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Note

High-performance liquid chromatographic analysis of licorice extracts

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Licorice root is the root or storon of *Glycyrrhiza* spp. which is used mainly as a sweetening agent and a drug. Several species of licorice are marketed as a drug in China, for example, Tongpei, Si-pei, Shingkiang, Russian, Iranian, Iraqi and Spanish licorice. They can be identified chemically by the presence of characteristic chalcones, flavanones, isoflavanones, coumestans, flavonols, isoflavans, isoflavenes, retrochalcones and glycyrrhizin analogues. Although several reports[1–4] have been made on the high-performance liquid chromatographic (HPLC) separation of licorice constituents, they are mostly concerned with glycyrrhizin and its analogues. Shibata and Saitoh[5] described a thin-layer chromatographic (TLC) profile analysis of licorice extracts with the aim of identifying licorice species on the market, but it was a qualitative semi-micro analysis in which, for example, liquiritin and isoliquiritin were not separated. We have now developed an HPLC method which employs an ODS column with a gradient solvent system for evaluation of licorice roots of various species and determination of their chemical constituents.

EXPERIMENTAL

Chromatograph

The HPLC system consisted of a Spectra Physics SP-8700 solvent-delivery system and UV detector (UV-1500; Sensyu Scientific, Tokyo, Japan) and integrator 7000A (System Instruments, Tokyo, Japan). The column was a Sensyu-Pak SSC-ODS-432 (C_{18}, particle size 7 μm, 25 cm × 4.6 mm I.D.; Sensyu Scientific).

Solvent system

The solvent gradient was as follows: initial state, acetonitrile–3% acetic acid (1:4); final state, acetonitrile–3% acetic acid (9:1); linear gradient, 60 min; flow-rate 1 ml/min.

The solvents used were all HPLC grade.

Extraction

Licorice root (Si-pei licorice, 0.5 g) was extracted with 20 ml of hot methanol for 3 h. The extract was dissolved in 5 ml of methanol for injection.

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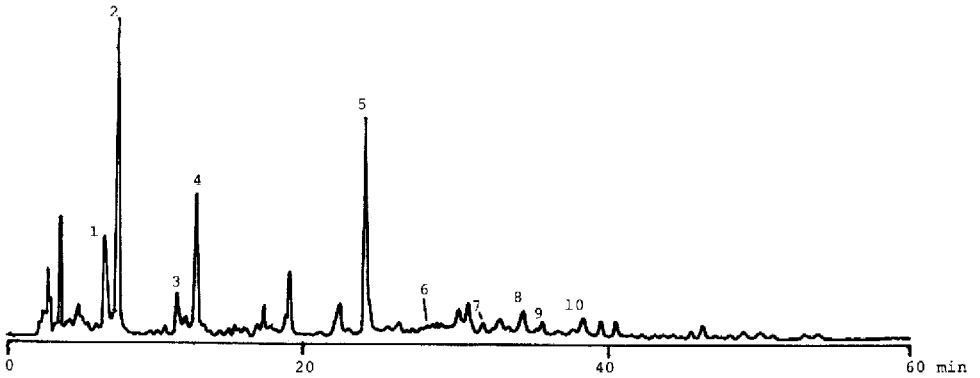

Fig. 1. HPLC separation of an extract of Si-pei licorice. For conditions, see Experimental. Peaks: 1 = neoliquiritin; 2 = liquiritin; 3 = neoisoliquiritin; 4 = isoliquiritin; 5 = glycyrrhizin; 6 = licoisoflavone C; 7 = kumatakenin; 8 = licoisoflavone A and licochalcone A; 9 = glycyrol; 10 = licoflavonol.

RESULTS AND DISCUSSION

In order to analyse licorice components of various polarities, methanolic extracts of licorice, without any pretreatment, were subjected to chromatography on an ODS (7 μm) column (25 cm × 4.6 mm I.D.) using gradient elution. The retention time of each standard sample of licorice (Table I) is reproducible in spite of the long analysis time (60 min) and repeated trials (Fig. 1).

TABLE I

RETENTION TIMES OF LICORICE COMPONENTS ON AN ODS COLUMN

Linear gradient of acetonitrile–3% acetic acid (1:4) to acetonitrile–3% acetic acid (4:1) over 60 min, flow-rate 1 ml/min.

Component	*Retention time (min)*
Neoliquiritin	6.69
Liquiritin	7.50
Neoisoliquiritin	11.57
Isoliquiritin	12.79
Glycyrrhizin	24.33
Isoliquiritigenin	24.66
Licoisoflavone C	28.88
Licoflavone B	32.14
Glabrene	32.45
Kumatakenin	33.13
Licoisoflavone A	34.40
Licochalcone A	34.50
Glycyrol	35.85
Glabridine	37.31
Glabrone	37.80
Licoflavonol	38.33
Glycyrin	39.57
Licoisoflavone B	39.90
Glabrol	40.50

In the retention time region of 6–13 min, each pair of flavanone (neoliquiritin and liquiritin) and chalcone glycosides (neoisoliquiritin and isoliquiritin) were separated on the chromatogram. Glycyrrhizin, a characteristic saponin of licorice, showed a retention time of 24.33 min. After the elution of glycyrrhizin, in the case of Si-pei licorice, non-glycosidic components were eluted in the following sequence: kumatakenin (flavonol), licoisoflavone C (isoflavone), glycyrol (coumestan), licoflavonol (flavonol), licoisoflavone B (isoflavone).

This separation method could be used not only for analysis or chemical evaluation of licorice roots, but also for detecting new minor chemical constituents. The quantitative analysis of licorice is in progress using this HPLC system. The practical application of this method to the evaluation of various species of licorice will be reported elsewhere.

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