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Note

Separation method for sennosides

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Rhubarb and senna have been used in the traditional and folklore medicines of both the east and west as laxatives, stomachic medicines and antidotes. Anthraquinones and bianthranyls and their glycosides and tannins have been found to be constituents, with the bianthranyl glucosides, sennosides being the principal purgative agents[1–4].

Several quantitative analyses of sennosides by high-performance liquid chromatography (HPLC) using gel-permeation chromatographic (GPC) and octadecylsilyl columns have been reported[5–9]. However these were very inefficient for practical analyses, involving long run-times and complicated pretreatments.

We have now developed a new analytical procedure for sennosides using HPLC on a dimethylamino column with a simple pretreatment using a Waters Sep-Pak C_{18} cartridge.

Materials

All solvents were of analytical-reagent grade and were obtained from Kanto Kagaku (Tokyo, Japan).

Apparatus

The liquid chromatograph used consisted of an SP-800-150SD (Sensyu Scientific, Suginamiku, Tokyo) solvent pumping system, a UVILOG-5III (Oyobunko) UV detector, and a Rheodyne Model 7125 injection valve. Chromatograms were recorded on a Seconic SS-250f recorder.

Experiments were performed on a Sensyu-Pak SN-342N column (25 cm × 8.0 mm I.D.) consisting of Nucleosil 5 $N(CH_3)_2$ (Macherey, Nagel & Co., Düren, G.F.R.).

The mobile phase consisted of tetrahydrofuran (THF)–water–acetic acid (8:2:1).

Pretreatment

Each 0.5 g sample of finely powdered rhubarb and senna was extracted with 10 ml of aqueous methanol (70%) for 30 min on a water bath at 40°C. The extract was evaporated and dried at 50°C and the residue was dissolved in methanol–water–acetic acid (27:73:1) (10 ml). A Waters Sep-Pak C_{18} cartridge was conditioned with 10 ml of the above solvent system before use. The sample solution (0.5 ml) was

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charged on to the cartridge and chloroform–methanol (8:1) (4 ml) was passed through the cartridge to remove any anthraquinone derivatives. The sennoside fraction was obtained using methanol–water–acetic acid (27:73:1) as eluent. The eluent was evaporated and dried at 50°C *in vacuo*. The residue was then dissolved in 10 ml of the mobile phase.

RESULTS AND DISCUSSION

The quantitative analysis of sennoside in rhubarb, which necessarily involves a good baseline separation, is rather difficult, because rhubarb, compared with senna, contains a greater number of anthraquinones and other compounds. Thus a pretreatment is required. In the GPC method, a complicated treatment was needed before using thin-layer chromatography (TLC) or a cellulose column, while the same problem still existed with the pretreatment using Sep-Pak cartridge silica owing to the poor recovery of sennosides.

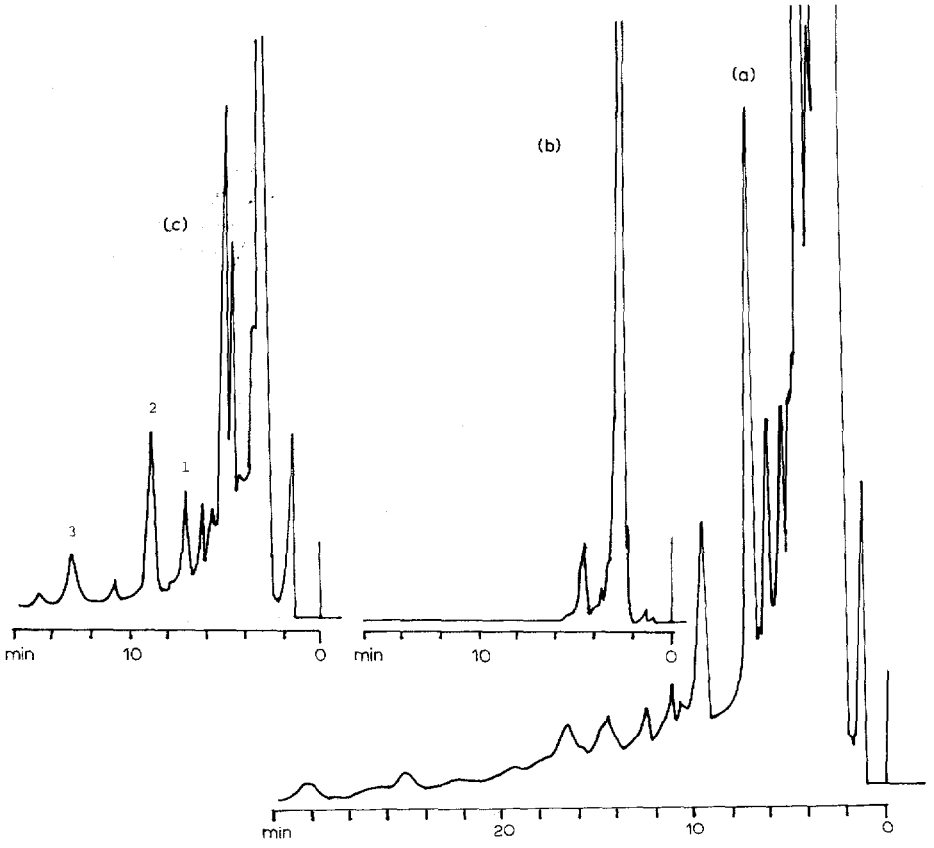

Fig. 1. HPLC of 70% methanol extracts of rhubarb (JP XI). Column, Senshu Pak SN-342N (25 cm × 8.0 mm I.D.); mobile phase, THF–water–acetic acid (8:2:1); flow-rate, 3.5 ml/min; detection, UV 280 nm (a) without pretreatment; (b) the first fraction after pretreatment with Waters Sep-Pak C_{18} cartridge; and (c) the second fraction after pretreatment with Waters Sep-Pak C_{18} cartridge. Peaks: 1 = Sennoside C; 2 = Sennoside A; 3 = Sennoside B.

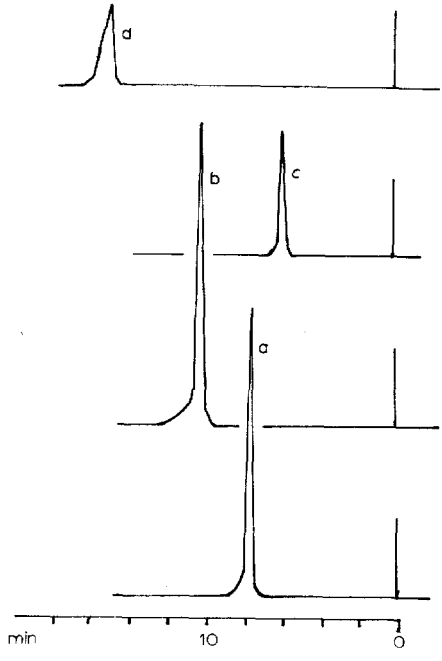

Fig. 2. HPLC of standard samples of sennosides. Column, Senshu Pak SN-342N; mobile phase, THF–water–acetic acid (8:2:1); flow-rate: 3.5 ml/min; detection, UV 280 nm. (a) sennoside A; (b) sennoside B; (c) sennoside C; (d) sennoside E.

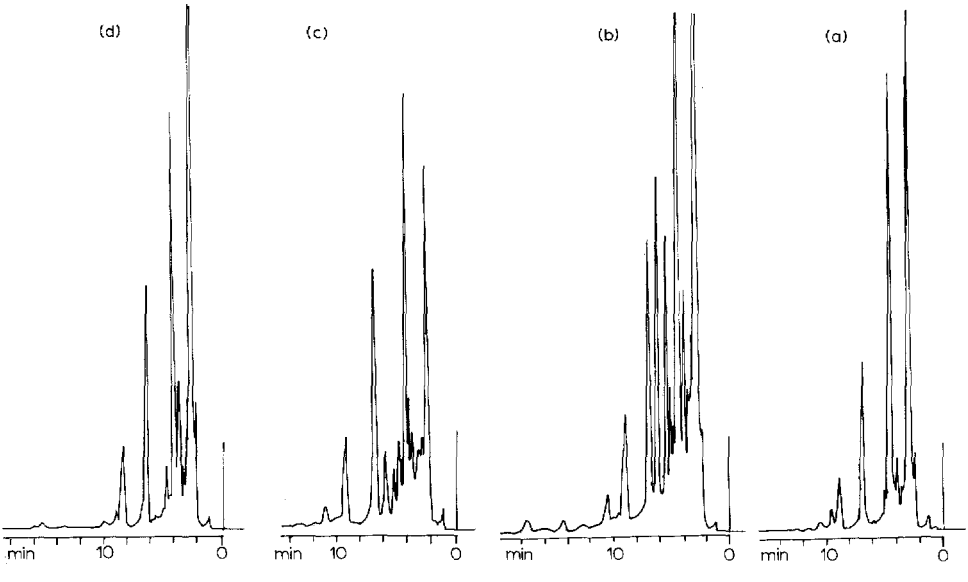

Fig. 3. HPLC elution of the methanolic extracts of various species of rhubarb. Pretreatment with Waters Sep-Pak C_{18}. Column, SN-342N (25 cm × 8.0 mm I.D.); mobile phase, THF–water–acetic acid (8:2:1); flow-rate, 3.5 ml/min; detection, UV 280 nm. (a) *Rheum palmatum var. tanguticum;* (b) *Rh. coreanum;* (c) *Rh. palmatum;* (d) *Rh. coreanum* × *Rh. palmatum* (Shinshu-rhubarb).

The GPC method also encounters difficulties in the baseline separation, and for preparative use a long time is needed for complete separation. Paired ion chromatography shows good results for qualitative analysis, but again is no use for preparative separations. The ODS method can only use a UV wavelength of 340 nm for detection, which is unsuitable for baseline separation and for the preparative isolation of sennosides.

We have now improved the pretreatment procedure by using a Waters Sep-Pak C_{18} (reversed-phase) cartridge to separate easily the sennoside fraction from the extracts of rhubarb and senna with excellent yields. After the preatreatment, the sennoside fraction was charged on to a Senshu-Pak SN-342N column with UV detection at 280 and 360 nm to demonstrate the excellent separation of all the sennosides within 20 min (Figs. 1 and 2).

The above process can be scaled up (Senshu-Pak SN-432N column; 20 cm × 20

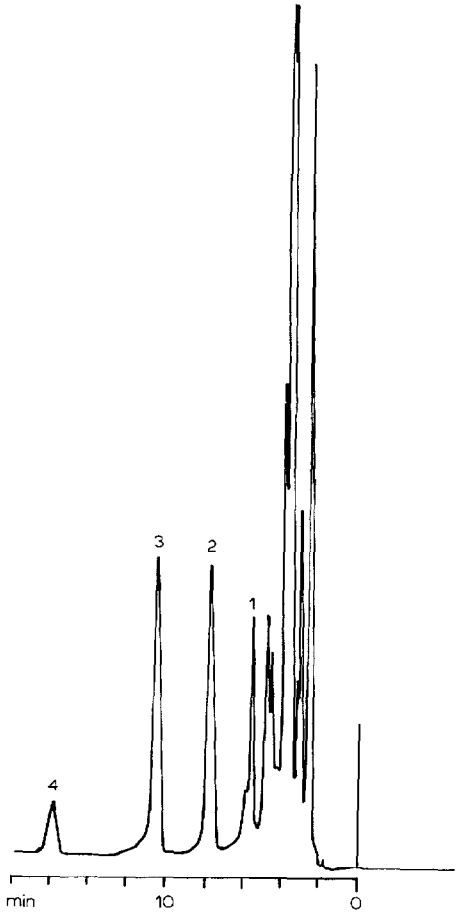

Fig. 4. HPLC elution profile of methanolic extracts of senna leaves. Pretreatment with Waters Sep-Pak C_{18}. Column, Senshu Pak SN-342N (25 cm × 8.0 mm I.D.); mobile phase, THF–water–acetic acid (8:2:1); flow-rate: 3.5 ml/min; detection: UV 280 nm. Peaks: 1 = Sennoside C; 2 = Sennoside A; 3 = Sennoside B; 4 = Sennoside E.

mm I.D.) for the preparative separation of sennosides from rhubarb and senna using a volatile mobile phase.

Satisfactory results were obtained by the present procedure and are shown in Figs. 3 and 4. By the present procedure a simultaneous HPLC analysis of the constituents of rhubarb is possible in a single operation.

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