

0063 - Senna for Sennosides by HPLC

Botanical Name: *Cassia angustifolia* Vahl, *Cassia acutifolia* Delile, *Senna alexandrina* Mill., *Cassia senna* L.

Common Names: Alexandrian senna, Indian senna, Tinnevely senna

Parts of Plant Used: Leaves and pods

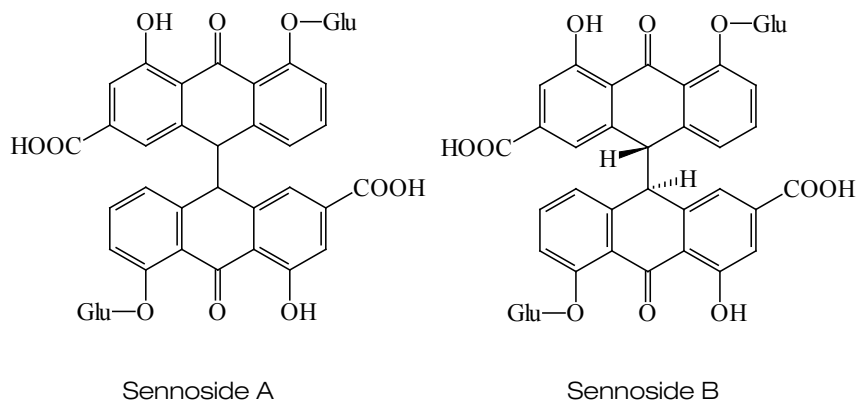
Uses: As a laxative

Modes of Action:

Senna is widely used as herbal laxative. Sennosides, diglucosides of sennidins, are the purgative principles of this plant. Sennosides are metabolized to anthrones and anthranols in the human intestine. The interaction between anthrones and immune cells of the colon has been suggested to be the basis for its laxative activity.^{1,2}

Chemical Markers:

The active components in senna leaves were first identified as sennosides A and B (dimeric products of emodin and/or rhein), which, when hydrolyzed, give the aglycones sennidins A and B and two molecules of glucose. Later work demonstrated the presence of sennosides C and D, small quantities of monomeric glycosides, and free anthraquinones. The active constituents of the pods are similar to those of the leaves but in larger quantities. Other compounds such as flavonoids and sterols also have been isolated from senna. The major flavonoids were found to be diglucosides of quercetin, kaempferol, and isorhamnetin.^{3,4} Recently, three unique compounds, tinnevellin-8-glucoside, cassiaphenone A-2-glucoside, and cassiaphenone B-2-glucoside, were identified in senna.³ Sennosides A and B are used as the marker compounds for quality control of senna products.



Methods of Analysis

Various methods have been used to analyze sennosides A and B for quality control of senna, including fluorometry, spectrophotometry, HPLC, and capillary electrophoresis.^{2,5-7} Currently, USP uses a fluorometric method to analyze the active components in senna preparations, but this method can determine only the total amount of dianthrone and anthraquinone glucosides and does not provide a detailed analysis.

Method 1:

The method of Bala et al.⁵ was used.

Sample Preparation:

Powder 1 g of leaves and extract with hexane (3 × 25 mL); discard the hexane extracts. Extract the remains with 25 mL of methanol–water (7:3) overnight at room temperature, then extract with methanol–water (7:3) (2 × 25 mL). Combine the extraction solutions and dilute to 100 mL with methanol–water (7:3).

Chromatography:

Column: Waters Symmetry C18, 5 µm, 150 × 4.6 mm.

Mobile phase: Solvent A = methanol–water–acetic acid (20:80:0.1 vol/vol/vol, pH 4), solvent B = methanol–water–acetic acid (80:20:0.1 vol/vol/vol, pH 4).

Gradient:

Time (minutes)	%A	%B
0	80	20
5	80	20
25	0	100
35	0	100
40	80	20

Flow rate: 0.6 mL/minute for the first 20 minutes, increasing to 1.0 mL/minute at 30 minutes.

Injection volume: 10 µL

Detection wavelength: 285 nm

Column temperature: 25°C

Validation Data:

Linearity: 2 to 50 mcg for sennosides A and B.

Accuracy: Not specified

Precision: Not specified

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: 0.2 mcg/mL for sennoside A and 0.1 mcg/mL for sennoside B.

Method 2:

The method of Sun and Su⁸ was used.

Sample Preparation:

Grind 10 tablets of senna preparation to powder. Dissolve 5 to 15 mg in 5 mL of sodium bicarbonate solution (1 g in 1000 mL of water).

Chromatography:

Column: Phenomenex Hypersil C18, 5 μ m, 250 \times 4.6 mm.

Mobile phase: pH 6 acetate buffer (prepared using 1 M sodium acetate and 1 M acetic acid). This buffer was diluted 10 times and mixed together with acetonitrile (70:30 vol/vol). To 1000 mL of the mixed solution, 2.17 g of tetrahexylammonium bromide was added and the resulting solution was used as the mobile phase.

Flow rate: 1.0 mL/minute

Injection volume: 20 μ L

Detection wavelength: 275 nm

Column temperature: 40°C

Validation Data:

Linearity: 30 to 70 mcg/mL for sennosides A and B with correlation coefficients over 0.9997.

Accuracy: The percent recoveries were 101.73 and 101.81 for sennosides A and B, respectively.

Precision: The RSD was less than 0.4%.

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: Not specified

Method 3:

The unpublished method of Mingfu Wang was used.

Sample Preparation:

Add 100 mg of herb powder or extract to a 50-mL volumetric flask and add 35 mL of water. Sonicate for 40 minutes and shake for 20 minutes. Cool the sample to room temperature and fill to volume.

Chromatography:

Column: Phenomenex Phenylhexyl, 3 μ m, 150 \times 4.6 mm.

Mobile phase: Solvent A = water (0.2% phosphoric acid), solvent B = acetonitrile.

Gradient: 10%B to 30%B in 20 minutes, then wash with 90%B for 10 minutes.

Flow rate: 1.0 mL/minute

Injection volume: 10 μ L

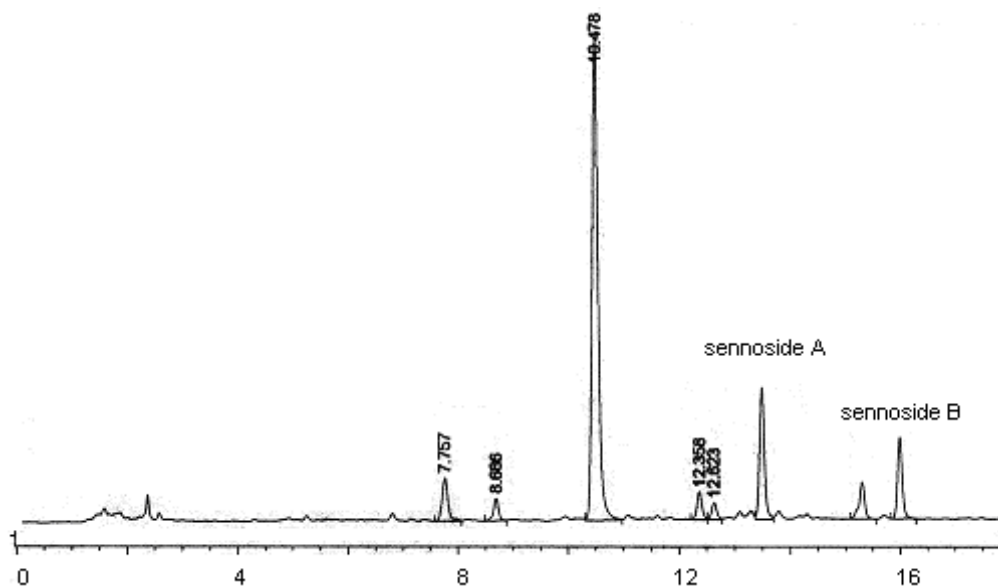
Detection wavelength: 360 nm

Column temperature: Ambient

Validation Data:

Not available

Representative HPLC Chromatogram Run by Method 3



References:

1. Lemli J. The mode of action of the sennosides. *Bull Acad Natl Med.* 1995;179(8):1605–11.
2. Djozan DJ, Assadi Y. Determination of anthraquinones in rhubarb roots, dock flowers and senna leaves by normal-phase high performance liquid chromatography. *Talanta.* 1995;42(6):861–5.
3. Terreaux C, Wang Q, Ioset JR, et al. Complete LC/MS analysis of a *Tinneveli senna* pod extract and subsequent isolation and identification of two new benzophenone glucosides. *Planta Med.* 2002;68(4):349–54.
4. Franz G. The senna drug and its chemistry. *Pharmacology.* 1993;47(S1):2–6.
5. Bala S, Uniyal GC, Dubey T, et al. An improved method for the analysis of sennosides in *Cassia angustifolia* by high-performance liquid chromatography. *Phytochem Anal.* 2001;12(4):277–80.
6. Weng WC, Sheu SJ. Separation of anthraquinones by capillary electrophoresis and high-performance liquid chromatography. *J High Resolut Chromatogr.* 2000;23(2):143–8.
7. Sugiyama A, Nakashima M, Nishida S. Rapid analysis of sennoside A and B in health teas by capillary electrophoresis. *J Health Sci.* 1999;45(5):293–6.
8. Sun SW, Su HT. Validated HPLC method for determination of sennosides A and B in senna tablets. *J Pharm Biomed Anal.* 2002;29(5):881–94.