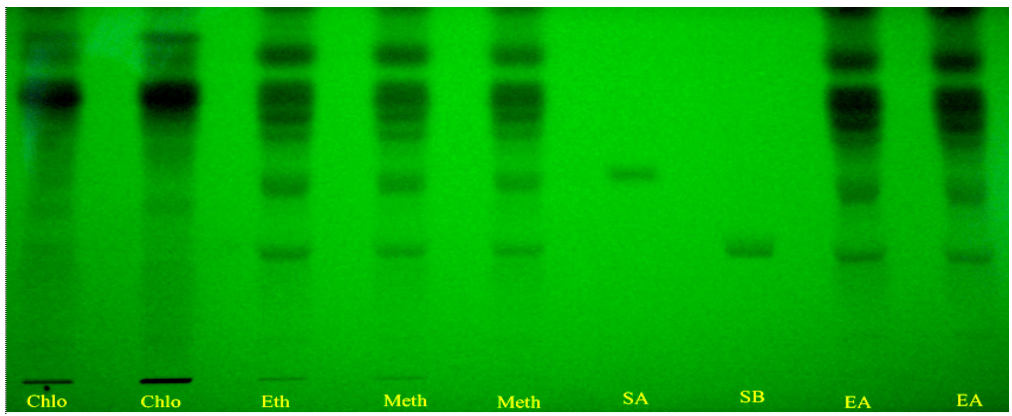



Fig. 3. HPTLC fingerprint profile of different samples of Cassia angustifolia leaves procured from different solvents along with the active constituents Sennoside A and B (Chlo – chloroform, Eth – ethanol, Meth – methanol, SA- sennoside A, SB – sennoside B, EA – ethyl acetate).

varied. Different solvents of varying polarities were used for the extraction of sennosides from the plant material and the averages of three replicates are presented in Table 2. Less extraction of sennosides was obtained with chloroform and ethyl acetate. More efficient extraction was obtained with ethanol and methanol, the maximum being with the latter solvent. So methanol was found to be the most appropriate solvent for the maximum extraction of sennosides (Fig 3). Different methods were used for the extraction of sennosides from the plant material and it was found that the reflux method was best. Similar results were reported by Paiva et al. (2004).

## CONCLUSION

The proposed HPTLC method can be used for determination of sennoside A and B in various commercial samples for quality evaluation. The method is very simple, rapid and suitable for rapid screening of plant materials. Different solvents of varying polarity have been applied for the extraction and methanol was found suitable for the most efficient extraction. Among the various methods used, the reflux method was the best one. The proposed solvent system and the scanning wavelength were found suitable to identify and estimate sennosides.

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