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Reverse-phase High Performance Liquid Chromatography of Sennosides in *Cassia angustifolia*

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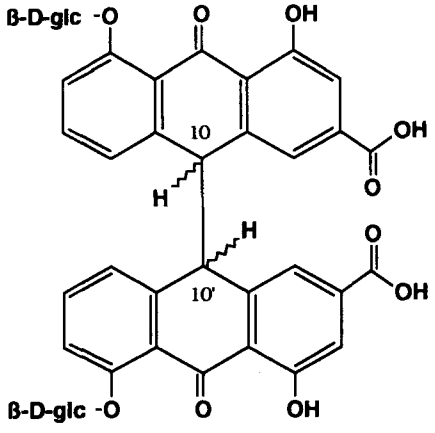
A rapid and simple reverse-phase high performance liquid chromatographic method has been developed for the quantitative estimation of the medicinally useful laxative principles sennoside A and sennoside B in senna. Using a Bondapak C_{18} column eluted with methanol:water:acetic acid:tetrahydrofuran (60:38:2:2) both compounds were well resolved with recoveries of 97 and 96% respectively.

Keywords: laxative; sennoside A; sennoside B; reverse phase high performance liquid chromatography; *Cassia angustifolia*; Caesalpinaceae.

INTRODUCTION

Leaves and pods of *Cassia angustifolia* Vahl (syn. *C. senna* L.) Caesalpinaceae are used as laxatives. Sennoside A (**1**) and sennoside B (**2**), the stereoisomers of rhein dianthrone with two glucose molecules, are the major compounds



	C10	C10'
(1) Sennoside A	*R*	*R*
(2) Sennoside B	*R*	*S*

which impart strong laxative properties. Although, a number of synthetic laxatives are available, sennoside-containing preparations are still the most widely used (Atzorn *et al.*, 1981). *C. angustifolia* is cultivated extensively in the Ram Nath Puram district of Tamilnadu (Husain *et al.*, 1992). During our crop improvement programme a quick, sensitive and accurate analytical method is required for the analysis of a large number of plant samples. Different techniques such as thin layer chromatography (TLC) (Lemmens, 1977), gas–liquid chromatography (Baars *et al.*, 1976), radio-immunoassay (Atzorn *et al.*, 1981) and high-performance liquid chromatography (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* spp. Many of these procedures, however, are time consuming and lack precision. Our continued interest in plant drug analysis using HPLC (Gupta *et al.*, 1993; Verma *et al.*, 1990) led us to develop a rapid, sensitive and accurate reverse-phase HPLC technique for the analysis of **1** and **2** in *C. angustifolia*. The method was applied to the analysis of a number of genotypes identified during our crop improvement programme for senna cultivated in Lucknow representing North Indian subtropical conditions.

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EXPERIMENTAL

Plant material. Seeds of *C. angustifolia* were collected from Trinell Veli, Tamilnadu for crop propagation at the experimental field station of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow.

Reagents. The reagents used were HPLC-grade (Spectrochem, Bombay, India) and were filtered through a Millipore filter (0.5 μm) as required. Sennosides A and B were obtained from Sandoz (Charlotte, NC, USA). Water used was deionized and double distilled using a glass apparatus.

Apparatus. A Waters (Milford, MA, USA) modular HPLC system consisting of a U6K injector, an M-6000 A pump, an M-450 variable wavelength detector and an M-730 data module was used. Analysis was performed on a reverse-phase μ-Bondapak C_{18} column (300 × 3.9 mm i.d.; particle size 10 μm; Millipore-Waters).

Chromatographic conditions. The composition of the mobile phase was optimized by varying the percentage of methanol and water. The following conditions gave the best results: mobile phase—methanol:water:acetic acid:tetrahydrofuran (60:38:2:2, v/v/v/v); flow rate—0.8 mL/min; column temperature—ambient (25 °C); detector wavelength—254 nm; detector sensitivity—0.04 aufs.

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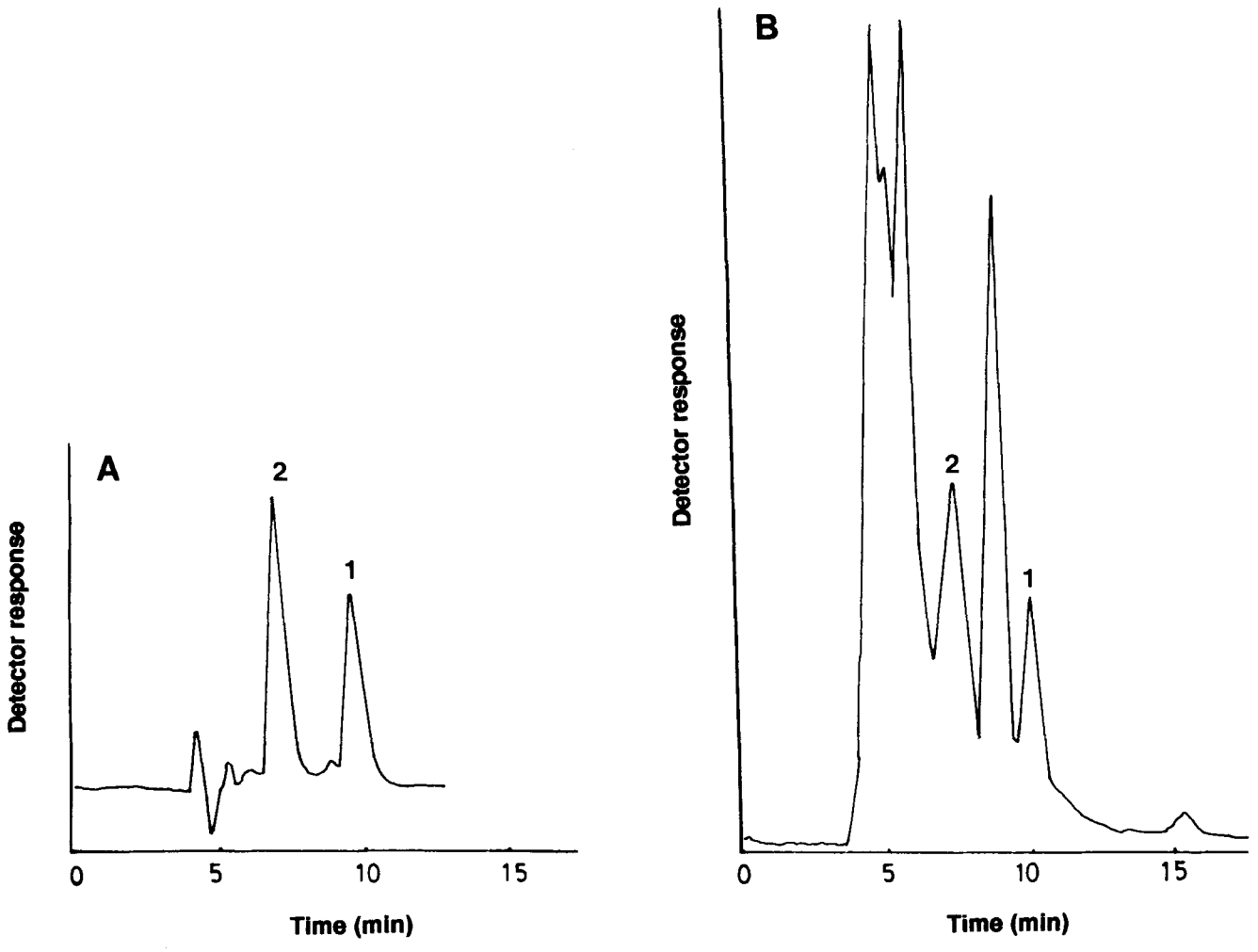

Figure 1. HPLC separation of sennoside A (**1**) and sennoside B (**2**) on a μ-Bondapak C_{18} column eluted with methanol:water:acetic acid:tetrahydrofuran (60:38:2:2): UV detection at 254 nm. **A**, Chromatogram, obtained with pure standards; **B**, chromatogram of a extract of *Cassia angustifolia*. (For chromatographic conditions see Experimental section.)

Calibration graphs. Standard solutions (1 mg/10 mL) of **1** and **2** were prepared in methanol. Different amounts of these standards were injected into the HPLC using the chromatographic conditions described above. The area counts of peaks (y) and the corresponding concentrations (x) were used to plot the calibration graphs. The graphs were linear in the range of 2–50 μg for both **1** and **2**. The regression equations were $y=53.15x+21.3 (r=0.99)$ for sennoside A (**1**) and $y=152.05x+22.1$ ($r=0.99$) for sennoside B (**2**).

Extraction procedure. Samples of plants of different genotypes (1.0 g each) were finely powdered and extracted with water (3 × 25 mL each). The extracts thus obtained were made up to 100 mL with water. Samples were filtered through a Millipore filter (0.5 μm) and a known amount of each extract was subjected to HPLC analysis under the above conditions. The contents of **1** and **2** were calculated using calibration graphs of each compound.

RESULTS AND DISCUSSION

Different compositions of the mobile phase were tested and the desired resolution of the sennosides with symmetrical and reproducibile peaks and a stable baseline was achieved by using methanol:water:acetic acid:tetrahydrofuran (60:38:2:2) as mobile phase (Fig. 1). Peaks corresponding to sennoside A (**1**) and sennoside B (**2**) were sharp and well resolved with retention times of 9.88 and 7.22 min respectively. The resolution factor between the two peaks was 0.52. As an effective measure of column performance, the number of theoretical plates for **1** and **2** were 579 and 1084, respectively. Recoveries of sennoside A and sennoside B were calculated by spiking the extract with a stock solution in the mobile phase and were found to be 97% and 96%, respectively. The sennoside contents in different genotypes of senna cultivated in Lucknow are given in Table 1.

In conclusion, the HPLC method described here is efficient and simple for the separation and determination of sennoside A and sennoside B in plant extracts. The method is an improvement over previous methods since the retention times of both compounds is reduced, with

Table 1. Contents of sennoside A and sennoside B in five genotypes of *Cassia angustifolia*

Genotype	Sennoside A(%)[a]	Sennoside B(%)[a]
12-121	2.44±0.03	1.06±0.03
12-7	1.03±0.02	ND[b]
CIMAP-L	1.26±0.01	1.03±0.02
G19	1.49±0.01	0.88±0.03
G27	0.89±0.04	ND

[a] Percentage composition on dry weight basis±SE: $n=5$.
[b] ND, none detected.

excellent resolution, which is desirable for column-life and for assay efficiency.

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