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Analytical Studies on the Active Constituents in Crude Drugs. IV.[1] Determination of Sennosides in Senna and Formulations by High-Performance Liquid Chromatography[2]

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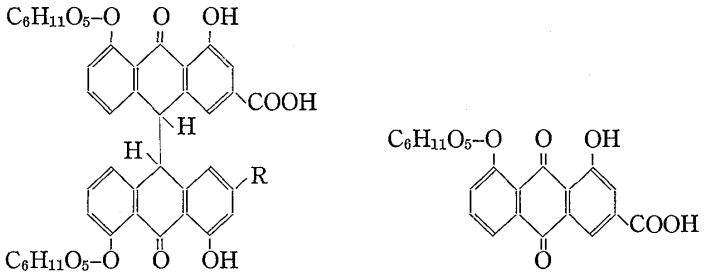
A high-performance liquid chromatographic method for the separation of 8-glucosyl-rhein, sennosides A, B, C, and a new compound, sennoside G, was established. The extraction of sennosides from senna was also investigated. Sennosides, extracted from senna or pharmaceutical preparations, were directly injected and separated using a Permaphase ODS column or a Lichrosorb Rp-18 column, and the sennosides contents in *Sennae* folium pulv., *Sennae* folium, and pharmaceutical preparations containing sennoside Ca were determined.

Keywords——high-performance liquid chromatography; sennosides: determination; *Sennae* folium pulv.; *Sennae* folium; sennoside Ca

Senna is a crude drug which has been used for a long time as a purgative; it appears in several pharmacopoeias (*e.g.* British pharmacopoeia 1973, European pharmacopoeia 1975, DAB 7, Japanese pharmacopoeia 1976, Soviet pharmacopoeia 1961). The active constituents of senna are known to be sennosides A, B, C, and D, while 1,8-dihydroxyanthraquinone derivatives such as chrysophanol, aloe-emodin, rhein, and their glucosides are also present.



Sennoside A: R=COOH
Sennoside B: R=COOH
Sennoside C: R=CH_2OH
Sennoside D: R=CH_2OH

8-glucosyl rhein

Chart 1

For the separation and quantitative determination of sennosides in senna, procedures involving paper chromatography, low-voltage[4] or high-voltage paper-electrophoresis,[5] and thin-layer chromatography (TLC)[6] have been reported, but no reports have appeared concern-

1) Part III: S. Ogawa, A. Yoshida, and R. Kato, *Chem. Pharm. Bull.*, **25** (5), 904 (1977).
2) This work was reported in part at the 96th Annual Meeting of the Chemical and Pharmaceutical Society of Japan, Nagoya, April 1976.
3) Location: *1-8-1, Tatsuminishi, Ikunoku, Osaka.*
4) G.M. Nano, *Pharm. Acta Helv.*, **35**, 451 (1960).
5) J.H. Zwaving, *J. Chromatogr.*, **97**, 109 (1974).
6) S.M. Khafagy, A.N. Girgis, S.E. Khayyal, and M.A. Helmi, *Planta Med.*, **21**, 304 (1972).

ing the application of high-performance liquid chromatography (HPLC) until our presentation at the Annual Meeting of the Chemical and Pharmaceutical Society of Japan.[2)]

Recently Komolafe[7)] reported the detection and separation of sennosides A and B by HPLC, but it was not always satisfactory.

The present paper presents a simple, rapid and accurate high-performance liquid chromatographic method to determine sennosides in *Sennae* folium pulv., *Sennae* folium, and pharmaceutical preparations containing sennoside Ca, which is a kind of extract of senna.

Experimental

Apparatus——A Du Pont-Shimadzu high-performance liquid chromatograph (model 830, 841) equipped with a variable wavelength ultraviolet spectromonitor (Shimadzu UV 202) set at 220 nm was used.

Conditions——The operating conditions were as follows. (A): column, Permaphase ODS, 3 mm i.d. × 50 cm (Umetani glass column (GCT-03), dry-packed with an Umetani auto dry-packer); column temperature, room temp.; mobile phase, 2% dioxane/pH 2.2 Britton–Robinson buffer; flow rate, 1.2 ml/min; sample size, 5 μl; detector, UV 220 nm; sensitivity, 0.08 Aufs.

(B): column, Permaphase ODS, 3 mm i.d. × 50 cm; mobile phase, pH 4.10 Britton-Robinson buffer; flow rate, 2.0 ml/min, other conditions were as in A.

(C): column, Lichrosorb RP-18, 4 mm i.d. × 15 cm; mobile phase, 30% MeOH/pH 3.4 succinate–borate buffer.

Reagents and Materials——All solvents used in this study were of analytical reagent grade. Sennosides A and B were purchased from Sandoz Ltd. (Basel, Switzerland). Sennoside C and the new compound sennoside G were isolated from sennoside Ca and recrystalized from 70% acetone. The color, crystal form and mp (dec.) of the sennosides were as follows: sennoside A, yellow plates, 219—229; sennoside B, yellow needles, 196—209; sennoside C, yellow needles, 197—205; sennoside G, yellow plates, 162—176. *Sennae* folium pulv. (JP), *Sennae* folium (JP), and sennoside Ca were purchased from Japan Powder Co., Ltd. Britton–Robinson buffer, pH 2.2 was prepared from 0.2 N NaOH and 0.04 M acid mixture composed of phosphoric acid, acetic acid, and boric acid. Britton–Robinson buffer, pH 4.10, was prepared from 0.05 N NaOH and 0.01 M acid mixture.

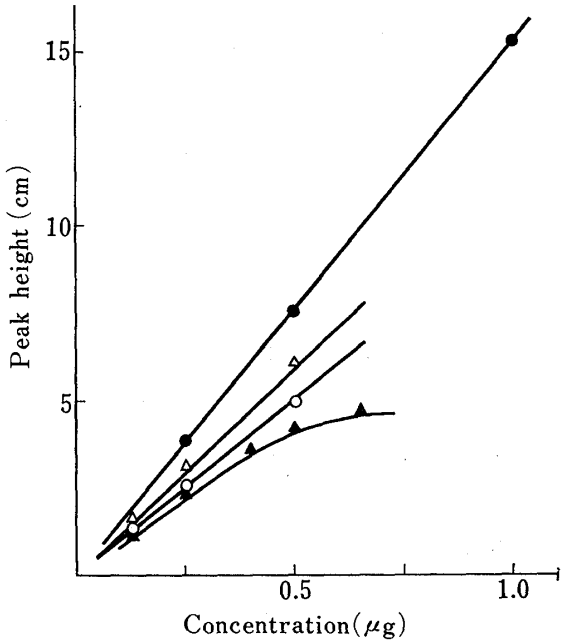


Fig. 1. Calibration Plots

▲—▲ : sennoside A, △—△ : sennoside C,
●—● : sennoside B, ○—○ : sennoside G.

Preparation of a Standard Solution——About 50 mg of sennoside A, 100 mg of sennoside B, 10 mg of sennoside C, and 25 mg of sennoside G were weighed accurately into a 50 ml volumetric flask and diluted to volumn with water; 5.0 ml of this solution was pipetted into a 50 ml volumetric flask and diluted to volume with water.

Preparation of Sample Solution——Senna: About 400 mg of *Sennae* folium pulv. or finely powdered *Sennae* folium was weighed accurately into a 50 ml centrifuge tube with a glass stopper and shaken for 30 min or 60 min (in the case of *Sennae* folium) with 20 ml of water. After centrifugation, the supernatant was filtered through a G-4 glass filter, and 5 μl of the filtrate was subjected to HPLC.

Sennoside Ca: About 80 mg of sennoside Ca was weighed accurately into a 100 ml volumetic flask and diluted to volume with water.

Pharmaceutical Preparations containing Sennoside Ca: Each tablet or capsule was extracted by shaking with 10 ml of water for 60 min, then the extract was filtered through a G-4 glass filter, and the filtrate was used as a sample solution.

Assay Procedure——Five μl of each standard or sample solution was accurately injected into the column and the peak height or area (sennoside A) on the chromatogram was calculated. The quantity of sennosides in sample solutions was calculated by reference to the standard solutions.

7) O.O. Kcmolafe, *J. Chromatogr. Sci.*, **16**, 496 (1978).

Calibration Curves——A calibration curve for each sennoside was constructed by dissolving sennosides in water at various concentrations in the range of 0.01—0.4 mg/ml and injecting 5 μl of each standard under conditions A. Good linearity was established between the concentration of each sennoside and the peak height or area (Fig. 1).

Results and Discussion

Separation of Sennosides

Kuriyama *et al.*[8] has reported an HPLC method for the determination of sennosides in rhubarb and its pharmaceutical preparations. Many interfering substances are present in rhubarb, so the extract cannot be subjected directly to HPLC; separation of the sennosides fraction by pretreatments such as TLC or column chromatography is required, and two types of mobile phase must be used for the complete separation of sennosides under their HPLC conditions.

Several column packing materials and mobile phases were examined, and a reversed phase partition chromatography using a Permaphase ODS column (3 mm i.d. × 50 cm) with a mobile phase of 2% dioxane/pH 2.2 Britton–Robinson buffer was selected for the present purpose.

Chromatograms of sennosides determined under these conditions (conditions A) are shown in Fig. 2. Although the peak of sennoside A is not symmetrical, as shown in Fig. 2, and the retention volume changes with its concentration, it can be estimated quantitatively by measuring the peak area, since good linearity was obtained between the concentration and the peak area, and it was separated sufficiently from adjacent peaks.

Fig. 2 shows the chromatogram of sennosides in *Sennae* folium pulv., and sennoside Ca. When sample solutions of senna were allowed to stand for 5—10 days at room temperature, the greater parts of these peaks disappeared, as shown by the dotted line in Fig. 2. However,

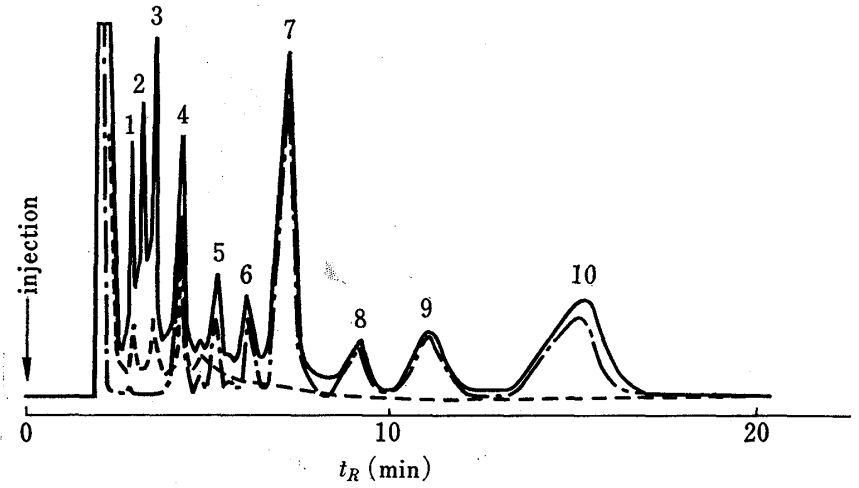


Fig. 2. Chromatograms of Sennosides in *Sennae* Fol. Pulv. (JP) and Sennoside Ca

——— : *Sennae* Fol. Pulv. (JP)
– – – – : *Sennae* Fol. Pulv. (JP) after 5 days
—·—·— : Sennoside Ca
3 : 8-glucosylrhein 7 : sennoside B 8 : sennoside C
9 : sennoside G 10 : sennoside A
Conditions : Permaphase ODS (3mm i.d. × 50cm), room temp.; mobile phase, 2% dioxane/pH 2.2 buffer; flow rate, 1.2ml/min; detector, UV 220 nm; sensitivity, 0.08 Aufs.

8) M. Kuriyama, J. Takahashi, K. Inoue, M. Muramatsu, T. Sado, K. Kawai, and Iwata, "Koseikagaku Kenkyuhokoku," 1975, p. 235—260.

if the sample solutions were filtered through a membrane filter, no chromatographic changes were observed. This suggests that a β-glucosidase in senna was extracted together with sennosides, and hydrolyzed them; thus, great care is required in sample preparation.

Ultraviolet absorption (UV) spectra of the ten peaks shown in Fig. 2 were measured by a wavelength scanning method at the top of each peak. Fig. 3 shows the UV spectrum of each peak. Peaks 5—10 showed similar spectra, characteristic of sennosides, and that of peak 3 coincides with the spectrum of standard 8-glucosylrhein. The spectra of peaks 7, 8, and 10 were identical with those of sennosides B, C, and A, respectively.

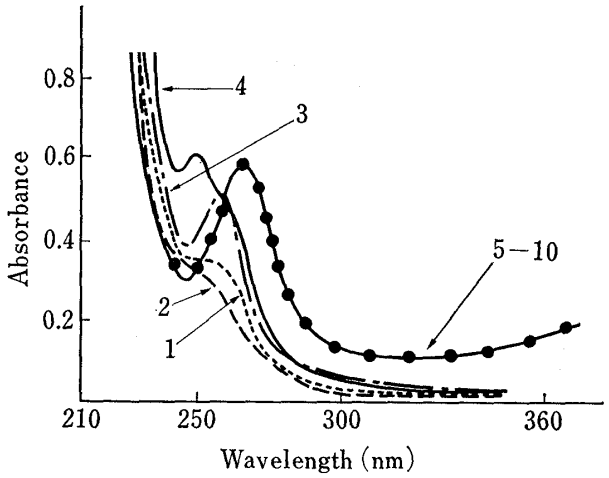


Fig. 3. UV Spectra of Peaks 1—10 in Fig. 2

Each spectrum was taken at the top of the peak in Fig. 2 by a wavelength scanning method.

When sennoside C was treated with sodium bicarbonate, peak 5 appeared in a chromatogram. This indicates peak 5 to be sennoside D, since sennoside C is known to isomerize to sennoside D in alkaline solution. On the other hand, peaks 6 and 9 gave the same UV spectrum as sennosides A—D and were hydrolyzed similarly during standing, so they appeared to be new sennosides. The compound corresponding to peak 9 was isolated from sennoside Ca by diethylaminoethylcellulose chromatography; its infrared and UV spectra (in 0.5% $NaHCO_3$) were very similar to those of sennoside B. The *Rf* value on TLC [Wako gel B-5F; *n*-PrOH–AcOEt–water (4: 4: 3)] was 0.25, and the spot turned yellow when sprayed with an alkaline solution.

Oshio *et al.*[9] reported the isolation of the new sennosides E and F from rhubarb, but they showed different behavior from this compound in HPLC. We therefore named this compound sennoside G.

A chromatogram of sennoside Ca dissolved in water is shown in Fig. 2, and is characterized by its simplicity owing to the disappearance of peaks attributed to anthraquinones.

Extraction of Sennosides from Senna

The extraction of sennosides from *Sennae* folium pulv. and *Sennae* folium was studied, and the results are shown in Table I. Extraction by shaking with water or pH 6.5 phosphate buffer seemed appropriate. Fig. 4 shows the effect of extraction time, indicating that the degree of extraction of sennosides A—C and G was unchanged between 15 and 90 min. As shown in Fig. 5, the degree of extraction was unchanged by increasing the number of extraction procedures. These results show that extraction by shaking for 30 min with water or pH 6.5 phosphate buffer is most effective. In the case of *Sennae* folium, the efficiency of extraction decreases, and it must be finely powdered and shaken for 1 hr.

Khafagy *et al.*[6] reported that 70 per cent methanol was the best solvent for extraction from senna leaves or pods, but we obtained a different result.

Determination of Sennosides

Analytical results for sennosides in commercially available *Sennae* folium pulv., *Sennae* folium, and sennoside Ca determined by the present method are summarized in Table II.

9) H. Oshio, S. Imai, S. Fujioka, T. Sugawara, M. Miyamoto, and M. Tsukui, *Chem. Pharm. Bull.*, **20**(3), 621 (1972); *idem*, *ibid.*, **22**(4), 823 (1974).

Table I. The Effects of Various Extraction Solvents and Methods

	Shaking time (min)	Percent extracted (%)		Reflux time (min)	Percent extracted (%)	
		S-A	S-B		S-A	S-B
Water	30	99	98	30	89	71
pH 6.5 buffer	30	100	100	30	86	78
70% MeOH	30	23	17	30	23	15

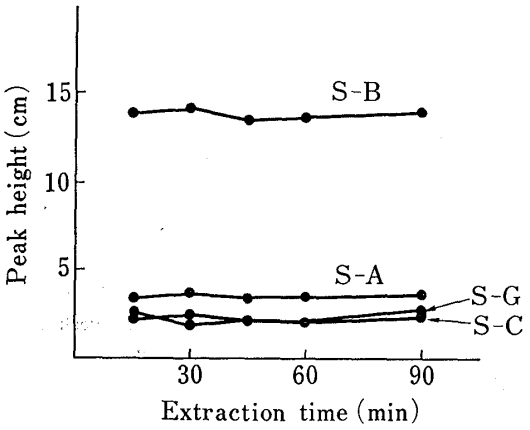


Fig. 4. The Effect of Time of extraction of sennosides A-C and G from *Sennae* Fol. with water

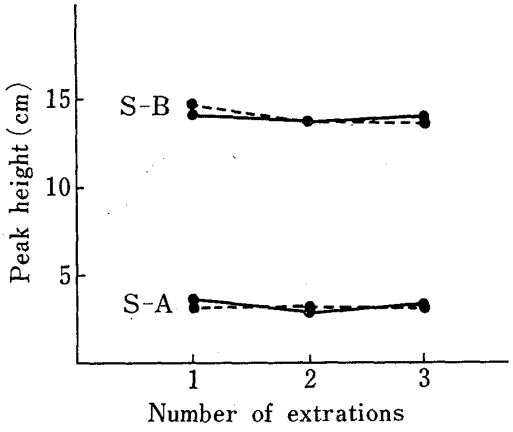


Fig. 5. The Effect of Number of Extraction

Sennosides A and B were extracted from *Sennae* Fol. with water and pH 6.5 phosphate buffer.
—— : water ---- : pH 6.5 phosphate buffer

As standards were available for sennosides A, B, C, and G, the contents of these compounds were determined. The total content of sennodises A—C and G in senna was about 1.7%, while that in sennoside Ca was about 47%. In all cases, the content of sennoside B was the highest and amounted to twice that of sennoside A.

Table III shows the analytical values for sennosides in pharmaceutical preparations containing sennoside Ca as an active ingredient, demonstrating that these laxatives include 3.8—9.0 mg of sennosides A—C and G per tablet or capsule.

Additional HPLC Conditions

The determination of sennosides in senna and pharmaceutical preparations can be achieved by the above HPLC method, but there are problems in that continually decreasing retention times and resolution are obtained during the use of the column; that is the column performance deteriorates. This is especially undesirable in routine analysis of pharmaceutical preparations.

Table II. Sennosides Contents in *Sennae* Fol. (JP), *Sennae* Fol. Pulv. (JP), and Sennoside Ca

Sample	Content (w/w%)				
	S-A	S-B	S-C	S-G	Total
Senn. Fol. (JP)	0.458	0.838	0.149	0.214	1.659
Senn. Fol. Pulv. (JP) 1	0.546	0.894	0.172	0.199	1.811
Senn. Fol. Pulv. (JP) 2	0.512	0.780	0.164	0.202	1.658
Sennoside Ca 1	11.0	26.8	2.0	6.3	46.1
Sennoside Ca 2	17.8	23.1	2.0	6.9	49.8

Table III. Sennosides Contents in Pharmaceutical Preparations Containing Sennoside Ca

Laxatives (Sennoside Ca)	Content (mg/tab.)					(w/w %)	
	S-A	S-B	S-C	S-G	Total	S-A	S-B
1 (12 mg/Tab.)	2.81	3.25	0.24	1.05	7.35	23.4	27.1
2 (12 mg/Tab.)	2.21	2.54	0.39	0.81	5.95	18.4	21.2
3 (10 mg/Tab.)	1.11	2.84	0.19	0.45	4.59	11.1	28.4
4 (20 mg/Cap.)	2.12	5.14	0.40	1.39	9.05	10.6	25.7
5 (8 mg/Tab.)	0.92	2.19	0.20	0.55	3.86	11.5	27.3

Further investigations of the HPLC conditions were therefore carried out. The effective life of an HPLC column depends on both the system and the conditions under which the column is operated. One factor decreasing the efficiency of the Permaphase ODS column was presumed to be the pH of the mobile phase used, so pH 4.10 Britton–Robinson buffer (conditions B) was substituted for 2% dioxane/pH 2.2 buffer. Under conditions B, sennosides in sennoside Ca could also be separated (Fig. 6), and column damage was reduced. However, conditions B could not be applied for the determination of senna due to incomplete separation of sennosides and other interfering constituents.

When a Lichrosorb Rp-18 column was used (conditions C) instead of Permaphase ODS, sennosides were strongly retained and sufficiently separated with 30% MeOH/pH 3.4 succinate and borate buffer (Fig. 7); moreover, the life of the column was greatly increased. However, conditions C require a long operating time (over 30 min) and a high pressure (300 kg/cm^2), and way not be suitable for routine work.

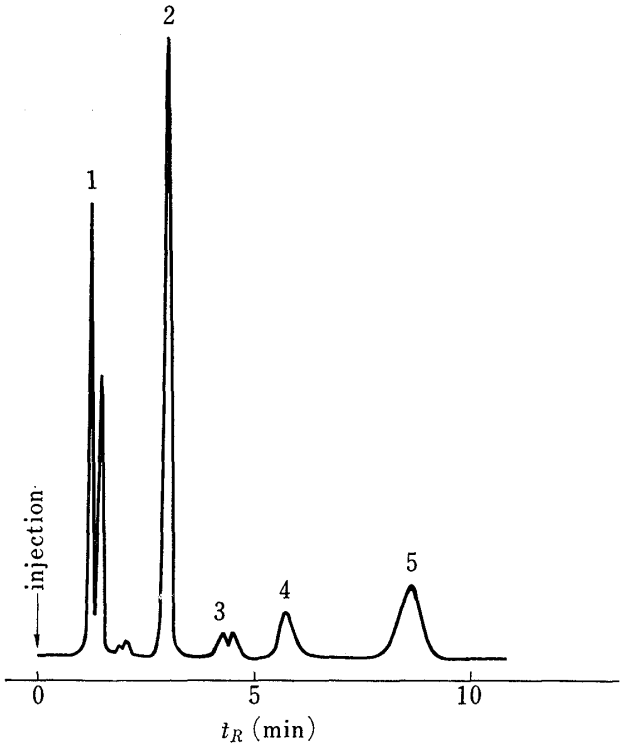


Fig. 6. Chromatogram of Sennosides in Sennoside Ca

1 : 8-glucosylrhein 2 : sennoside B 3 : sennoside C
4 : sennoside G 5 : sennoside A
Conditions: Permaphase ODS (3 mm i.d. × 50 cm), room temp.; mobile phase, pH 4.10 buffer; flow rate, 2.0 ml/min; detector, UV 254 nm; sensitivity, 0.04 Aufs.

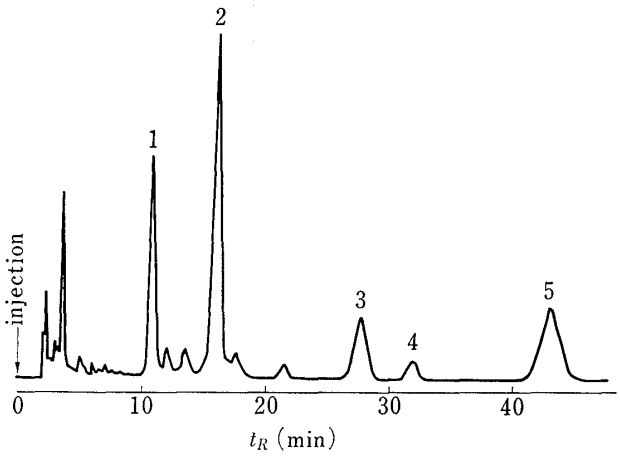


Fig. 7. Chromatogram of Sennosides in Sennoside Ca

1 : 8-glucosylrhein, 2 : sennoside B 3 : sennoside G,
4 : sennoside C 5 : sennoside A
Conditions: Lichrosorb RP-18 (4 mm i.d. × 15 cm), room temp.; mobile phase, 30% MeOH/pH 3.4 buffer; detector, UV 254 nm; sensitivity, 0.08 Aufs.

Consequently, for the simple, rapid and accurate determination of sennosides in *Sennae* folium pulv., *Sennae* folium, and pharmaceutical preparations, conditions A using a Permaphase ODS column with a mobile phase of 2% dioxane/pH 2.2 buffer are useful, while for routine analysis of pharmaceutical preparations containing sennoside Ca, conditions B can be recommended.

The present method provides a useful tool for the quantitative determination of sennosides A, B, C, and the new compound sennoside G, as well as for the identification of 8-glucosylrhein in senna or its formulations.

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