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Sennosides

DEFINITION

Change to read:

Sennosides is a partially purified natural complex of anthraquinone glucosides, isolated from senna leaflets and/or senna pods, *Senna alexandrina* Mill. [syn. *Cassia acutifolia* ▲Delile▲2S *(USP41)* or *C. angustifolia* ▲Vahl] (Family Fabaceae),▲2S *(USP41)* as calcium salts. It contains NLT 90.0% and NMT 110.0% of the labeled amount of sennosides. The labeled amount is NLT 60.0% (w/w), calculated on the dried basis.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Solvent: Ethyl acetate, *n*-propyl alcohol, and water (1:1:1). Shake well, and discard the upper layer.

Standard solution: 1 mg/mL of USP Sennosides RS in *Solvent*

Sample solution: 1 mg/mL of Sennosides in *Solvent*

Chromatographic system

(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Ethyl acetate, *n*-propyl alcohol, and water (4:4:3)

Analysis: Apply the solutions, as 1-cm bands, on a line 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Develop and dry. Examine the plate under long-wavelength UV light. Expose the plate to ammonium hydroxide vapor until color develops (about 5 min). Cover the plate with a piece of glass, and heat at 120° for 5 min.

Acceptance criteria: The two most prominent spots of the *Sample solution* correspond in color and position to those of the *Standard solution*.

COMPOSITION

• **Content of Total Sennosides**

Buffer: Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of the dibasic sodium phosphate solution. Adjust, if necessary, with the dibasic sodium phosphate solution to a pH of 7.0.

Borate solution: 37.9 g/L of sodium borate in water

Sodium dithionite solution: 15 g/L of sodium dithionite in water

Standard solution: 1 mg/mL of USP Sennosides RS in *Buffer*. Dissolve with the aid of an ultrasonic bath.

Sample solution: 1 mg/mL of Sennosides in *Buffer*

Instrumental conditions

(See *Fluorescence Spectroscopy ⟨853⟩*.)

Mode: Fluorescence

Excitation wavelength: 392 nm

Emission wavelength: 505 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 1-mL portions of the *Standard solution* and the *Sample solution* into separate 100-mL volumetric flasks, and dilute with *Borate solution* to volume. Transfer 5.0-mL portions of each of the resulting solutions to separate, low-actinic glass, 50-mL volumetric flasks. Add 15.0 mL of *Borate solution* and 15.0 mL of *Sodium dithionite solution*. Pass nitrogen through the solutions, seal the flasks with nitrogen-filled balloons, and heat in a water bath for 30 min. Cool the flasks for 15 min in a water bath thermostatically controlled at 20°. Dilute the solutions with *Borate solution* to volume. Determine, without delay, the fluorescence intensities of the resulting solutions, for which the time elapsed between the addition of *Sodium dithionite solution* and the measurement is the same.

Calculate the percentage of the labeled amount of sennosides in the portion of the *Sample* taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times (100/L)$$

I_U = fluorescence value observed in the *Sample solution*

I_S = fluorescence value observed in the *Standard solution*

C_S = concentration of USP Sennosides RS in the *Standard solution*, corrected for loss on drying (mg/mL)

C_U = concentration of Sennosides in the *Sample solution* (mg/mL)

L = labeled amount of total sennosides (mg/mg)

Acceptance criteria: 90.0%–110.0% of the labeled amount of sennosides

Change to read:

• **Content of Sennosides A and B**

Solvent: 1% sodium acetate in water

Buffer solution: Dissolve 3.6 g of dibasic sodium phosphate dodecahydrate in 50 mL of water, add to a solution of 6.2 g monobasic sodium phosphate dihydrate in 200 mL of water, mix, and adjust the pH to 5.0. Dilute the final solution (1:10) in water.

Solution A: Use a filtered and degassed mixture of *Buffer solution* and acetonitrile (1:1), containing 0.5% of benzyldimethylstearylammonium chloride.

Solution B: Use filtered and degassed acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Elution
0–35	100	0	Isocratic
35–40	100→30	0→70	Linear gradient
40–50	30	70	Isocratic
50–55	30→100	70→0	Linear gradient
55–60	100	0	Isocratic

Standard solution A: 0.1 mg/mL of USP Sennoside A RS in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Standard solution B: 0.1 mg/mL of USP Sennoside B RS in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

▲**Standard solution C:** 0.3 mg/mL of USP Sennosides RS in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.▲2S *(USP41)*

Sample solution: 0.3 mg/mL of Sennosides in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: About 1 mL/min, adjusted so the retention time of the sennoside B peak is 30 min

Injection volume: 10 µL

System suitability

Sample: ▲*Standard solution C*▲2S *(USP41)*

Suitability requirements

Resolution: ▲NLT 1.5 between the sennoside B and preceding peaks, between the sennoside B and sennoside A peaks, and between the sennoside A and subsequent peaks▲2S *(USP41)*

Relative standard deviation: NMT 2.0% determined for the sum of the areas of the sennoside A and sennoside B peaks in replicate injections

▲**Chromatogram similarity:** The chromatogram obtained is similar to the reference chromatogram provided with the lot of USP Sennosides RS being used.▲2S *(USP41)*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of sennosides A and B in the portion of Sennosides taken:

$$\text{Result} = (r_U/r_S) \times (C \times V/W) \times 100 \times F$$

r_U = peak area of relevant sennoside in the *Sample solution*

r_S = peak area of relevant sennoside in the corresponding *Standard solution*

C = concentration of relevant sennoside in the corresponding *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Sennosides, corrected for the loss on drying, taken to prepare the *Sample solution* (mg)

F = conversion factor for the molecular weights of sennoside A or sennoside B to the corresponding calcium salt, 1.044

Calculate the total percentage of sennoside A and sennoside B relative to the labeled amount of total sennosides:

$$\text{Result} = [(A + B)/L] \times 100$$

A = percentage of sennoside A

B = percentage of sennoside B

L = labeled amount of total sennosides (%)

Acceptance criteria: The total percentage of sennosides A and B is NLT 60% of the labeled amount of total sennosides, calculated on the dried basis.

CONTAMINANTS

Delete the following:

▲• **Heavy Metals,** *Method II*⟨231⟩**:** 60 µg/g▲ (Official 1-Jan-2018)

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 6.3–7.3, in a 100-mg/mL solution

• **Residue on Ignition** ⟨281⟩**:** 5.0%–8.0%, ignited at 800 ± 25°, the use of sulfuric acid being omitted

• **Loss on Drying** ⟨731⟩

Analysis: Dry under vacuum at 100° to constant weight.

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store protected from light and moisture, at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Sennoside A RS

USP Sennoside B RS

USP Sennosides RS

Topic/Question	Contact	Expert Committee
SENNOSIDES	Nam-Cheol Kim Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines
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Chromatographic Database Information: Chromatographic Database

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