Journal of Chromatography, 360 (1986) 303–306
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 550

Note

High-performance liquid chromatographic separation of rhubarb constituents

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(Received January 21st, 1986)

Sennosides are the purgative principles of rhubarb and senna, which have been in common use since ancient times as medicaments. Previously we reported[1,2] an efficient procedure for the separation of sennoside A, B, C, D, E and F using high-performance liquid chromatography (HPLC) on a dimethylamino-bonded silica gel column, which is generally applicable for the separation of acidic compounds. This method was also applied to the qualitative and quantitative determination of sennosides in the extracted preparations of Chinese medical recipes containing rhubarb[3].

Rhubarb contains a wide variety of components, including monomeric anthraquinones, their glycosides and dimeric anthrone glycosides. For the simultaneous separation of these constituents, we have developed an HPLC procedure using a gradient solvent system. Three-dimensional UV absorbance–HPLC pattern and contour line map displays have also been developed. The above three-dimensional HPLC display is useful for the identification of the chemical types of the components from their UV absorption curves revealed simultaneously on the chromatogram.

EXPERIMENTAL

We used our new program and the MCD-350 system (Union Giken, Kyoto, Japan) for displaying three-dimensional patterns and contour maps of HPLC. The microcomputer used in the MCPD-30 consisted of SORD M223 MKV, CRT, an 8-in. twin disk drive system and printer–plotter. The basic compiler used was CBASIC Program 04E.

Extraction

Rhubarb (1.0 g) was extracted with hot chloroform and 70% methanol, and after evaporation of the extract to dryness the residue was dissolved in 2 ml of methanol. A 10-μl volume of the solution was used for a single HPLC operation.

Authentic samples

All the compounds were individually identified in comparison with authentic samples.

0021-9673/86/$03.50

Instruments

The HPLC system consisted of a Spectra-Physics SP-8700 solvent-delivery system with a Rheodyne 7125 injection valve and the MCPD-350 system (Union Giken) coupled with a microcomputer system. Our new program for the presentation of contour maps and three-dimensional UV absorbance–HPLC traces (90° display) was used.

The following conditions were used for the HPLC of rhubarb extracts: column, Senshu Pak SN-352N (Senshu Scientific, Tokyo, Japan) (dimethylamino-bonded), 15 cm × 4.6 mm I.D.; eluent, gradient conditions with 15% acetic acid–tetrahydrofuran (THF), 0 min 0:10, 25 min 1:9 and 60 min 6:4; and flow-rate, 1.0 ml/min.

All the solvents employed were of HPLC grade.

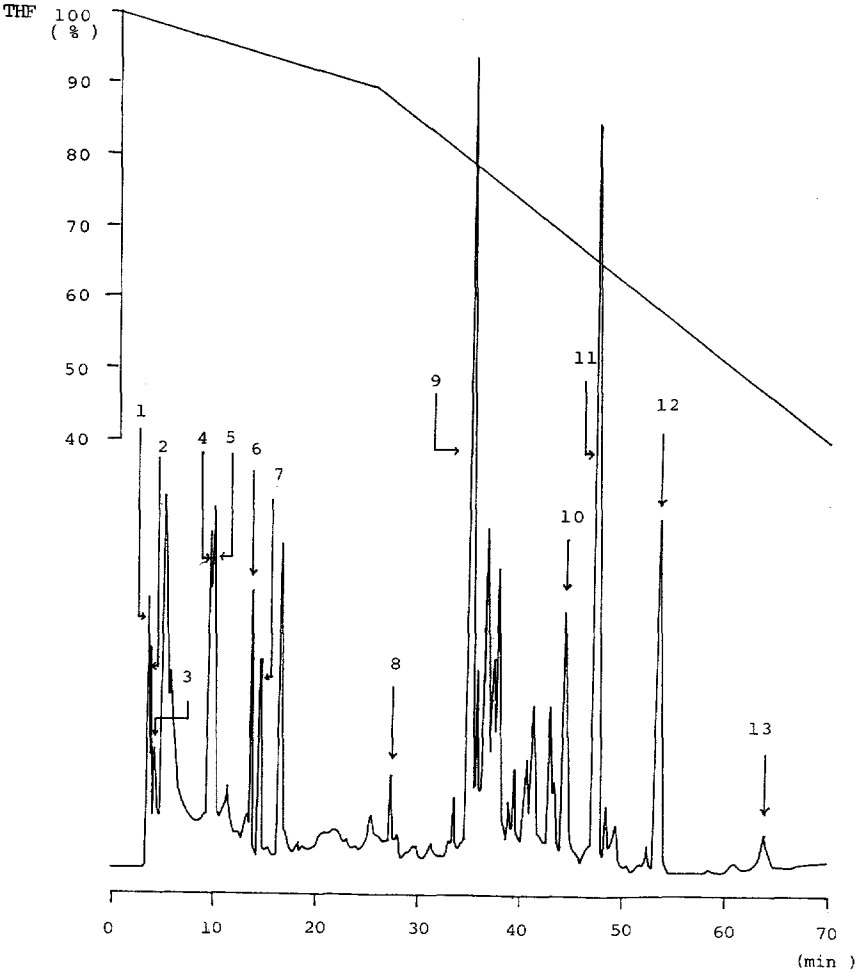

Fig. 1. HPLC elution profile of methanol extracts of rhubarb. Column, Senshu Pak SN-352N (dimethylamino-bonded (15 cm × 4.6 mm I.D.) flow-rate, 1.0 ml/min; detector, UV (361 nm). Peaks: 1 = chrysophanol–physcion mixture; 2 = emodin; 3 = aloe-emodin; 4 = chrysophanol-1-glucoside–8-glucoside mixture; 5 = aloe-emodin-8-glucoside; 6 = citreorosein; 7 = emodin-1-glucoside; 8 = rhein; 9 = rhein-8-glucoside; 10 = sennoside C; 11 = sennoside A; 12 = sennoside B; 13 = sennoside E.

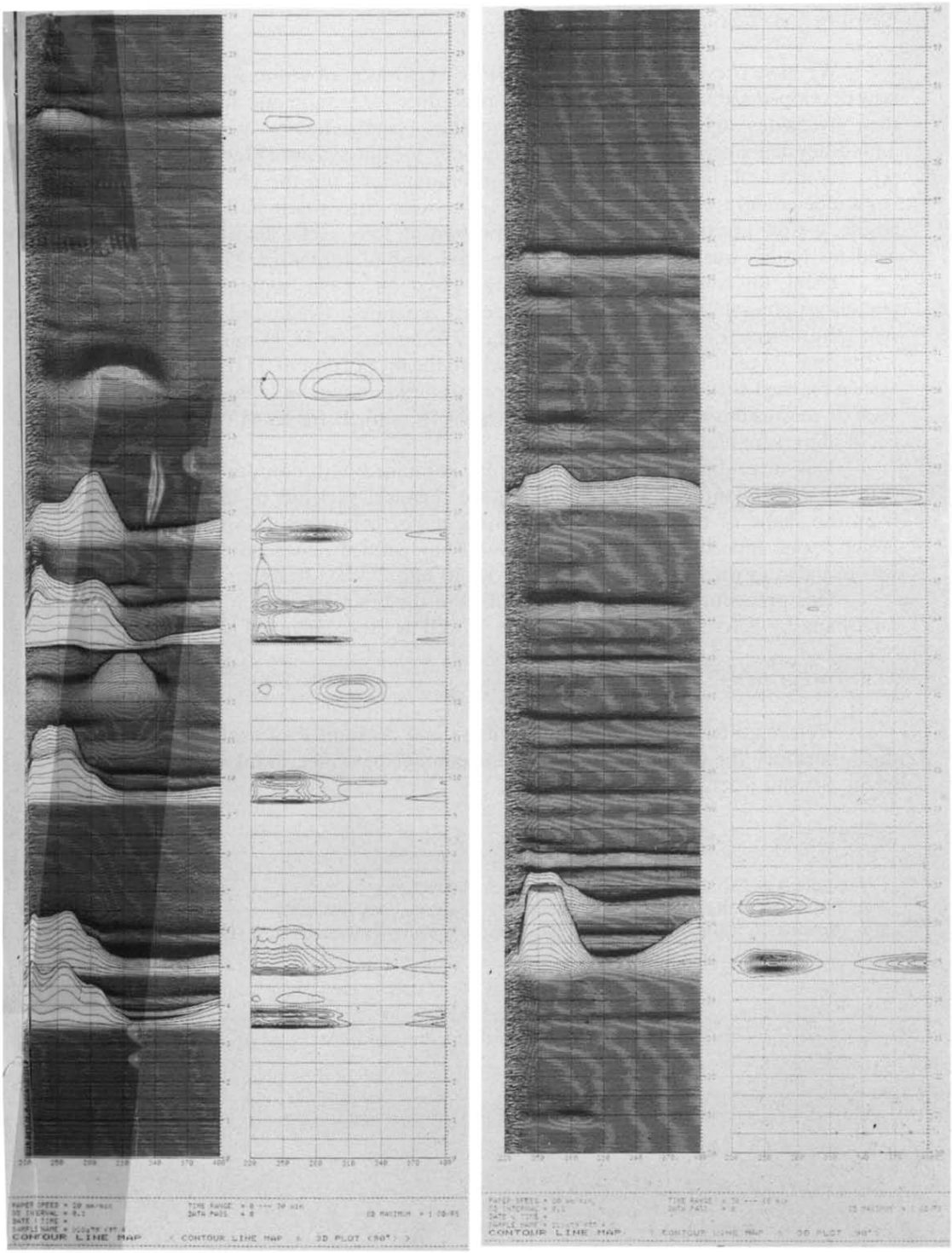

Fig. 2. Three-dimensional plot (90° and contour line map of rhubarb extract. Retention times (min) are indicated in parentheses. Chrysophanol and physcion (3.52); emodin (3.80); aloe-emodin (4.18); chrysophanol-1- and -8-glucoside (9.48); aloe-emodin-8-glucoside (9.91); citreorosein (13.67); emodin-1-glucoside (14.53); rhein (27.25); rhein-8-glucoside (34.99); sennoside C (36.44); sennoside A (47.18); sennoside B (53.44).

RESULTS AND DISCUSSION

We have previously reported the HPLC separation of sennoside[1–3], and have now developed a simultaneous separation procedure for monomeric anthraquinones, their glycosides and dimeric anthrone glycosides (sennosides) by using a dimethylamino-bonded silica gel column and a gradient solvent system (Figs. 1 and 2). To separate most efficiently the monomeric anthraquinones and the glucosides of rhubarb, aloe-emodin, emodin, aloe-emodin-8-glucoside, citreorosein and emodin-1-glucoside, a slow linear increase of the proportion of 15% acetic acid in THF in the gradient system was applied, reaching the final ratio of 1:9 within 25 min.

Using this system, chrysophanol and physcion or chrysophanol 8-glucoside and chrysophanol 1-glucoside were not clearly separated. Rhein and rhein 8-glucoside were detected at retention times (t_R) of 27.25 min and 34.99 min, respectively. In the final stage of the gradient elution, where the proportion of THF in the solvent system reached 40%, more acidic monomeric anthraquinones and dimeric anthrones, such as sennosides C (t_R 36.48 min), A (t_R 47.18 min), B (t_R 53.44 min) and E (t_R 63.76 min) were eluted in this sequence.

This three-dimensional HPLC display combined with contour map graphics (Fig. 2) was advantageous in identifying the chemical skeleton of the constituents from the shape of the peaks and in locating minor components in the shadow of larger peaks. Indeed, the presence of several unidentified new sennoside analogues was revealed in the retention time range 36–50 min.

This procedure can usefully be applied to the practical evaluation of rhubarb and senna by determination of sennoside as well as other chemical constituents.

ACKNOWLEDGEMENTS

We are grateful to Professor S. Shibata and Asistant Professor Y. Hiraga of this laboratory for their kind advice and encouragement. We thank Mr. S. Yamaguchi, Senshu Scientific Co.

REFERENCES

1 Y. Ohshima and K. Takahashi, *J. Chromatogr.*, 258 (1983) 292.
2 K. Takahashi, H. Kaizuka and Y. Ohshima, *J. Chromatogr.*, 268 (1983) 522.
3 Y. Ohshima, K. Takahashi, Y. Hiraga and S. Shibata, *Shoyakugaku Zasshi*, 37 (1983) 217.